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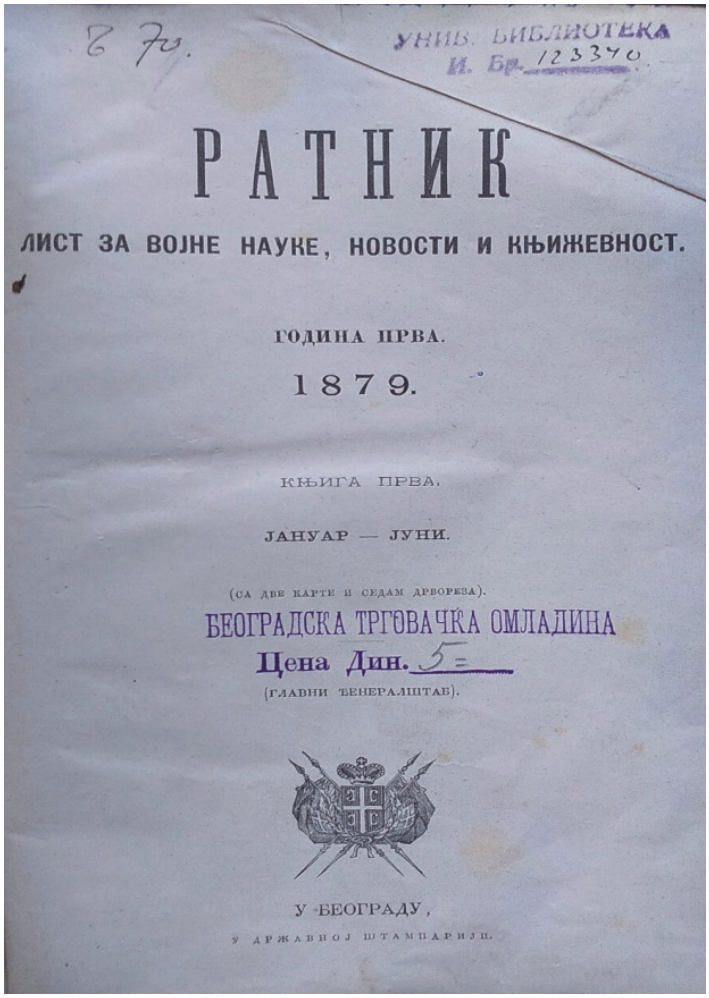

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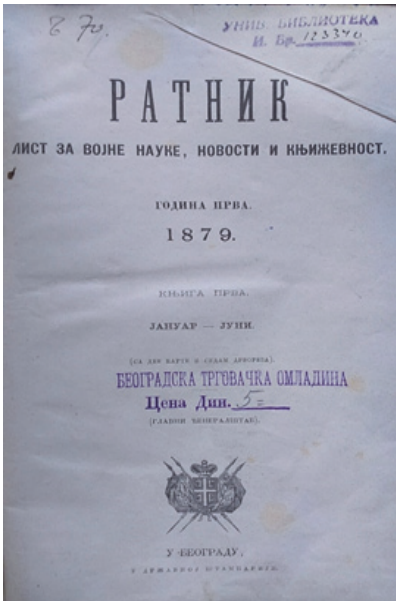
ORIGINAL ARTICLES / ORIGINALNI RADOVI



Naslovna strana prvog broja časopisa „Ratnik", prvog naučno-stručnog časopisa Vojske Srbije koji je izlazio od januara 1897. godine do marta 1941. godine, sa prekidom u toku Prvog svetskog rata. U njemu su objavljivani aktuelni prilozi iz različitih vojnih disciplina, kao i teme iz istorije ratovanja. Do pojave „Vojnosanitetskog glasnika", preteče „Vojnosanitetskog pregleda", kao specijalizovanog vojnomedicinskog časopisa, u „Ratniku" su objavljivani i sadržaji iz domena vojnog zdravstva.
U ovom broju časopisa „Vojnosanitetski pregled" nalazi se članak o zdravstvenom stanju u srpskoj vojsci krajem 19. veka, nastao na osnovu analize tekstova objavljenih u tom periodu u časopisu „Ratnik" (vidi str. 450–454).

Cover page of the first issue of the Warrior ("Ratnik" in Serbian), the first scientific and professional journal of the Serbian Armed Forces, published from January 1897 to March 1941, with interruption during the First World War. It published articles on current topics from various military disciplines as well as topics in the history of warfare. Until publication of the Military Medical Courier ("Vojnosanitetski glasnik" in Serbian) which preceded the Military Medical Review ("Vojnosanitetski pregled" in Serbian) as a specialized military medical journal, contents in the field of military health were published in the "Ratnik".
This issue of the "Vojnosanitetski pregled" contains an article on health status in the Serbian Army at the end of the 19th century, based on an analysis of texts published during that period in the "Ratnik" (see pp. 450–454).

ORIGINAL ARTICLES
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Factors predicting rehabilitation outcome in patients after unilateral transtibial amputation due to peripheral vascular disease

Prediktivni faktori ishoda rehabilitacije kod bolesnika posle jednostrane transtibijalne amputacije zbog periferne vaskularne bolesti

Biljana Majstorović*†, Mladen Pešta*

***Institute for Physical Medicine and Rehabilitation „Dr Miroslav Zotović“, Banja Luka, Bosnia and Herzegovina; University of Banja Luka, †Faculty of Medicine, Banja Luka, Bosnia and Herzegovina**

Abstract

Background/Aim. The primary rehabilitation in the prosthetic phase after amputation of lower extremities is of great importance for the improvement of the activities of daily living (ADLs) of persons with amputation and their successful social reintegration. The aim of this study was to examine the influence of independent predictors (age, gender, duration of rehabilitation, time between the amputation and the mounting of the prosthesis) on the success of the primary rehabilitation in the prosthetic phase after amputation of lower extremities. **Methods.** This retrospective clinical study included patients who underwent the primary rehabilitation in the prosthetic phase at the Institute for Physical Medicine and Rehabilitation "Dr Miroslav Zotović", Banja Luka, in 2015. A total of 75 patients with unilateral transtibial amputation were included. Etiologically, these transtibial amputations occurred as a consequence of vascular complications of diabetes mellitus or peripheral occlusive arterial disease. Evaluation of the success of rehabilitation was performed at the end of the primary rehabilitation in the prosthetic phase and 3 months after the end of the treatment by means of K-levels classification system and Locomotor Capabilities Index (LCI) scale. Depending on the distribution of data, univariate and multivariate multiple regression analysis, *post hoc* Mann-Whitney test, Spearman's correlation coefficient and Wilcoxon test were used for statistical analysis. Statistical significance of the found differences was set at $p < 0.05$. **Results.** A total of 75 patients, 55 (73.33%) men and 20 (26.67%) women, were included in this clinical trial. Average age of all participants was 63.5 ± 9.06 years, 61.8 ± 9.34 years for males and 68.1 ± 6.4 for females ($p < 0.01$). Average duration of rehabilitation was 27.69 ± 7.39 days in men and 33.9 ± 6.89 days in women ($p < 0.01$). Male patients had better functional results compared to females obtained by all analysed outcome measures ($p < 0.01$). Younger patients achieved better results, with the degree of statistical significance ranging between $p < 0.05$ and $p < 0.001$. The time from the amputation to the mounting of prosthesis and the duration of rehabilitation had no influence on the rehabilitation outcome. **Conclusion.** The present study identified age and gender of patients as relevant independent predictors of the success of rehabilitation. Although it was initially expected, this clinical trial did not prove the importance of the time from the amputation to the start of the primary rehabilitation in the prosthetic phase. In the future research other independent predictive factors, such as comorbidities, first and foremost cardiovascular diseases, medication, laboratory parameters and mental status, should be taken into account.

Key words:
amputees; peripheral vascular diseases; prostheses and implants; prognosis; rehabilitation; treatment outcome.

Apstrakt

Uvod/Cilj. Primarna protetička rehabilitacija posle amputacije donjih ekstremiteta ima izuzetan značaj za poboljšanje samostalnosti u aktivnostima svakodnevnog života osoba sa amputacijom i za njihovu uspešnu društvenu reintegraciju. Cilj rada bio je da se ispita uticaj nezavisnih prediktivnih faktora (pol, starost, dužina trajanja rehabilitacije, dužina čekanja na početak primarne protetičke rehabilitacije) na uspešnost primarne protetičke rehabilitacije posle amputacije donjih ekstremiteta. **Metode.** Ovom retrospektivnom studijom obuhvaćeni su bolesnici koji su uspešno završili primarnu protetičku rehabilitaciju u Zavodu za fizikalnu medicinu i rehabilitaciju „Dr Miroslav Zotović“, Banja Luka, u 2015. godini. U studiju je bilo uključeno 75 bolesnika sa jednostranim transtibijalnim amputacijama. Etiološki,

Correspondence to: Biljana Majstorović, Institute for Physical Medicine and Rehabilitation „Dr Miroslav Zotović“, 78 000 Banja Luka, Bosna and Herzegovina. E-mail: biljanamajstorovic72@gmail.com

radilo se o potkolenim amputacijama nastalim kao posledica vaskularnih komplikacija dijabetes melitusa ili periferne okluzivne arterijske bolesti. Procena uspešnosti primarne protetičke rehabilitacije vršena je na otpustu (po završetku rehabilitacije) i na kontroli, tri meseca posle protetisanja, a kao mere uspešnosti korišćeni su K-nivoi funkcionalnog klasifikacionog sistema i *Locomotor Capabilites Index* (LCI) skala. Shodno distribuciji podataka izvršene su odgovarajuće statističke analize: univarijantna i multivarijantna multipla regresiona analiza, *post hoc* Mann-Whitney test, Spearmanov koeficijent korelacije i Wilcoxonov test. Nivo statističke značajnosti nađenih razlika utvrđen je za verovatnoću $p < 0,05$. **Rezultati.** Studijom je obuhvaćeno 75 bolesnika, 55 (73,33%) muškaraca i 20 (26,67%) žena, prosečne starosti 63,5 ± 9,06 godina. Prosečna starost bolesnika muškog pola iznosila je 61,8 godina, a bolesnika ženskog pola 68,1 godinu ($p < 0,01$). Prosečno trajanje rehabilitacije kod muškaraca iznosilo je 27,69 dana, a kod žena 33,9 dana ($p < 0,01$). Bolesnici muškog pola ostvarili su bolje funkcionalne rezultate, u odnosu na žene, kroz sve analizirane merne instrumente ($p < 0,01$). Mlađi bolesnici ostvarili su bolje rezultate, a utvrđena je statistička značajnost u rasponu od $p < 0,05$ do $p < 0,001$. Analizirana uspešnost rehabilitacije posmatrana kroz faktore vremena početka protetisanja u odnosu na amputaciju, kao i trajanje rehabilitacije nisu pokazali statističku značajnost. **Zaključak.** Studija je identifikovala pol i starost kao relevantne nezavisne prediktore uspešnosti protetičke rehabilitacije. Iako je bilo očekivano, ova studija nije pokazala značaj koji ima vreme od amputacije do započinjanja primarne protetičke rehabilitacije. U narednim istraživanjima treba uzeti u obzir i uticaj drugih nezavisnih prediktivnih faktora kao što su pridružene bolesti, pre svega kardiovaskularna oboljenja, medikamentna terapija, laboratorijski parametri i mentalni status.

Ključne reči:
amputacija; krvni sudovi, periferni, bolesti; proteze i implantati; prognoza; rehabilitacija; lečenje, ishod.

Introduction

The primary rehabilitation in the prosthetic phase, as part of the rehabilitation medicine, is not sufficiently present in Bosnia and Herzegovina. It is a complex treatment, the realization of which is stipulated by the existence of the multi-professional and well-coordinated prosthetic team. This work is based on the experience in prosthetic rehabilitation of the personnel of the Institute for Physical Medicine and Rehabilitation "Dr Miroslav Zotović" in the City of Banja Luka, capital of Republic of Srpska, Bosnia and Herzegovina. The Institute is a referral tertiary institution that performs the primary rehabilitation in the prosthetic phase in patients after amputation of lower extremities in Republic of Srpska, the population of which is estimated at 1.5 million. Diabetes mellitus with its late complications, including the complications in peripheral blood vessels in the lower extremities, as well as the peripheral occlusive arterial disease (POAD), are main etiological causes of amputations, which was clearly confirmed in this investigation, and which is also corroborated by the world statistical data [1–3]. Besides, our experience, as well as some international studies [4, 5], show that amputations are more frequent among the male diabetic patients.

Adequate estimate of the prosthetic potential and influence of the analysed independent predictive factors (gender, age, time after start of prosthetic rehabilitation after amputation and the duration of the primary rehabilitation in the prosthetic phase) is very important for the outcome of the prosthetic management of patients. Besides, existence of the adequate outcome measures is necessary for the evaluation of success of the primary rehabilitation in the prosthetic phase.

This is the first clinical trial in the Republic of Srpska to analyse prosthetic rehabilitation as means of medical rehabilitation aimed to improve the quality of this segment of medicine on one hand, but also to enable adequate tracking and comparison of our results and methods with the results in other institutions engaged in prosthetic rehabilitation. Since this study was a retrospective one, we chose the four independent parameters as the ones available in the medical charts of patients, which does not mean that in the future, prospective studies, other independent predictors will not be analysed, too.

Majority of the other studies suggest worse outcome in patients of older age at the moment of the lower limb amputation [6–13]. Similar studies, however, in principle, do not identify patient gender as a relevant determinant of success of the prosthetic rehabilitation process [3, 14]. Minority of studies found different level of mobility between the patient of different gender and different success rates between the two genders – sometimes better results were found in men [15–17], and sometimes in women [18].

There are discrepancies in the current literature regarding the choice of basic instruments that would enable to estimate the rehabilitation potential in the pre-prosthetic phase and to adequately verify the success of the prosthetic rehabilitation. Some of the most frequently used outcome measures used in the evaluation of the patients with amputations are: Time Up and Go; 10 m walk test; 2-min walk test, mobility grades [such as Special Interest Group of Amputee Medicine (SIGAM) and K-levels], Barthel index, Functional Independence Measure (FIM), Locomotor Capabilities Index (LCI) scale, Houghton Scale, Prosthetic Evaluation Questionnaire-Mobility Scale (PEQ-MS) and Amputee Mobility Predictor (AMP) [19].

The aim of this study was to examine the influence of independent predictors (age, gender, duration of rehabilitation, time between the amputation and the mounting of the prosthesis) on the success of the primary rehabilitation in the prosthetic phase after amputation of lower extremities.

Methods

This was a retrospective clinical study including the patients underwent the primary rehabilitation in the prosthetic phase at the Institute for Physical Medicine and Rehabilitation

"Dr Miroslav Zotović", Banja Luka, Bosnia and Herzegovina, in 2015. A total of 75 patients of both genders were included.

Inclusion criteria were: patients of both genders with unilateral transtibial amputations caused by peripheral vascular disease as late complications of diabetes mellitus and POAD. Non-inclusion criteria were: patients in whom amputations were performed due to malignant diseases, injuries, patients with bilateral transtibial amputations, patients with transfemoral amputations and patients without adequate prosthetic potential.

All the patients signed the informed consent at the beginning of rehabilitation, in a form of a general document consenting to permit use of their medical data for the purpose of research. This is the routine procedure for all the admitted patients in the Institute and not the concrete study-oriented document, although it covered the present study, too. The primary rehabilitation in the prosthetic phase programme was not time-limited; each patient was treated for as long as the prosthetic team saw fit. Patients after amputation performed in the regional general hospitals or clinical centres received an information to report to the Institute after their sutures had been removed and following the complete healing of their postoperative wounds at the amputated limb. Our health insurance system cannot allow for all the patients to be directly transferred to our Institute – hence the wide range of times elapsing between the discharge after amputation and admission at the Institute.

Evaluation of the success of rehabilitation was at the end of the primary rehabilitation in the prosthetic phase and 3 months after the end of the treatment with K-levels classification system and LCI scale. LCI at discharge and on the control examination was not performed as interview; it was tested through the requested activities and it was performed by the same therapist at discharge and on the control examination in order to eliminate subjectivity.

K-levels classification system was developed in the USA in 1995 by the Medicare programme, as a functional classification system that makes a triage of all patients in one of the five levels of mobility, depending on their functional status. Marks were from 0 to 4, with higher mark meaning better patient functionality [20]. Use of K-levels classification system during the study enabled the monitoring of the mobility level of the patients without prosthesis, with prosthesis at the end of the prosthetic rehabilitation and during the control examination 3 months following the end of the treatment.

LCI scale consists of 14 questions, each question is scored on scale from 0 to 4. The questions are divided into two groups. Each group consists of 7 questions, the first covering the basic activities and the second one covering the advanced activities [21]. Maximum score, for basic and advanced activities alike is 28 points, depending on the performance of the tested activities.

Review of the clinical studies addressing the outcome analysis of prosthetic rehabilitation appraises the LCI scale as content-consistent, reliable during testing and re-testing, which recommends the test for clinical use and its usage as an investigational tool [14].

The calculated descriptive statistical parameters included mean value ± standard deviation (SD) as well as minimal and maximal values.

Based on the distribution of the obtained data that was checked by the Kolmogorov-Smirnov test, adequate statistical analysis was performed with Mann-Whitney and Wilcoxon test. Influence of certain predictors on the values of the used outcome measures at discharge was checked by means of the univariate and multivariate multiple regression analysis.

Statistical significance of the found differences was set at $p < 0.05$. Complete statistical analysis of the data was performed by use of the commercial statistical software SPSS Statistics 18.

Results

A total of 75 patients, 55 (73.33%) men and 20 (26.67%) women, with average age of 63.51 ± 9.06 years, were enrolled in this study. Average age of male and female patients was 61.84 ± 9.34 years and 68.10 ± 6.40 years, respectively. Average time from amputation to mounting of the prosthesis was 5.15 ± 2.08 months (range 2–11 months), (5.05 ± 2.24 months for men and 5.40 ± 1.60 month for women) while the average duration of the rehabilitation was 29.35 ± 7.68 days, range 11–53 days (27.69 ± 7.31 days for men and 33.90 ± 6.89 days for women). Duration of rehabilitation was significantly longer in women than in men ($p < 0.01$) and women were significantly older ($p < 0.01$).

A significant difference between the genders was found for all the outcome measures at discharge, with all parameters being better in men than in women ($p < 0.01$ to $p < 0.001$) and also a significant difference among men between the results obtained at the control and at discharge. In women the same could be applied for LCI basic and advanced activities, while no significant difference could be found regarding the K level values (Figure 1).

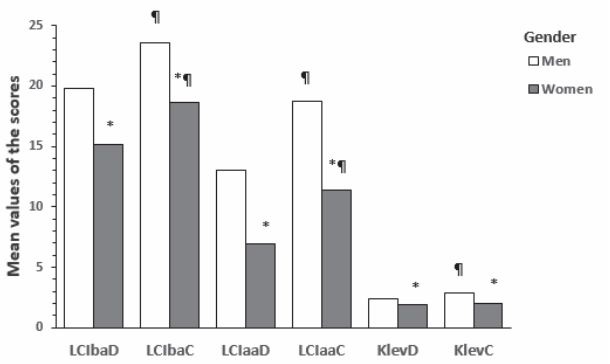

Fig. 1 – Mean values of the scores at discharge and at the control examination in patients with transtibial amputation. LCI – Locomotor Capabilities Index; LCIbaD – LCI basic activities at discharge; LCIbaC – LCI basic activities at control; LCIaaD – LCI advanced activities at discharge; LCIaaC – LCI advanced activities at control; KlevD – K-levels at discharge; KlevC – K-levels at the control examination.
***$p < 0.01$ – difference between mean values of the outcome measures in women compared to men (Mann-Whitney test).**
¶$p < 0.01$ – difference between mean values of the outcome measures at the control examination compared to the corresponding discharge values (Wilcoxon test).

Table 1
Summary of univariate/multivariate multiple regression analyses with the Locomotor Capabilities Index (LCI) activities at discharge as dependent variable

Variables	Standardized coefficients β	*t*-value	*p*
Basic			
Univariate analyses			
gender	-0.519	5.186	0.001
age	-0.376	3.472	0.001
time (amputation-prosthesis)	-0.044	0.377	0.708
duration of rehabilitation	-0.232	2.040	0.045
Multivariate analyses			
gender	-0.440	4.067	0.001
age	-0.237	2.282	0.025
duration of rehabilitation	-0.018	0.166	0.868
Advanced			
Univariate analyses			
gender	-0.477	4.633	0.001
age	-0.354	3.232	0.002
time (amputation-prosthesis)	-0.003	0.022	0.983
duration of rehabilitation	-0.169	1.466	0.147
Multivariate analyses			
gender	-0.406	3.850	0.001
age	-0.229	2.176	0.033

Basic/advanced – influence of independent variables on LCI basic/advanced activities.

Table 2
Summary of univariate and multivariate multiple regression analyses with the K-levels at discharge as dependent variable

Variables	Standardized coefficients β	*t*-value	*p*
Univariate analysis			
gender	-0.369	-3.388	0.001
age	-0.404	-3.769	0.001
time (amputation-prosthesis)	-0.074	-0.634	0.528
duration of rehabilitation	-0.190	-1.650	0.103
Multivariate analysis			
gender	-0.270	-2.481	0.015
age	-0.321	-2.947	0.004

The influence of important demographic and clinical parametres as independent variables on values of the LCI basic activities was determined at discharge from the hospital by means of the univariate and multiple regression analyses (Table 1).

Among the parametres investigated, the important factors were gender, age and duration of rehabilitation. Time from amputation to mounting of the prosthesis was not a significant independent variable (Table 1).

The mutual influence and importance of the individually significant parameters, analysed together, on the values of the LCI basic activities was analysed by the multivariate multiple regression analysis (Table 1).

Based on the multivariate regression analysis, gender and age remained significant predictors of the LCI basic activities values, while the duration of rehabilitation lost importance in interaction with the gender and age (Table 1).

Gender and age were defined as important predictors for the LCI advanced activities at discharge (Table 1).

Gender and age were defined as significant predictors of K-levels at discharge, too (Table 2).

A negative, highly significant correlation was found between the age and the values of all the used outcome measures. Older age of patients was associated with smaller values of the outcome measures at discharge ($p < 0.001$) (Table 3).

Table 3
Correlation parameters of LCIbaD, LCIaaD and KlevD with patient's age

Score	*ρ*	*p*
LCIbaD	-0.448	< 0.001
LCIaaD	-0.408	< 0.001
KlevD	-0.404	< 0.001

LCI – Locomotor Capabilities Index;
LCIbaD – LCI basic activities at discharge;
LCIaaD – LCI advanced activities at discharge;
KlevD – K-levels at discharge;
***p* – Spearman's correlation coefficient.**

Discussion

In this study the influence of several factors as predictors of the outcome of the prosthetic rehabilitation were analysed: patient age, gender, time between the amputation and the mounting of the prosthesis, and duration of rehabilitation. The initial hypothesis that the predictive factors mentioned above have a significant influence on the outcome of rehabilitation was partly confirmed, i.e., for two independent variables – gender and age of patients. However, for the time elapsed from the amputation to the mounting of the prosthesis and the duration of rehabilitation, a significance was not confirmed. A possible explanation of this result would be that the duration of rehabilitation was set on an individual basis and the patients used to finish their treatment after reaching the maximum level of functionality, which was ascertained by the prosthetic team.

Mean age of patients was 63.5 ± 9.06, which is in accordance with the mean age of patients at the Institute during the latest five-year period. It was also confirmed that the success of rehabilitation decreased with age. Better results in younger patients were maintained at the control examination three months after the rehabilitation. The aim of the control testing was to obtain a more adequate estimate of the improvement functionality in activities of daily life.

This study included patients of both genders. Male patients were dominant (73.33%), which is in accordance with the gender structure of lower limb amputees rehabilitated at the Institute over the last five years, as well as with the data from other countries that were available in the literature [3, 22]. Male patients had much better rehabilitation results than the female ones. This difference could be explained by the fact that men were on average 7 years younger than women. This is why better results obtained in men in the present study could be primarily ascribed to their younger age, although the significant levels obtained in the univariate and multivariate multiple regression analyses indicate that gender of patients may be an age-independent predictor of the success of rehabilitation in amputees. This result could have been attributable to the insufficiently large sample that could not allow for a more adequate analysis of the rehabilitation success within the same age groups between male and female patients.

In the present study, time from the amputation to the mounting of the prosthesis and the duration of rehabilitation were analysed as possible predictors of success of the prosthetic rehabilitation. Although some studies report worse results in patients with a delayed start of rehabilitation, the results of the present study did not confirm the importance of time from the amputation to the mounting of the prosthesis. This factor was considered relevant in some clinical trials, which probably indicates the adequate triage of patients during their rehabilitation [23].

The patients were evaluated by LCI scale and K-levels classification system. Relevant publications from this field report on use of a larger number of tests, but warn to the absence of clear guidelines on the choice of the optimal outcome measures [3, 19]. The choice of the outcome measures in the present study was made based on their content, practicability of their implementation and capability of the monitoring of the registered results.

The experience with use of K-levels classification system were according to the other reports that justified its use during the prosthetic rehabilitation [19].

Single use of the K-levels test as a predictor of the success of the prosthetic rehabilitation does not offer the detailed estimate of the capabilities of the patients, which is a consequence of the general character of its content. Some other publications also did not recommend use of the K-levels alone [24]. A more complete estimate of the patient's capabilities would be obtained by a combined use of outcome measures, based on complementarity of their content. The present study confirmed the content consistency and analytical usefulness of the LCI scale in the continuous follow-up of the monitored results. It makes the LCI scale an adequate outcome measure during the rehabilitation phase with a prosthesis.

A limitation of this research could be a small number of outcome measure used for the evaluation of patients and for this reason in the future research a special attention will be paid to the inclusion of more measurable and mutually complementary outcome measures.

Future studies should also include a larger number of potential predictive factors, since in this study, due to its retrospective nature, we did not have an access to any additional predictors, other than the four ones mentioned above.

Conclusion

Success of the prosthetic rehabilitation is based on the adequate estimation of the rehabilitation potential of patients. Measurement of the success of rehabilitation at the end and at the control examinations is possible by using the adequate outcome measures.

The present study identified age and gender of patients as relevant independent predictors of the success of rehabilitation. Although this study failed to show statistical significance of the time elapsing from the amputation to the start of the primary rehabilitation in the prosthetic phase on the rehabilitation success, this factor should be paid attention in the forthcoming prospective studies. In the future clinical studies other independent predictive factors should also be taken into account, such as comorbidities, first and foremost cardiovascular diseases, medication, laboratory parametres and mental status.

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Hyperbaric oxygenation in prevention of amputations of diabetic foot

Hiperbarična oksigenacija u prevenciji amputacija dijabetičkog stopala

Zvezdan Stefanović*†, Branislav Donfrid*†, Tomislav Jovanović‡, Zoran Zorić§, Radmila Radojević-Popović‖, Uroš Zoranović¶**

Zvezdara University Medical Center, *Clinic for Surgery, Belgrade, Serbia; University of Belgrade, †Faculty of Dental Medicine, ‡Faculty of Medicine, §Faculty of Veterinary Medicine, Belgrade, Serbia; ‖Center for Hyperbaric Medicine, Belgrade, Serbia; University of Defence, ¶Faculty of Medicine of the Military Medical Academy, Belgrade Serbia; **Military Medical Academy, Clinic for Vascular Surgery, Belgrade, Serbia

Abstract

Background/Aim. Diabetic foot is the term for the pathological changes on foot in patients with diabetes. It is caused by diabetic angiopathy, polyneuropathy and osteoarthropathy. The treatment is complex and long-term and often leads to the loss of the extremity. The appliance of hyperbaric oxygen therapy (HBOT) has a lot more important place in adjuvant treatment of this disease. The aim of this study was to determine the influence of HBOT on the wound healing in comparison with the conventional treatment, the possibility of shortening the time of the treatment in patients with diabetic foot. **Methods.** In a five-year period a retrospective-prospective multicentric study, involving 60 patients with diabetic foot divided into two groups, was performed. The first group (group A) consisted of 30 patients treated by combined therapy (with medications, surgical therapy and HBOT). All the patients were receiving HBOT in the Special Hospital for Hyperbaric Medicine, CHM Hollywell-Neopren in Belgrade. The control group (group B) also consisted of 30 patients treated with medications and surgical therapy, but without HBOT. **Results.** The demographic data, the types of diabetes, as well as the Wagner classification stage of diabetic ulcers and radiography scans of changes in bones were equal in both groups. The median healing time of the Wagner grade III ulcer in the group A was 37.36 days [mean ± standard deviation (SD) = 65.6 ± 45.8 days], and in the group B 99.78 days (mean ± SD = 134.8 ± 105.96 days) and it was statistically significant (p = 0.074). The median time of recovery in patients of the group A with the Wagner grade IV was 48.18 days (mean ± SD = 49.7 ± 33.8 days), and in the group B 85.05 days (mean ± SD = 86.7 ± 71.6 days) and that was statistically significant (p = 0.121). The foot amputations were performed in both groups in 3 (10%) patients. In the group A there were no high amputations, whereas in the group B there were 4 (13.33%) below-knee amputations and 4 (13.33%) above-knee amputations which was highly statistically significant ($p < 0.0001$). **Conclusion.** In this study, HBOT definitely showed positive adjuvant role in the treatment of diabetic foot. For the good treatment result it is essential the timely and successful surgical treatment of the ulcer and the use of bandage with the healing dressings. In case of the clear signs of local infection, the antibiotic therapy according to the antibiogram is necessary.

Key words:
diabetic foot; hyperbaric oxygenation; amputation; wound healing.

Apstrakt

Uvod/Cilj. Dijabetičnim stopalom nazivamo patološke promene na stopalu kod bolesnika koji boluju od šećerne bolesti, a uzrokovane su dijabetičkom angiopatijom, polineuropatijom i osteoartropatijom. Lečenje je kompleksno i dugotrajno i često dovodi do gubitka esktremiteta. Primena hiperbarične oksigene terapije (HBOT) ima sve značajnije mesto u adjuvantnom lečenju ovog oboljenja. Cilj ovog rada bio je utvrđivanje uticaja HBOT na efikasnije zarastanje rane u poređenju sa konvencionalnim lečenjem i mogućnost skraćenja vremena lečenja bolesnika sa dijabetičkim stopalom. **Metode.** U petogodišnjem periodu urađena je retrospektivno-prospektivna multicentrična studija, koja je obuhvatila 60 bolesnika podeljenih u dve grupe. Prva grupa (grupa A) od 30 bolesnika lečena je kombinovanom terapijom (medikamentoznom, hirurškom i HBOT). Svi bolesnici dobijali su HBOT u Specijalnoj bolnici za hiperbaričnu medicinu, CHM Hollywell-Neopren u Beogradu. Kontrolna grupa (grupa B), takođe od 30 bolesnika sa dijabetičkim stopalom, lečena je medikamentozno i hirurški, ali bez HBOT. **Rezultati.** Demografski podaci, tip dijabetesa,

Correspondence to: Branislav Donfrid, Zvezdara University Medical Center, Clinic for Surgery, Dimitrija Tucovića 161, 11 000 Belgrade, Serbia. E-mail: branislav.donfrid@gmail.com

stadijum dijabetičkih rana prema Wagner-u i nalazi radiografskih promena na kostima bili su jednaki u obe grupe. Medijana vremena za sanaciju rane III stadijuma po Wagner-u u grupi A iznosila je 37,36 dana [srednja vrednost ± standardna devijacija (SD) = 65,6 ± 45,8 dana], a u grupi B 99,78 dana (srednja vrednost ± SD = 134,8 ± 105,96 dana) (p = 0,074). Bolesnici u IV stadijumu po Wagner-u u grupi A imali su medijanu vremena za sanaciju rane od 48,18 dana (srednja vrednost ± SD = 49,7 ± 33,8 dana), a u grupi B 85,05 (srednja vrednost ± SD =86,7 ± 71,6 dana) (p = 0,121). Amputacije stopala bile su izvršene u obe grupe kod tri (10%) bolesnika. U grupi A nije bilo ni jedne visoke amputacije, a u grupi B su bile izvršene četiri (13,33%) potkolene i četiri (13,33%) natkolene amputacije, što je bilo visoko statistički značajno (p < 0,0001). **Zaključak.** HBOT u ovoj studiji kao i kod većine drugih autora definitivno je pokazala pozitivnu adjuvantnu ulogu u lečenju dijabetičkog stopala. Za dobar rezultat lečenja potrebna je pravovremena i sukcesivna hirurška obrada rane i zavoj lekovitim oblogama. U slučaju pojave jasnih znakova lokalne infekcije potrebna je antibiotska terapija prema antibiogramu.

Ključne reči:
dijabetesno stopalo; hiperbarična oksigenacija; amputacija; rana, zarastanje.

Introduction

Diabetic foot is the term for the pathological changes on foot caused by ischaemia as a consequence of microangiopathy, the late notice of soft tissue damage and slow ulcer healing as a result of polyneuropathy as well as the uneven pressure of footwear due to the deformation of foot because of diabetic osteoarthropathy [1, 2]. The curing demands a complex multimodal treatment, including regulation of glycaemia, antibiotic therapy, local treatment of the ulcer, as well as surgical or endovascular revascularization in patients with macro-occlusive artery disease. The healing of diabetic foot ulcer is longterm and in 60% of patients it lasts about one year. All this is accompanied by high treatment costs and additional social problems [3]. In the most of European countries 10% of health care costs are expended on diabetes treatment, and 68% of those are spent on the curing the disease complications.

In the newer literature hyperbaric oxygen therapy (HBOT) has a lot more significant place in an adjuvant treatment of this disease [4]. HBOT means a breathing 100% oxygen in a special chamber, in higher ambient pressure conditions (2.0–2.9 Kpa), determined by the particular protocols. The oxygen content in plasma increases from 0.3 to 5.62 volume percents. The average number of treatments is 20 (from 15 to 30). In normal conditions haemoglobin-bound oxygen is transported to the cells in erythrocytes. In the hyperbaric pressure conditions, according to the laws of physics, there is the increased dissolution of molecular oxygen in plasma which enables the oxygen supply even there where the blood vessels are narrowed (the capillary lumen is smaller than the erythrocytes' diameter) or occlusive [5, 6]. In patients with diabetic foot HBOT ameliorates the peripheral tissue oxygen supply, and in addition to that oxygen has antibacterial (for anaerobic flora it is bactericidal), anti-inflamatory and imunosuppressive effects [7, 8]. These effects are made by the inhibition of prostaglandins, interfereon gamma (IFNG), interleukin-1 and interleukin-2 [9]. The hyperbaric oxygenation is beneficial for wound healing due to stimulation of fibroblast proliferation and differentiation, and rapid collagen synthesis [10, 11]. The neovascularization is stimulated and the energy metabolism of peripheral cells is increased.

The aim of this study was to determine the significance of HBOT as an adjuvant therapy that may influence on: the efficient healing of diabetic foot ulcer in comparison to the conventional type of treatment (with medications and surgical treatment); the possibility of shortening the time of diabetic foot healing and reducing the treatment costs in patients with diabetic foot.

Methods

In a five-year period a retrospective-prospective multicentric study was conducted which involved 60 patients divided into two groups. The first group (group A), consisted of 30 patients, was treated by combined therapy (with medications, surgical therapy and HBOT). There were 25 patients from the Clinic for Surgery "Zvezdara" in Belgrade and the rest 5 of them were from The Clinic for the Vascular and Endovascular Surgery, Clinical Center of Serbia, Belgrade. All the patients were receiving HBOT in Special Hospital for Hyperbaric Medicine, CHM Hollywell-Neopren in Belgrade.

The control group (group B), also consisted of 30 patients, was treated with medications and surgical therapy, but without HBOT. Twenty three patients were treated in the Clinic for Surgery "Zvezdara" in Belgrade and the remaining 7 patients in the Clinic for the Vascular and Endovascular Surgery, Clinical Center of Serbia, Belgrade.

Only the patients with diabetic foot in whom magistral arteries were passable and surgical or endovascular revascularization was not indicated, as proved by non-invasive examination (Color duplex sonography – CDS, Ankle brachial index – ABI), were included in the study. Before the treatment, radiography scans were made to all the patients and the wound smear was taken for the bacteriological examination.

The inclusion criteria for the study were: palpable pedal pulses; an ancle-brachial index (ABI) higher than 0.75; three-phase spectrogram on pedal arteries.

The surgical interventions were performed in both groups depending on the type of diabetic foot lesions and with: ulcers – necrectomia; phlegmons – incision, contra-incision, drainage; osteomyelitis – incision, contra-incision, sequestrectomia; gangrene – necrectomia or amputation.

The transplantation of skin (Thiersch) was performed in a few patients with the amputation of foot in the joint line (Chopart or Lisfranc) in order to shorten the healing period.

The complete recovery considered the state of full epithelialization of the wound or recovery of inflammatory changes (the soft tissue and the bone). In patients with the amputation the recovery considered the full healing of the amputation stump.

Statistical analysis

Descriptive statistics were used for the processing demographic and clinical characteristics of patients in both groups. Categorical variables were compared by using χ^2 test. Continuous variables were compared by ANOVA test, or Median test (for variables without normal distribution). A significance of 0.05 was required. Means ± standard deviations (SD) and medians with the corresponding 95% confidence intervals (CI) were estimated. Analyses were performed using SPSS for Windows, Version 22 (SPSS, Inc., Chicago, IL).

Results

Patients characteristics in both groups are presented in Tables 1 and 2.

The Group A was treated by combined therapy (with medications, surgical therapy and HBOT), and the Group B was treated in the same way, but without HBOT. It was shown that there were no statistically significant differences in demographic data between patients in the Groups A and B (Table 3).

In the group A there were 11 patients with type 1 diabetes and 19 with type 2 diabetes. In the control group there were 12 patients with type 1 diabetes and 18 with type 2 diabetes. There was no statistically significant difference between groups regarding diabetes type. Among 30 patients in the group A, 12 were with the Wagner grade III ulcers and 18 with the Wagner grade IV ulcers. In the control group (group B) there were 10 patients with ulcers of the grade III in the Wagner classification system, and 20 with the Wagner grade IV ulcers (Table 3).

Based on the foot radiography, the patients were divided into subgroups with osteoporosis, osteoarthropathy, osteomyelitis and the normal finding of foot bones. In the group A the normal result was found in 50% of the patients, and in the group B in 60% of the patients (Table 3).

The most frequent pathological result was osteomyelitis, which was diagnosed in 30% of the patients in the group A and in 26.67% of the patients in the group B. Radiography results of foot bones did not differ significantly between groups.

The type of surgical intervention depended on the local result (Table 3). Incision and drainage were performed in 5 patients in total, in the group A in 3 (10%) patients, whereas in the group B in 2 (6.7%) patients.

Necrectomia was the most frequent intervention in the group A (in 17 patients or 56.7%) while in the group B just in 3 (10%) patients. The finger amputations were conducted in 7 (23.3%) patients of the group A and in 14 (46.7%) patients of the group B. The foot amputations (transmetatarsal, in Chopart and Lisfranc's joint line) were performed in 3 (10%) patients in each the group. There were no high amputations in the group A, but there were 4 (13.3%) below-knee and 4 (13.3%) above-knee amputations in the group B ($p < 0.0001$).

In this study the mean (± SD) healing time of the Wagner grade III ulcer in the group A was 65.6 (± 45.8) days whereas in the group B it was 134.8 (± 105.96) days ($p = 0.074$).

In the group A, the patients with the Wagner grade IV ulcers had the mean time of healing 49.7 (± 33.8) days, and in the group B 86.7 (± 71.6) days ($p = 0.121$) (Tables 4).

The first control examination was carried out immediately after the healing process was finished, the second one was after a month and later on, the examinations were carried out in three months. In case of deterioration of the local result the examinations were carried out more frequently.

In patients treated with HBOT the most common side effects were discomfort and ear pain (17–20%) and claustrophobia (13%). The cases of pneumothorax and neurological disturbances were not noticed.

Ten patients from the group A had some problems after healing of diabetic foot lesions: one patient – foot pain and discomfort during walking; five patients – ulcer appearing at the different place on the same foot, or ulcer appearing on the other foot; four patients – foot deformation after the surgical interventions and discomfort during walking; four patients died within a year; 7 patients did not come for the control examination and their state was not known.

Discussion

The reasons for the bad outcomes of the diabetic foot ulcer healing are combined influences of ischaemia with hypoxia of soft tissues, prolonged wound healing due to existing polyneuropathy and propensity to infection [12]. Many authors report about positive influence of oxygen therapy in hyperbaric conditions on the healing or reducing the major complications of diabetic foot ulcer. In this study the effects of treatments on the Wagner grades 3 and 4 ulcers in two groups of patients with diabetic foot were compared [13]. The first group of 30 patients was treated with HBOT and medication and surgical methods (group A), whereas the the control group (group B) was treated with medication and surgical methods in the same way, but without HBOT.

In regards to significant parameters, this study showed the positive influence of HBOT on diabetic foot ulcer healing, especially in regard to the most important result – high amputation. Moreover, there were no above-knee and below-knee amputations whereas there were 8 amputations in the control group and that was highly significant ($p < 0.0001$).

The most patients in the group A well tolerated HBOT. The most common side effects were discomfort and ear pain (17–20%) and after that claustrophobia (13%). The cases of pneumothorax and neurological disturbances were not noticed.

Table 1

Demographic and clinical characteristic of patients treated by conventional therapy + hyperbaric oxygen therapy (HBOT) – the group A

Patients	Gender	Age	Diabetes Type	Ulcer grade (wagner)	Foot x ray scan at the beginning of the study	Treatment before entering the study	Surgical intervention	Number of HBOT treatments	Healing Period
1	M	65	I	3	*Osteolysis dig. V ped. l. dex. Osteoporosis digitorum ped. dex.*	30 days	*Amputatio dig. V Inciharacteristics sio, contraincisio et drainage ped. l. dex.*	30	150 days
2	M	69	I	4	*Osteomyelitis dig. II, III et IV ped. l. dex.*	30 days	*Amputatio ped. l. dex. (Chopart)*	20	120 days
3	M	64	II	3	/	7 days	*Incisio et contraincisio ped. l. sin.*	20	21 days
4	M	67	II	4	*Osteoporosis ped. dex.*	5 days	*Amputatio hal. l. dex. Necrectomia et incisio*	20	31 days
5	M	64	II	4	*Osteomyelitis hall. l. dex. St. p. fracturam hall. l. dex.*	4 days	*Incisio et contraincisio hall. l. dex. Necrectomia*	30	20 days
6	M	30	II	3	/	15 days	*Necrectomia*	30	40 days
7	F	64	II	3	*Osteomyelitis dig. V et osteoporosis gravis ped. dex.*	10 days	*Amputatio ped. dex. (Lisfrank) Thiersch*	30	130 days
8	F	71	II	4	/	35 days	*Necrectomia ped. l. sin.*	30	25 days
9	M	61	II	4	/	5 days	*Necrectomia et incisio ped. l. sin.*	30	90 days
10	M	50	II	4	*Osteolysis dig. II ped. l. sin. DOAP ped. l. sin. Fractura patologica digitorum ped. l. sin.*	5 days	*Incisio et contraincisio ped. l. sin. Necrectomia*	30	45 days
11	M	70	I	4	*Osteomyelitis dig. IV ped. dex.*	30 days	*Amputatio dig. IV ped. l. sin.*	15	15 days
12	M	73	II	4	/	10 days	*Amputatio dig. IV ped. l. dex.*	20	30 days
13	F	61	I	4	/	120 days	*Amputatio ped. l. sin. (Chopart) Thiersch*	20	130 days
14	M	74	II	3	*Osteomyelitis hall.dex. DOAP ped. l. dex.*	10 days	*Incisio et contraincisio hall. l. dex.*	20	35 days
15	M	63	I	4	*DOAP pedis l. dex.*	5 days	*Amputatio hall. dex.*	20	30 days

Table 1 (continued)

Patients	Gender	Age	Diabetes Type	Ulcer grade (wagner)	Foot x ray scan at the beginning of the study	Treatment before entering the study	Surgical intervention	Number of HBOT treatments	Healing Period
16	M	51	II	3	*Osteomyelitis hall. sin.*	30 days	*Amputatio hall. et incisio ped. l. sin.*	30	60 days
17	F	80	I	4	/	19 days	*Necrectomia ped. l. dex.*	20	60 days
18	M	64	II	3	*Osteomyelitis hall. sin.*	45 days	*Incisio Amputatio hall. sin.*	15	20 days
19	F	65	II	3	/	15 days	*Incisio ped. l. sin.*	10	30 days
20	M	70	I	3	/	7 days	*Incisio Necrectomia ped. l. dex.*	20	30 days
21	M	66	II	4	*Osteoporosis ped. dex.*	10 days	*Necrectomia ped. l. dex.*	20	60 d days
22	M	50	II	4	/	60 days	*Necrectomia ped. l. dex.*	20	20 days
23	M	57	I	3	*Osteomyelitis phal. dis. hall. l. sin.*	15 days	*Incisio et contraincisio hall. sin. Extirpatio phal. dist. hall. ped. l. sin.*	30	120 days
24	F	69	I	4	/	3 days	*Necrectomia ped. l. sin.*	10	62 days
25	M	46	I	4	/	30 days	*Necrectomia hall. l. dex.*	30	60 days
26	M	43	II	4	/	150 days	*Necrectomia hall. l. dex.*	20	21 days
27	F	75	II	4	*Osteoporosis ped. l. dex.*	180 days	*Necrectomia dig. V ped. dex.*	30	30 days
28	M	66	II	4	*Osteomyelitis hall. dex.*	10 days	*Necrectomia hall. l. dex.*	30	45 days
29	M	72	I	3	/	120 days	*Necrectomia ped. l. dex.*	20	90 days
30	M	60	II	3	/	10 days	*Necrectomia ped. l. sin.*	30	61 days

In the Group A, 24 patients were treated as the inpatients, whereas 6 patients were outpatiently treated. The number of HBOT treatments depended on the Wagner classification grade of ulcers as well as on the approval of the treatment extension by National Health Insurance Fund; DOAP – *A. dorsalitis pedis.*

Table 2

Demographic and clinical characteristic of patients in the control gropu treated by conventional therapy only (the group B)

Patients	Gender	Age	Diabetes Type	Ulcer grade (wagner)	Foot x ray scan at the beginning of the study	Treatment period before entering the study	Surgical intervention	Healing Period
1	M	58	I	3	*Osteomyelitis ped. l. sin. Arthodesim mediotarsalis l. sin.*	720 days	*Amputatio ped. l. sin. (Chopard) Thiersch*	150 days
2	M	64	II	3	/	7 days	*Amputatio dig. IV ped. dex.*	244 days
3	M	71	II	4	*Osteomyelitis dig. II ped. l. sin*	31 days	*Amputatio dig. II et III ped. l. sin.*	240 days
4	M	73	II	4	/	30 days	*Amputatio hall. l. sin.*	120 days
5	F	74	I	3	*DOAP gr. III ped. l. sin.*	15 days	*Amputatio dig. IV ped. l. sin.*	38 days
6	M	58	II	3	/	10 days	*Amputatio transmetatharsalis ped. l. dex.*	360 days
7	M	69	I	4	*Osteomyelitis hall. dex.*	153 days	*Amputatio hall. l. dex.*	81 days
8	M	62	I	4	/	7 days	*Amputatio dig. I–III ped. dex.*	91 days
9	M	83	II	4	/	30 days	*Amputatio cruris l. sin.*	35 days
10	M	52	II	4	/	7 days	*Amputatio femoris l. dex.*	26 days
11	M	65	I	4	/	30 days	*Amputatio femoris l. sin.*	15 days
12	M	63	II	4	*DOAP ped. l. sin. Osteoporosis ped. l. sin.*	75 days	*Incisio et contraincisio ped. l. sin. Necrectomia ped. l. sin.*	126 days
13	M	50	I	3	/	15 days	*Amputratio dig. IV ped. dex.*	60 days
14	M	46	II	4	/	21 days	*Amputatio hall. l. sin.*	66 days
15	F	71	II	4	*Osteomyelitis dig. II ped. dex.*	21 days	*Amputatio dig. II ped. dex.*	121 days

Table 2 (continued)

Patients	Gender	Age	Diabetes type	Ulcer grade (Wagner)	Foot x- ray scan at the beginning of the study	Treatment period before entering the study	Surgical intervention	Healing Period
16	M	55	II	4	*DOAP ped. l. sin.*	15 days	*Amputatio dig. V ped. sin.*	61 days
17	M	65	II	3	*Osteomyelitis dig. V ped. sin.*	120 days	*Incisio et contraincisio ped. l. sin.*	90 days
18	F	51	II	4	/	10 days	*Amputatio dig. IV ped. dex.*	60 days
19	M	67	I	3	*Osteomyelitis hall. l. sin.*	30 days	*Amputatio cruris l. sin.*	28 days
20	F	76	I	4	/	35 days	*Amputatio ped. l. dex. (Chopart)*	210 days
21	M	53	I	4	*Osteoporosis ped. l. sin.*	30 days	*Amputatio cruris l. sin.*	21 days
22	M	49	II	3	*Osteomyelitis metatharsalis V ped. l. sin.*	15 days	*Incisio et curettage ped. l. sin.*	180 days
23	M	78	II	4	/	60 days	*Amputatio femoris l. dex.*	18 days
24	M	66	II	4	*Osteomyelitis hall. dex.*	60 days	*Amputatio hall. dex.*	51 days
25	F	71	II	4	/	10 days	*Amputatio dig. II ped. l. sin.*	100 days
26	F	68	I	4	/	15 days	*Amputatio dig. II ped. l. dex.*	240 days
27	F	70	I	4	/	90 d days	*Amputatio femoris l. dex.*	34 days
28	F	82	II	3	/	7 days	*Incisio, contraincisio et necrectomia ped. l. dex.*	48 days
29	M	79	I	4	/	360 days	*Amputatio cruris l. dex.*	18 days
30	M	37	II	3	/	7 d days	*Necrectomia ped. l. dex.*	150 days

All the patients in the control group were admitted to the hospital, and the duration of their stay depended on the healing period; DOAP – *A. dorsalitis pedis.*

Table 3
Summary of patients demographic characteristics, diabetes mellitus (DM) type and the ulcer grade (Wagner classification)

Characteristics	Group A	Group B	*p*
Number of patients	30	30	
Age (years), mean ± SD	62.67 ± 10.71	64.20 ± 11.35	0.592
median	64.5	65.5	
range	30–80	37–83	
Gender, n (%)			
female	7 (23)	8 (27)	0.766
male	23 (77)	22 (73)	
Type of DM, n (%)			
I	11 (37)	12 (40)	0.791
II	19 (63)	18 (60)	
Wagner classification, n (%)			
3	12 (40)	10 (33.3)	0.592
4	18 (60)	20 (66.7)	
Radiographic findings, n (%)			
without pathological result	15 (50.0)	18 (60.0)	
osteoarthropathy	1 (3.3)	3 (10.0)	0.262
osteomyelitis	9 (30.0)	8 (26.7)	
osteoporosis	5 (16.7)	1 (3.3)	
Intervention type, n (%)			
incision	3 (10.0)	2 (6.7)	
necrectomia	17 (56.7)	3 (10.0)	0.0001
finger amputation	7 (23.3)	14 (46.7)	
foot amputation	3 (10.0)	3 (10.0)	
high amputation	0 (0.0)	8 (26.7)	

Group A – patients treated by combination of conventional therapy + hyperbaric oxygen therapy (HBOT); Group B – patients treated by conventional therapy only; SD – standard deviation.

Table 4
Treatment period before entering the study and the healing period in patients with diabetic foot

Treatment period (days)		n	Mean ± SD	95% CI		Min.	Max.
				lower bound	upper bound		
Group A							
before entering the study	Wagner 3	12	26.2 ± 31.7	6.0	46.3	7.0	120.0
	Wagner 4	18	39.5 ± 54.0	12.7	66.3	3.0	180.0
	Total	30	34.2 ± 46.2	16.9	51.4	3.0	180.0
healing period	Wagner 3	12	65.6 ± 45.8	36.5	94.7	20.0	150.0
	Wagner 4	18	49.7 ± 33.8	32.8	66.5	15.0	130.0
	Total	30	56.0 ± 39.1	41.4	70.6	15.0	150.0
Group B							
before entering the	Wagner 3	10	94.6 ± 222.4	-64.5	253.7	7.0	720.0
study	Wagner 4	20	54.5 ± 80.1	17.0	92.0	7.0	360.0
	Total	30	67.9 ± 141.2	15.2	120.6	7.0	720.0
healing period	Wagner 3	10	134.8 ± 106.0	59.0	210.6	28.0	360.0
	Wagner 4	20	86.7 ± 71.6	53.2	120.2	15.0	240.0
	Total	30	102.7 ± 85.9	70.7	134.8	15.0	360.0

SD – standard deviation; CI - confidence interval.

Baroni et al. [14] were among the first who published treatment outcomes with HBOT. In their study, when comparing the two groups of patients (the group treated with HBOT and the group without receiving HBOT) the statistical analysis using χ^2 test demonstrated highly significant difference ($p = 0.001$) in favour of HBOT. In regards to the most significant parameter, the limb amputation, HBOT drastically reduced the percentage of amputations. These results coincide with our experience.

Kalani et al. [15] from Karolinska Hospital, Stockholm, Sweden, in their study followed-up the treatment results of two groups of patients with diabetic foot (treated with and without HBOT) during 3 years. Seventy six percent of patients treated with HBOT had healed ulcer lesion and intact skin, whereas in the group of patients treated conventionally that effect was obtaind in 48% of the patients. The amputation had to be performed just in 12% of the patients in the HBOT group and in 33% of the conventionally treated patients [15].

The mechanisms by which HBOT acts positively on diabetic foot ulcer healing are the reducing of wound exudate and stimulation of granulation process. The values of partial oxygen pressure in the wound surrounding during HBOT may indicate the future treatment outcome. There is positive correlation between transcutanous oxygen pressure (TcPO2) values and the speed of the wound size and exudate reduction, and epithelialization [16]. A negative correlation between TcPO2 values and parameters of wound healing was determined in the group of patients whose treatment ended with high amputations [17].

The authors who have compared the patients with the Wagner grades III and IV diabetic foot ulcer conclude that HBOT after 30 sessions greatly contributes to prevention of amputations and the healing of the wound by epithelialization, but an antibiotic therapy has alsorole in the healing process [18].

In comparison with the results of Fedorko et al. [19] who randomly chosen 103 patients divided into two groups (49 in the HBOT group and 54 in the control group), our results are far better regarding amputations. They had 22.4% of high amputations in each group. In HBOT group 11 out of 49, and in the control group 13 out of 54 patients with the Wagner grades III and IV diabetic foot ulcer had underwent amputations.

Conclusion

HBOT definitely has positive adjuvant role in managing diabetic foot. For the optimal treatment results successful surgical ulcer treatment is necessary and the use of bandage with the healing dressings, as well as the treatment with HBOT. In case of the clear signs of local infection, antibiotic therapy according to the antibiogram is necessary.

The medical practitioners, the patients and policy creators should define good clinical practice guidelines of Shared Decision Making for appliance of hyperbaric oxygen therapy as the additional treatment for diabetic foot management. The future researches should be aimed at the improvement of methods for choosing patients, testing various protocols of treatment and improvement of trust in those assessments. The routine implementation of transcutaneous oximetry imposes itself as a simple, cheap and reliable method for early assessment of HBOT efficacy and the patients are not needlessly exposed to the efforts which exist at some degree (arrival from their home to Centre for baromedicine or organizing transport from their hospital to the Centre). The special problem is the treatment cost which should be paid by the Health Insurance Fund without interference with ethical principles that every patient should have the same right on treatment if that treatment is a proper one.

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Application of a geographic information system in the study of spatial aspects of cervical cancer incidence in Belgrade

Primena geografskog informacionog sistema u istraživanju prostornih aspekata obolevanja od raka grlića materice u Beogradu

Nevenka Pavlović*, Ljiljana Marković Denić†, Katarina Vojvodić*

***Institute of Public Health of Belgrade, Belgrade, Serbia; University of Belgrade, Faculty of Medicine, †Institute of Epidemiology, Belgrade, Serbia**

RETRACTED

Abstract

Background/Aim. Cervical cancer is still an important public health problem in Belgrade. The aim of this study was to explore spatial patterns of cervical cancer, provision and accessibility of women's health service on the primary health care level in Belgrade, as well as the needs for improving cancer surveillance and preventive programs. **Methods.** This study applied a descriptive epidemiological method and a geographic information system based on data on cervical cancer diagnosed among female residents of Belgrade in 2006 and 2011. A map of the density of cases, with precise and complete data on the address of residence at the time of diagnosis, and a map of the distribution of gynecological practices in the primary health care in Belgrade, were generated through the process of georeferencing. **Results.** A total of 569 cases of cervical cancer were registered in 2006 and 2011, without significant differences. Significant associations were noticed for municipality of residence and year of diagnosis (χ^2= 42.99, df = 16, p = 0.000), and year of diagnosis and age groups 30–34 (p = 0.038, f = 3.998, df = 11, ANOVA), 40–44 (p = 0.001, f = 7.545 df = 13, ANOVA) and 45–49 (p = 0.046, f = 2.679, df = 15, ANOVA). The process of georeferencing covered a total of 466 (81.8%) cases with 97.4% of all cases diagnosed in 2006 and 68.6% in 2011. The generated maps showed similar spatial patterns of cases for both years: a higher density of cases with addresses in central parts of urban and suburban municipalities, as well as in parts of densely populated areas of urban municipalities. There was no regularity of grouping found for the cases in relation to the provision of women's health service, or of distance from the place of residence of cases to gynecological practices. **Conclusion.** Our results indicate possibilities for the perception of the spatial distribution of cervical cancer and needs for improving cancer surveillance and preventive programs on small geographical areas.

Key words:
epidemiologic methods; geographic information systems; primary health care; urban population; uterine cervical neoplasms.

Apstrakt

Uvod/Cilj. Rak grlića materice je još uvek značajan javnozdravstveni problem u Beogradu. Ciljevi ove studije bili su istraživanje prostornih obrazaca raka grlića materice, obezbeđenosti i dostupnosti ginekološke zdravstvene zaštite na primarnom nivou u Beogradu, kao i sagledavanje potrebe za unapređenjem epidemiološkog nadzora i programa prevencije. **Metode.** U radu je primenjen deskriptivni epidemiološki metod i tehnologija geografskih informacionih sistema. Uključeni su svi slučajevi raka grlića materice utvrđeni kod stanovnica Beograda u toku 2006. i 2011. godine. Mape gustine slučajeva i mape distribucije ginekoloških ordinacija domova zdravlja u Beogradu su generisane procesom geokodiranja preciznih i kompletnih podataka adrese stanovanja obolelih u vreme utvrđene dijagnoze i adresa ginekoloških ordinacija. **Rezultati.** U toku 2006. i 2011. godine registrovano je ukupno 569 slučajeva raka grlića materice, bez statistički značajne razlike. Statistička značajna povezanost je utvrđena za opštinu stanovanja i godinu utvrđene dijagnoze (χ^2 = 42,99 df = 16 p = 0,000), i godinu utvrđene dijagnoze i dobne grupe 30–34 (p = 0,038, f = 3,998, df = 11, ANOVA), 40–44 (p = 0,001, f = 7,545, df = 13, ANOVA) i 45–49 (p = 0,046, f = 2,679, df = 15, ANOVA). Procesom geokodiranja obuhvaćeno je ukupno 466 (81,8%) slučajeva obolevanja, među kojima 97,4% svih slučajeva utvrđenih u 2006. i 68,6% u 2011. godini. Generisane mape pokazuju slične prostorne obrasce obolevanja za obe posmatrane godine: veća gustina slučajeva među osobama sa adresom stanovanja u centralnim delovima opština gradskog i prigradskog područja, kao i u delovima sa većom gustinom naseljenosti u opštinama užeg gradskog jezgra. Nije uočena

Correspondence to: Nevenka Pavlović, Institute of Public Health of Belgrade, Bul. despota Stefana 54a 11 000 Belgrade, Serbia.
E-mail: nevenka.pavlovic@zdravlje.org.rs

pravilnost grupisanja slučajeva obolevanja u odnosu na obezbeđenost ginekološke zdravstvene zaštite na primarnom nivou ili udaljenosti mesta stanovanja obolelih do najbliže ustanove koja pruža ovaj vid zdravstvene zaštite. **Zaključak.** Naši rezultati ukazuju na mogućnost potpunijeg sagledavanja prostornih obrazaca distribucije obolevanja od raka grlića materice i potreba za unapređenjem nadzora i programa prevencija na majim geografskim područjima.

Ključne reči:
epidemiološki metodi; informacioni sistemi, geografski; zdravstvena zaštita, primarna; stanovništvo, gradsko; grlić materice, neoplazme.

Introduction

Cervical cancer is the fourth most common malignant tumor among women worldwide, with an expressed disparity in the burden and trends in various parts of the world. It is the second most frequent cancer in the less developed regions, and the eleventh in the more developed regions [1]. The burden on the European continent is increasing from west to east, and it is highest in Central and Eastern European countries (standardized incidence rate of 19.2/100,000 and mortality of 8.0/100,000). The values of the incidence and mortality for countries in the region are about twice those of Northern and Western European countries. Based on the incidence rate, Romania is in the first place (34.9/100,000), while Serbia (the region of Southern Europe) is in the fourth place (28.3/100,000) [2, 3]. Differences are also noted across smaller geographic areas. The area of Belgrade and eastern regions that gravitate towards the Romanian border have been the areas with the highest incidence rates in Serbia for years. Cervical cancer is in the third place regarding incidence and in the fourth place as the cause of death among women due to malignant tumors in Belgrade [3–6].

The differences in geographic burden of cervical cancer and high variation in incidence rate and mortality may arise due to multiple reasons. They mainly reflect the varied distribution of known risk factors, various host sensitivity, differences in the detection, treatment and monitoring of carcinoma patients, methods of registration and reporting system, as well as lack of health care, lack of screening or insufficient coverage of the population by preventive examinations [7–10].

Data on the incidence and mortality due to cervical cancer in most European countries, as well as in Serbia, are part of the surveillance of malignant diseases, and are found in the population cancer registries. Their completeness and quality are the basis for research activities, efficient planning and adaptation of the programs of prevention and suppression of the disease at all levels, from the national to the local one [10–13]. Aiming to provide a comprehensive overview of the epidemiological situation and assess further activities, an increasing level of attention today is dedicated to analyzing the spatial aspects of the disease, by combining a descriptive epidemiological method with the application of a geographic information system (GIS). Contemporary information technologies provide for the detection and visualization of spatial patterns that may be missed by applying the classical descriptive method or with tabular overviews of the results [14–17].

The aim of this study was to determine the spatial distribution of cervical cancer incidence, provision and accessibilty of women's health service on the primary health care level in Belgrade, carried out by descriptive epidemiological method and GIS.

Methods

Study location and population

This retrospective study used a descriptive epidemiological method and GIS based on data on cervical cancer diagnosed among female residents of Belgrade in 2006 and 2011.

The territory of Belgrade covers an area of 322,268 ha (the inner-city area covers 35,996 ha), administratively divided into 16 municipalities – 10 urban (Čukarica, Voždovac, Vračar, Novi Beograd, Palilula, Rakovica, Savski venac, Stari grad, Zemun, Zvezdara) and 6 suburban municipalities (Barajevo, Grocka, Lazarevac, Obrenovac, Mladenovac, Sopot). According to census data (from 2002 and 2011) Belgrade had a total of 828,270 female inhabitants with a median age of 41.6 (for 2006) and 873,614 female inhabitants with a median age of 43.2 (for 2011).

Data collection and management

The source of data was the Population Cancer Registry for Belgrade. The data analysis used the incidence of cervical cancer diagnosed in Belgrade for 2006 and 2011 (International Classification of Diseases, Injuries and Causes of Death, 10th revision, code C 53). We used proportions, crude, standardized and age-specific incidence rates per 100,000 female inhabitants. Crude and age-specific incidence rates were calculated using census data (2002 and 2011). Analysis of standardized incidence rates was performed using the direct method with world standard population [18].

The collected data set included information on the address of residence, year of diagnosis and age of patients at the time of diagnosis.

Maps of cervical cancer density and maps of the distribution of gynecological practices at Primary Health Care Centers (PHCCs) were generated through the process of georeferencing, using the precise data on residence at the time of diagnosis and the addresses of gynecological practices in 2006 and 2011.

Data for the provision of gynecological health care at the primary health care level were taken from the annual report on the plan of work of women's health Belgrade PHCCs in 2006 and 2011.

Provision of gynecological health care was expressed through the number of women per one gynecologist (6,500/1)

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among the total adult female population (age 15 and over at the municipality of the health care center), and interpreted according to the Rulebook for providing health services in health care institutions on a daily basis (the measure is 30 visits per day per gynecologist) [19].

Spatial accessibility of gynecological health care was examined as the geographical distance of the registered cases to the nearest gynecological practice in the municipality of residence at the time of established diagnosis (20 min walking distance).

Statistical analysis

The χ^2-test, Student's *t*-test and analysis of variance (ANOVA) were used to assess statistical significance.

Results

There were 569 registered new cervical cancer cases within the territory of Belgrade in 2006 and 2011 (263 and 306, respectively). Crude incidence rate in 2006 was 31.75/100,000, while the standardized one was 20.4 per 100,000. Crude incidence rate in 2011 was 35.0/100,000, and the standardized one was 22.9/100,000. Cervical cancer in 2006 was in the second place (9.3%) in frequency among all female cancers in Belgrade, after breast cancer (30.7%), while in 2011 it was in the third place (8.2%) after breast carcinoma (33.7%) and colorectal carcinoma (8.3%).

Significant differences were noticed regarding municipality of residence and year of diagnosis. Among the total number of registered cases of the disease in 2006, 45.6% were with residence data within 4 municipalities: Novi Beograd, Čukarica, Palilula and Voždovac (with uniform participation between 11.0% and 11.8%). During 2011 nearly all Belgrade municipalities registered a decrease or maintenance of values similar to those in 2006, with the exception of Zemun and Savski venac municipalities, where a nearly threefold increase in the number of female patients was registered (from 8.7% to 24.8%, and from 1.9% to 5.9%, respectively). This difference was statistically significant ($\chi^2 = 42.99$, df = 16, $p = 0.000$).

The municipalities with the highest incidence rate in 2006 were Mladenovac, Vračar, Rakovica and Voždovac, while in 2011 these were Savski venac, Zemun and Mladenovac. The highest increase in the incidence rate in 2011 was registered among the residents of the municipalities of Zemun and Savski venac. A decrease in the incidence rate in 2011 was registered among the residents of the municipalities of Rakovica, Vračar and Mladenovac (Figure 1).

The average age of female patients in 2006 was 54.9 years, while in 2011 it was 53.5 years. The highest age-specific incidence rates in 2006 were registered in the age groups of 55–59 (3.6/100,000) and 40–44 (3.2/100,000). Comparing 2011 with 2006, incidence rates were higher in nearly all age groups, except for 55–59, 65–69 and 70 and over. High values of age-specific rates were registered among women aged 35 to 64 years, with the highest values in the age groups of 40–44 (3.6/100,000) and 45–49 (3.5/100,000) (Figure 2). A statistically significant difference for age groups was noticed by ANOVA. The difference was noticed for the age groups 30–34 ($p = 0.038$, f = 3.998, df = 11), 40–44 ($p = 0.001$, f = 7.545, df = 13) and 45–49 years ($p = 0$ .046, f = 2.679, df = 15).

Among the total of 569 reported cases of cervical cancer during the two observed years, the process of georeferencing made it possible to capture data for 466 (81.8%) patients. Data for 103 patients was not included in the formation of case density maps, since they lacked precise and complete data on the place of residence at the time of diagnosis. The maps were created based on municipal administrative borders.

Among the 263 reported cases of cervical cancer in 2006 complete data was available for 256 (97.4%) and their spatial distribution is shown on a map of Belgrade (Figure 3). The highest density of cases was observed in central parts of urban and suburban municipalities. Nearly all municipalities exhibited areas without a single registered case of disease.

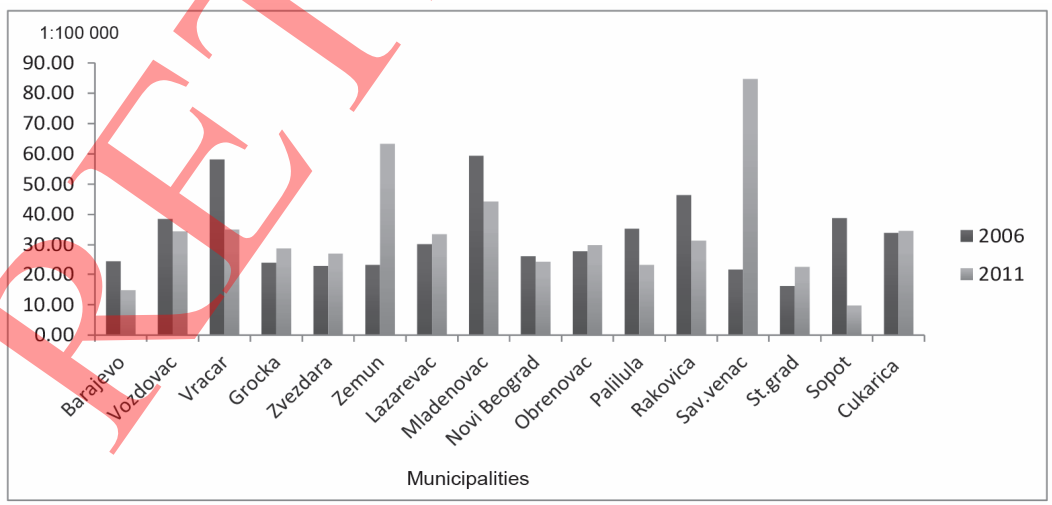

Fig. 1 – Cervical cancer incidence rates (per 100,000) by municipalities, Belgrade, 2006 and 2011.

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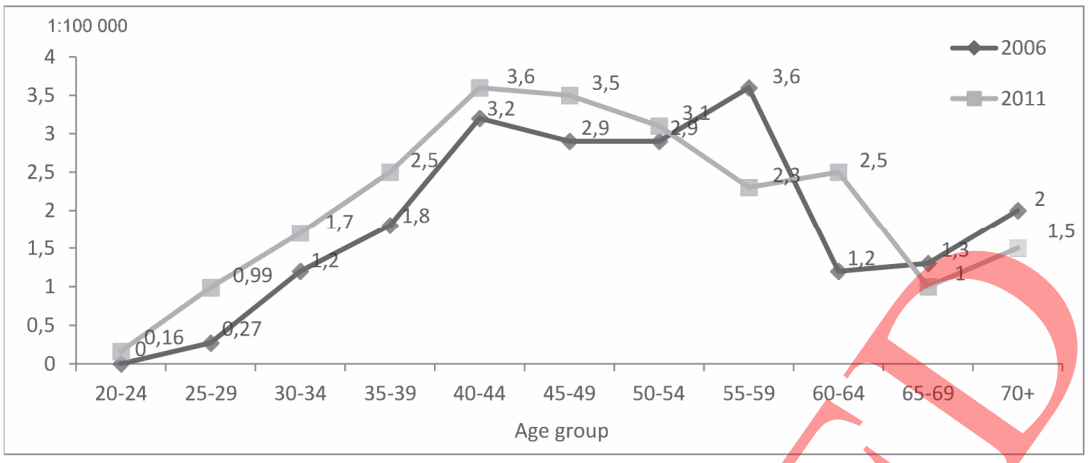

Fig. 2 – Age-specific incidence rates of cervical cancer (per 100,000) in Belgrade, 2006 and 2011.

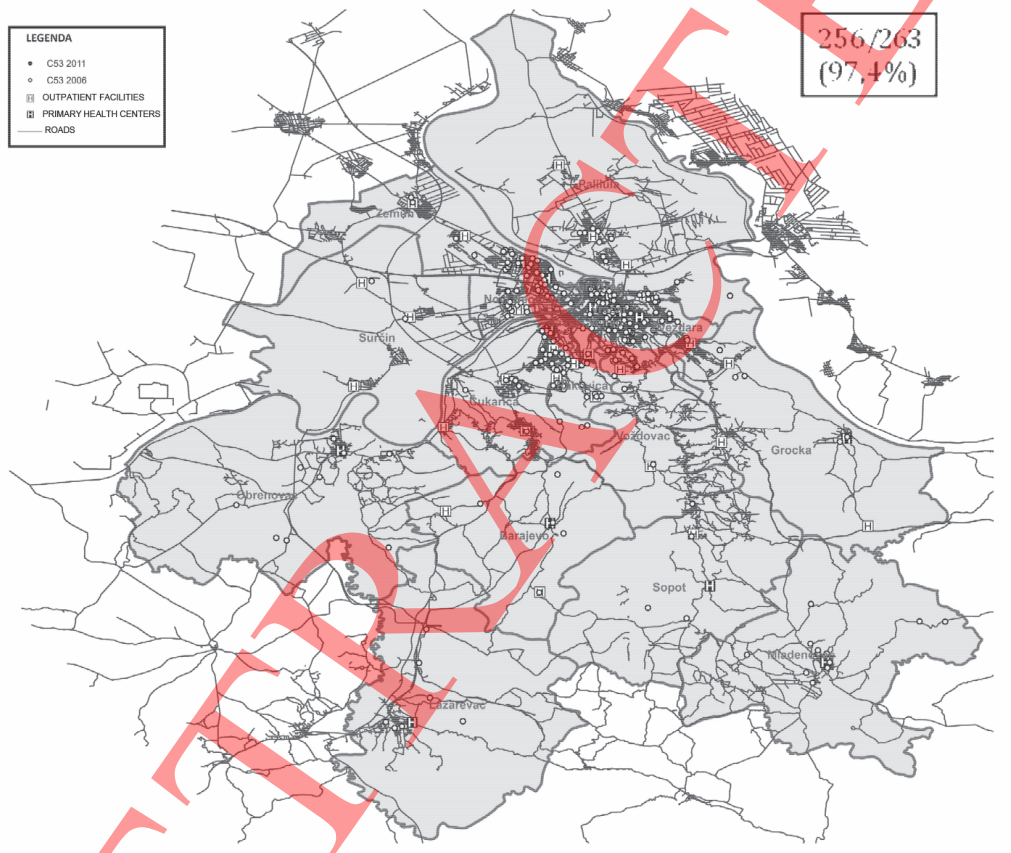

Fig. 3 – Spatial distribution of cervical cancer cases in Belgrade, 2006.
Note: For 256 out of 263 reported cervical cancer patients complete data was available.

The cervical cancer case density map for 2011 was formed by geocoding data for only 210 of the 306 reported cases of the disease. The necessary data was lacking for nearly one third of the cases (31.4%) (Figure 4). The highest number of reports with incomplete geocoding data was related to patients with addresses of residence in the municipality of Zemun (around 47.0%), Voždovac (9.4%), Savski venac and Palilula (8.3% each). The highest percentage of cases not shown regarding the number of registered cases per municipality of residence was in the municipalities of Zemun – 55.2% (42 of 76 cases), Savski venac – 45.0% (8 out of 18 cases) and Palilula -38.0% (8 out of 21 cases).

All usable data, shown simultaneously in a single map, displayed nearly similar spatial grouping patterns of cervical cancer cases in both observed years. A higher density of cases was registered among persons with an address of residence in the central parts of urban and suburban municipalities, as well as in parts of more densely populated urban municipalities (Figure 5). This map, along with the map for 2011, remained without the large amount of data that could affect the spatial distribution of the disease and case density within the territory of the city.

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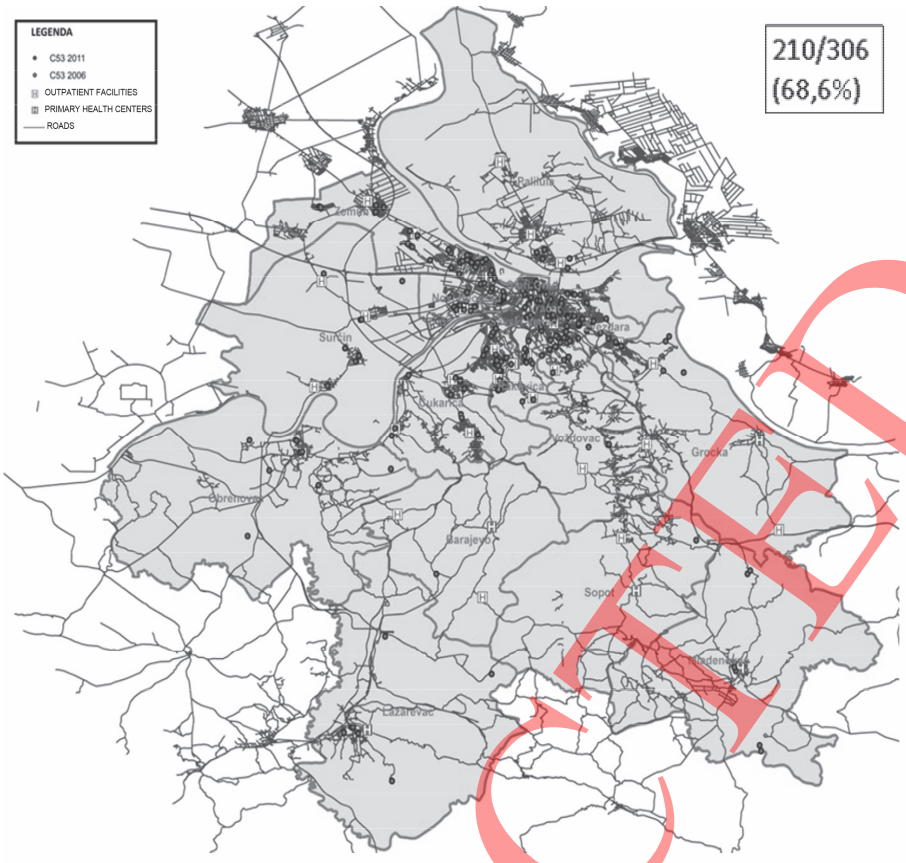

Fig. 4 – Spatial distribution of cervical cancer cases in Belgrade, 2011.
Note: For 210 out of 306 reported cervical cancer patients complete data was available.

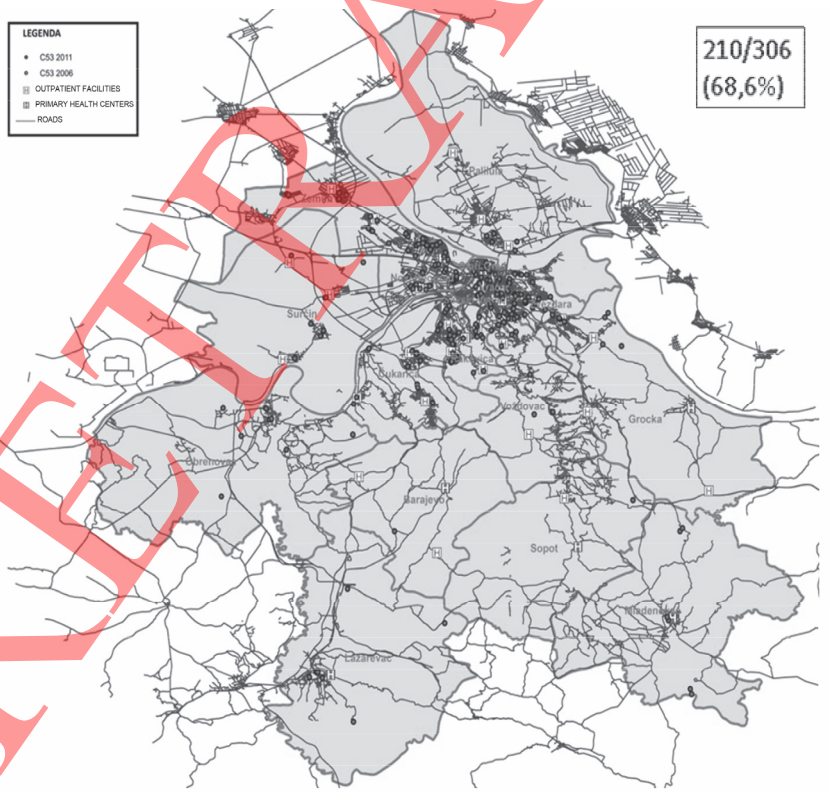

Fig. 5 – Spatial distribution of cervical cancer cases in Belgrade, 2006 and 2011 – a single map.
Note: All usable data simultaneously presented reveals a higher density of cervical cancer patients in the central parts of urban and suburban areas.

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A map was formed to analyze the accessibility of gynecological healthcare with data on the spatial distribution of registered cases of cervical cancer in both observed years and the networks of health care institutions of the primary health care where a gynecological examination can be made. The results of mapping the available data did not indicate a regularity of grouping regarding distance from the healthcare institution. Except for parts of municipalities with a higher case density near a health care institution, the registration of individual cases was also noted with residential addresses at up to 20 min of walking distance from the health care institutions, as well as a significant number of cases at destinations farther than the above measure.

The provision of gynecological health care in accordance with current norms for the primary health care during both observed years was met by 13 of 16 Belgrade PHCCs (Table 1). More than 6,500 women per one gynecologist were registered in 2006 in the PHCC in Lazarevac (8,281) and Novi Beograd (7,288), and in 2011 in the PHCC in Zvezdara (7,065), Čukarica (6,875) and Zemun (6,748). The number of women exceeding the amount of the established norm per gynecologist was noted to be higher during this year compared to the previous observed year, despite the average provisions at the city level being at nearly identical values (2006 : 2011 = 5,645 **:** 5,675).

The greatest daily gynecologist workload was reached in 2006 at the health care centers in Lazarevac (44.9), Voždovac (34.8) and Zemun (33.0). During 2011 this parameter of the workload indicator of gynecologists was above 30 visits per day almost only at a single health care centre (Zvezdara 30.5 and Stari grad 33.2) (Table 1). The average daily workload of gynecologists decreased in 2011 compared to 2006 (2006 : 2011 = 28.2 : 21.9).

Table 1

Provision of gynecological health care and daily workload of gynecologists at healthcare centers in Belgrade, 2006 and 2011

Primary Health Center	Number of patients per gynecologist		Average number of daily visits per gynecologist	
	2006	2011	2006	2011
Barajevo	5,282	5,845	22.8	16.7
Voždovac	5,405	5,695	34.8	21.7
Vračar	5,879	4,708	22.4	18.9
Grocka	4,554	5,123	22.2	19.3
Zvezdara	6,125	7,065	25.1	30.5
Zemun	6,546	6,748	33.0	20.8
Lazarevac	8,281	6,402	44.9	13.6
Mladenovac	4,579	4,682	30.4	19.3
Novi Beograd	7,288	5,340	29.1	20.6
Obrenovac	6,076	5,230	30.8	17.5
Palilula	5,037	6,078	25.3	20.2
Rakovica	4,975	5,531	30.5	23.2
Savski venac	2,929	3,794	15.3	19.1
Sopot	4,395	4,472	22.0	10.7
Stari grad	4,649	3,482	27.4	33.2
Čukarica	6,316	6,875	28.4	26.0

Discussion

According to the results of our study, both crude (35.0/100,000) and standardized rate of cervical carcinoma incidence in 2011 (22.9/100,000) was higher than in 2006, but this increase was not significant. The average standardized incidence rate of this malignant tumor among the population of women in Belgrade during the period 2006–2011 was at 21.6/100,000 [6]**.** Within the structure of malignant tumor cases, cervical cancer was in the third place in 2011, after breast cancer and colorectal cancer. Other parts of Serbia had also a higher frequency of colorectal cancer registered [5], explicable through risk factors such as, in addition to the aging population and family history of colorectal cancer, poor diet, smoking, and insufficient physical activity [20]. Similar findings have been noted in other countries, mostly due to the adaptation to lifestyles and behaviors commonly associated with westernization [9, 13, 21].

Incidence rates at the level of municipalities within the territory of Belgrade differed notably. Similar to the results of research abroad, data from research within our area indicates that women living in rural areas are at higher risk of cervical cancer compared to those living in urban areas. This risk is related to failure to undertake preventive examinations, but not because of their place of residence, but the lower level of education and poorer socioeconomic status [22–24]. According to the latest health survey in our country covering the period 2011–2013, the Papanicolaou (Pap) test was undertaken by 75.9% of women from the most prosperous group, 74.0% of highly educated, 72.5% of residents of Belgrade and 62.3% from urban settlements [25]. An unavoidable component was also the positive sum of migration for Belgrade, particularly during the period 1990-1999, when it was altered characteristics (forced migration). Between the last two census years immigration was particularly intensified in settlements outside the core urban area. The highest number of persons arriving found refuge in Zemun settlements, where they comprise around 11% of the population of this municipality [26].

The highest age-specific incidence rates in 2006 were registered in the age groups 55–59 and 40–44, while in 2011 this moved towards younger age groups, 40–44 and 45–49. During the two observed years a statistically significant difference was found for the age groups 30–34, 40–44 and 45–49, pointing towards a necessary analysis of a greater number of years. The risk of cervical cancer increases with age, and in our country the incidence rate was at its maximum between ages 45–49 and 50–54. The shift towards younger age groups can be related to changes in exposure to risk factors. Sexual habits have changed in the sense of earlier onset of sexual activity, a higher number of partners compared to older generations, and tobacco use which is, after oncogenic types of human papilloma virus (HPV), the second most important risk factor for the occurrence of cervical cancer [6, 27, 28].

Applying the process of georeferencing the available data on the precise addresses of residence of patients at the time of diagnosis with the gynecological offices of PHCCs, maps were generated indicating approximately similar spatial patterns of grouping of cervical cancer patients during both observed years.

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A higher density of cases was noted in more densely populated areas (in central zones of urban and suburban municipalities) and a lower number of cases in rural municipalities compared to urban parts of the city. Certain parts of the municipalities were with no registered a single case of the disease. During both observed years cases were registered in approximately similar locations compared to the place of residence

Regarding the distance of the place of residence of registered cases of the disease from PHCCs, no correlation or regularity of patient grouping was observed.

Our results are only in regards to the data available for 2006 and 2011, and do not preclude the potential for the existence of a different disease distribution pattern after accounting for data from a greater number of years. Similar study was conducted by researchers from Malaysia. They investigated the spatial distribution cases of colorectal cancer over a ten-year period and measured the distance from existing health facilities. They also had 17.6% of incomplete data. A part of the results revealed higher concentration of cases in major town centers. This concentration of cases is probably due to accessibility of the population to screening facilities. Authors of this study also discussed other results and considered limitations of the study and agreed that it is important to include spatial information as part of the Cancer Registry database. This information can be used for improve efficacy of public health promotion activities, as well as for planning health care delivery [29].

GIS has been used for the needs of the Cancer Registry for more than fifteen years in the USA. Data on all identified cases that are already in the Cancer Registry, are routinely entered into GIS. On that way, it is possible to correlate an incidence with geographical and environmental parameters and discover of the disease emergence patterns within an area [15, 30, 31].

The visualization of case distribution and evaluation of the accessibility of health care institutions can further be used for planning health care services (e.g. screening centers), both in regards to assessing the location of existing health care institutions, as well as to planning the opening of new ones in locations more favorable for the population [31, 32]. The experience of Australian researchers indicates the importance of the distance between the place of residence of those invited for a mammography screening and the place it is being held. A better response was obtained among women from areas where no mammography was organized up to that time and who did not do this preventive examination living up to 3 km from the nearest healthcare units (12%) than among those living at a greater distance (8%). They concluded that the response of the target population could be increased if the existing healthcare facilities were replaced with six new ones, located closer to the areas where the situation is "least favorable" [33]. Researches by a numerous authors produced assessments of the role of accessibility of health care in explaining variations in late-stage breast cancer, by applying GIS and spatial analysis. Researches have shown that poor geographic accessibility regarding distance and time necessary to reach the health care institution, as well as socioeconomic factors, all contribute to the higher development of the late stage of the disease. Similar conclusions were obtained in the studies of the impact of geographical and racial/ethnic variability in uptake of cervical cancer screening, incidence and mortality rate [31, 34–36].

We also analyzed the impact of providing gynecological health care and gynecologist workloads in Belgrade PHCCs during both observed years. The data indicated that the average annual values are below the values recommended by current norms prescribed for performing health care activities at the primary level [17]. Deviations were found in 3 health care centers during each year, with the daily number of gynecologist visits decreasing in 2011 (Table 1). During both observed years gynecological examinations aimed at early detection of malignant disease or examinations containing the Pap test covered around 19% of the population aged 25 and above (19.5% and 18.8%, respectively). Studies have shown that in countries where the incidence of cervical cancer is high, coverage of women through regular gynecological examinations is low. Also, they have shown that the Pap smear use as the primary test, and well organized screening program at the national level could play an important role. These measures could have a major effect on decline in cervical cancer incidence and mortality in the next few decades by detecting and treating precancerous lesions [37–41]. The implementation of organized screening program in Serbia started in December 2012 (in Belgrade at Voždovac, Palilula and Čukarica), but we still are dealing with obstacles, such as low percent of women of target population who have been screened within the program [42, 43].

Limitations of the study

This study had some limitations which have to be pointed out. Case density maps were shown for the two observed years (2006 and 2011), but for 82.0% of the total number of registered cases. For the rest of the cases, precise data on the place of residence at the time of diagnosis were not available. The share of incomplete data was particularly significant in the reports from 2011 (as many as 31.4% of the reports). This data limited the analysis of the spatial distribution of the disease.

Conclusion

The results of cervical cancer incidence mapping in Belgrade showed a greater density of cases among persons with a residential address in central parts of municipalities in the urban and suburban areas during both observed years (2006 and 2011), and identified zones without any registered cases of the disease in nearly all Belgrade municipalities. These zones were noticed due to the visualization method used, and other display methods would left them unrecognized.

Providing a more complete data on precise addresses of residence and expanding research to a wider range of years can initiate the application of other, analytical geographic information system functions. This should contribute to better insight into the epidemiological situation and improving the efficiency of prevention program implementation for cervical cancer.

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Long-term outcomes after catheter-ablation of atrioventricular nodal reentrant tachycardia: A ten-year follow-up

Dugoročni ishodi nakon kateter-ablacije atrioventrikularne nodalne *reentrant* tahikardije: desetogodišnje praćenje

Aleksandar Kocijančić*, Dragan Simić*†, Nebojša Mujović*†, Tatjana Potpara*†, Vladan Kovačević*, Milan Marinković*, Jelena Marić-Kocijančić‡, Dušica Kocijančić Belović§

Clinical Center of Serbia, *Clinic for Cardiology, §Clinic for Gynaecology and Obstetrics, Belgrade, Serbia; University of Belgrade, †Faculty of Medicine, Belgrade, Serbia; Military Medical Academy, ‡Clinic of Cardiology, Belgrade, Serbia

Abstract

Background/Aim. Atrioventricular nodal (AV) reentry tachycardia (AVNRT) is the most common form of supraventricular tachycardia. Treatment of choice is a catheter-ablation of the slow pathway of the AV node. The aim of the study was to present the outcomes of this procedure after ten years of follow-up. **Methods.** The catheter-ablation procedure was performed in 92 patients (30 men and 62 women, mean age 52.0 ± 13.3 years, range 19 to 76 years) with confirmed AVNRT during the electrophysiological examination, from 2007 to 2009. Out of these, 64 patients were followed-up for ten years by inviting them to clinical examinations regularly. The occurrence of AV block, arrhythmia and the use of antyarrhythmic drugs were the main outcomes of the ten-year follow-up. Multivariate logistic regression was applied to identify significant predictors of arrhythmia after a follow-up period. **Results.** The primary success of intervention was achieved in 91 (98.9%) patients. Third-degree AV block was registered in 1 (1.1%) patient after the intervention, which required the implantation of a pacemaker. After ten years of follow-up, AVNRT relapses were not registered. A total of 7 out of 64 (10.9%) patients died during the follow-up period, mostly due to non-cardiac causes. After ten years of follow-up, first-degree AV block was registered in six (10.5%) patients, whereas other arrhythmias were observed in 17 (29.8%) patients such as atrial fibrillation or flutter, atrial premature beats and sinus tachycardia. The number of antiarrhythmic drugs were reduced from 2.1 ± 1.2 at baseline to 0.5 ± 0.6 during follow-up, mostly beta-blockers, propafenone and amiodarone, and 33 (57.9%) patients were no longer using anti-arrhythmic therapy. Logistic regression identified participant's age above 55 years at baseline and re-intervention performed after the initial catheter-ablation as significant predictors of arrhythmia after a 10-year follow-up, independent from gender and arterial hypertension at baseline. **Conclusion.** The catheter-ablation of AVNRT represents a successful and safe procedure, from the perspective of ten-year follow-up.

Key words:
tachycardia, atrioventricular nodal reentry; catheter ablation; arrhythmias, cardiac; treatment outcome.

Apstrakt

Uvod/Cilj. Atrioventrikularna (AV) nodalna *reentrant* tahikardija (AVNRT) je najčešća forma supraventrikularne tahikardije. Lečenje izbora predstavlja kateter-ablacija sporog puta AV čvora. Cilj ove studije je bio prikaz ishoda AVNRT nakon desetogodišnjeg perioda praćenja. **Metode**. Procedura je urađena kod 92 bolesnika (30 muškaraca i 62 žene, prosečne starosti 52,0 ± 13,3 godina, raspon 19 do 76 godina) sa dokazanom AVNRT tokom elektrofiziološkog ispitivanja od 2007 do 2009. godine. Od toga, 64 bolesnika su praćena tokom deset godina dolaženjem na redovne kliničke preglede. Pojava AV bloka, aritmije i upotreba antiaritmika bili su glavni ishodi desetogodišnjeg praćenja bolesnika. Multivarijantna logistička regresija je primenjena kako bi se izdvojili značajni faktori kojima se može predvideti pojava aritmije nakon praćenja od deset godina. **Rezultati.** Primarni uspeh intervencije je bio postignut kod 91 (98,9%) bolesnika. Kod jednog (1,1%) bolesnika registrovan je atrioventikularni blok III stepena koji je zahtevao ugradnju pejsmejkera. Posle desetogodišnjeg praćenja, nisu registrovani recidivi AVNRT. Ukupno 7 od 64 (10,9%) bolesnika je umrlo u periodu praćenja, većina zbog nekardijalnih uzroka. Posle perioda praćenja utvrđeno je da je kod šest (10,5%)

Correspondence to: Aleksandar Kocijančić, Clinical Center of Serbia, Clinic for Cardiology, Dr Subtića 13, 11 000 Belgrade, Serbia.
E-mail: kocijancic@yahoo.com

bolesnika registrovan AV blok I stepena, dok su druge aritmije zabeležene kod 17 (29,8%) bolesnika, kao što su atrijalna fibrilacija i flater, pretkomorske ekstrasistole i sinusna tahikardija. Prosečan broj antiaritmika redukovan je sa 2,1 ± 1,2 leka, na početku, na 0,5 ± 0,6 leka u periodu praćenja, najčešće beta-blokatori, propafenon i amiodaron, pri čemu 33 (57,9%) bolesnika više nije koristilo antiaritmijsku terapiju. Logističkom regresijom izdvojili su se starost bolesnika preko 55 godina, na početku studije, i izvršena ponovna reintervencija posle kateter ablacije kao značajni predikotri pojave aritmije posle desetogodišnjeg praćenja, nezavisno od pola i postojanja arterijske hipertenzije na početku. **Zaključak.** Kateter-ablacija AVNRT predstavlja uspešnu i bezbednu proceduru iz perspektive desetogodišnjeg praćenja.

Ključne reči:
tahikardija, atrioventrikularna nodalna kružna; ablacija preko katetera; aritmija; lečenje, ishod.

Introduction

Atrioventricular (AV) nodal reentry tachycardia (AVNRT) is the most common form of supraventricular tachycardia (SVT). The prevalence of SVT is 2.25 per 1,000 persons and more than half of them have AVNRT [1]. The substrate for AVNRT is the existence of dual physiology of the AV node; the electrophysiological difference in the slow and fast pathway properties is a predisposition to trigger the circular motion mechanism of the impulse [2, 3]. The therapy of choice is a catheter-ablation of the slow pathway (SP) by radio frequency (RF) or cryo-energy, and this method is primarily successful in more than 95% of patients [4]. The most common procedural complication is I-III degree AV block that occurs in 1% of all procedures [5].

The aim of this study was to present the outcomes of the RF catheter-ablation after ten years of follow-up, in terms of the occurrence of AV block, occurrence of other arrhythmias and the use of antiarrhythmic therapy.

Methods

Study population

The study was done at the Clinic for Cardiology of the Clinical Center of Serbia, Belgrade in the period from 2007 to 2009. The study comprised a total of 92 consecutive patients diagnosed with AVNRT during the electrophysiological study; RF catheter-ablation of the slow pathway was performed in all patients. During the reception, patients were introduced to the method of the procedure, the rate of its success in our institution and possible complications. Given the fact that the RF catheter-ablation is an invasive cardiac procedure, patients were required to give their written consent before the procedure.

Study protocol

Antiarrhythmic therapy was discontinued 3–7 days before the procedure, in order to facilitate clinical tachycardia induction during the intervention. In all patients, electrophysiological study and catheter-ablation were performed during the same procedure. The intervention was performed in local anesthesia, except in rare cases when the patient was upset during the procedure due to the use of an intravenous analgesic, in the presence of anesthesiologist. Vascular access via the right femoral vein was used. Under the control of fluoroscopy, two catheters were placed through the 7Fr sheaths, via the inferior cava vein. Quadripolar Diagnostic Catheter (Medtronic MC XL or Biosense Webster) for the stimulation and ablation catheter (Medtronic MC) were used.

The diagnostic catheter was positioned at the right ventricular apex for continuous pacing in order to evaluate retrograde conduction. After the ventricular stimulation, the quadripolar catheter was positioned on the lateral wall of the right atrium for atrial stimulation. For the purpose of the induction of AVNRT, programmed stimulation was performed with one (S2) and two (S2, S3) extrastimuli. The ablation catheter was positioned in the coronary sinus for the anatomical marking of this structure and the detection of intracardial signals in the left heart, and then the position of His is marked. If the clinical tachycardia was induced, an anatomical mapping of the right atrium and coronary sinus was performed in order to establish the exact diagnosis of tachycardia. Ablation was mainly performed in the sinus rhythm with the prior detection of slow pathway potential (an integrated anatomical and electrophysiological approach), and in some patients it was performed during AVNRT with the goal of termination of tachycardia. In both cases, an accelerated nodal rhythm was recorded, a non-specific parameter of the site of the successful ablation. The use of RF pulses was limited to 50 Watts. At the end of the procedure, programmed stimulation was repeated under the same conditions and with the same stimulation protocol as well as pre-ablation. In addition to the inducibility of tachycardia, other electrophysiological parameters were compared with those obtained before the ablation itself.

After 10 years of follow-up, patients were invited to examination in order to obtain anamnestic symptom and occurrence of other arrhythmia data and to perform a 12-channel electrocardiogram to measure the PQ interval. Data on AVNRT relapse were reported and occurrence of other arrhythmias were observed in the meantime. Patients gave information on taking antiarrhythmic drugs.

Statistical analysis

Descriptive statistic was presented as mean values ± standard deviation (SD) for numeric variables, or as percents (relative numbers) for categorical variables. Multiple logistic regression analysis was used to compute adjusted odds ratios

(OR) for the prediction of permanent arrhythmia after a ten-year follow-up in relation to age, gender, arterial hypertension diagnosed at baseline, and the need for re-intervention. A probability level of less than 0.05 was accepted as significant. The SPSS 15.0 for Windows software (SPSS Inc. 1989–2006) was used.

Results

Basic demographic and clinical characteristics of the investigated patients at baseline are presented in Table 1. Most patients were female, aged 52 years, who had more than 82 paroxysms for more than 15 years. Common comorbidities were present in 38 (41.3%) patients. Structural heart disease was diagnosed in 14 (15.2%) patients, 33% of the patients had arterial hypertension and only 4% had diabetes at baseline. The average number of anti-arrhythmic drugs was 2.1 (range 0 to 6), and only 5% of the patients had no anti-arrhythmic therapy at baseline. Our data records showed that the patients were treated with anti-arrhythmic drugs such as amiodarone, propafenone, verapamil and different beta-blockers at baseline.

Table 1
Basic demographic and clinical characteristics of the investigated patients at baseline

Characteristics	Number (%) or mean ± SD
Number of patients	92 (100.0)
Men	30 (32.6)
Women	62 (67.4)
Age (years)	52.0 ± 13.3
Number of paroxysms	82.5 ± 125.5
Duration of paroxysms (years)	15.1 ± 10.2
Structural heart disease	14 (15.2)
Other diseases (co-morbidities)	38 (41.3)
Arterial hypertension	31 (33.7)
Diabetes mellitus	4 (4.4)
Number of anti-arrhythmic drugs	2.1 ± 1.2
Without anti-arrhythmic therapy	5 (5.4)

SD – standard deviation.

Cardiological characteristics of the investigated patients before, during and after the catheter-ablation are presented in Table 2. Less than 20% of the patients experienced atrial fibrillation or atrial flutter during the intervention, transitory AV or ventriculoatrial (VA) block during the intervention, or needed to be treated with atropine during the intervention. The success rate was 98.9%, and only 10.9% of the patients underwent a re-intervention. Permanent AV block of any degree after the intervention was present in only 3 patients. The average number of anti-arrhythmic drugs was 0.2 (range 0 to 2), and 80% of the patients had no anti-arrhythmic therapy after the ablation. Our patients were given the following anti-arrhythmic drugs after the ten-year follow-up: beta-blockers, amiodarone and propafenone.

Characteristics of the investigated patients after the 10-year follow-up period are presented in Table 3. The total number of patients followed for 10 years was 64; seven of them died during the follow-up period and 57 were still alive.

Table 2
Cardiological characteristics of the investigated patients before, during and after the intervention

Characteristics before, during and after the intervention	Number (%) or mean ± SD
Dual physiology of AV node before the intervention	90 (97.8)
Application of atropine during the intervention	15 (16.3)
Atrial fibrillation or atrial flutter during the intervention	16 (17.4)
Cycle of tachycardia (ms)	353.8±60.0
Number of RF applications	9.4 ± 6.8
Time of RF application (seconds)	444.4 ± 239.3
Total RF energy applied (Ws)	14123.0 ± 8514.5
Rtg time of exposure (seconds)	530.3 ± 315.8
Absorbed dose (mGy)	224.9 ± 194.4
Transitory AV block during the intervention	19 (20.7)
Transitory VA block during the intervention	20 (21.7)
Acute success of the intervention	91 (98.9)
Re-intervention rate	10 (10.9)
Dual physiology of AV node after the intervention	54 (58.7)
Atrial Echo impulse after the intervention	47 (51.1)
Effective refraction period after the intervention (ms)	282.9 ± 56.6
Weckenbach cycle after the intervention (ms)	376.8 ± 73.4
PQ interval after the intervention (ms)	160.3 ± 29.2
Permanent AV block after the intervention	3 (3.3)
Number of anti-arrhythmic drugs after the intervention	0.2 ± 0.5
Without anti-arrhythmic therapy after the intervention	74 (80.4)

RF – radio frequency; Rtg – Roetgen; AV – atrioventricular; VA – ventriculoatrial;
SD – standard deviation.

Table 3
Characteristics of the investigated patients after the ten-year follow-up

Characteristics	Number (%) or mean ± SD
Number of patients	64 (100.0)
Men	19 (29.7)
Women	45 (70.3)
Alive	57 (89.1)
Deceased	7 (10.9)
Recidivism of tachycardia	0
Permanent AV block	6 (10.5)
Paroxysmal arrhythmia	17 (29.8)
PQ interval (ms)	180.0 ± 30.9
Number of anti-arrhythmic drugs	0.5 ± 0.6
Without anti-arrhythmic therapy	33 (57.9)

SD – standard deviation.

Table 4

Multivariate logistic regression model for the prediction of arrhythmia after a ten-year follow-up

Variables	Probability coefficient	95% Confidence interval	Standard error	*p*
Male gender	0.638	0.137–2.975	0.785	0.567
Age above 55 years at baseline	3.945	1.022–15.223	0.689	0.046
Arterial hypertension at baseline	3.502	0.874–14.031	0.708	0.077
Re-intervention	5.437	1.028–28.740	0.850	0.046
Constant	0.137		0.556	0.000

None of the patients experienced recidivism of tachycardia, whereas permanent AV block of any degree was reported in 6 (10.5%) patients, and paroxysm of other arrhythmia was found in 17 (29.8%) patients. The average number of anti-arrhythmic drugs was 0.5 (range 0 to 2), and 58% of the patients had no anti-arrhythmic therapy after the 10-year follow-up period.

Univariate logistic regression models identified age (categorized as 0 – less than 54 years, and 1 – aged 55 years and older), arterial hypertension at baseline (categorized as 0 – not diagnosed, and 1 – diagnosed), and the need for re-intervention (categorized as 0 – not performed, and 1 – performed) as significant predictors for the paroxysmal arrhythmia after a 10-year follow-up.

A multiple logistic regression model was fitted, including gender and the above mentioned variables (Table 4). This model was statistically significant (χ^2 value = 12.069; $p = 0.017$) and explains between 18.8% and 26.4% of the variance in the occurrence of arrhythmia 10 years after the intervention. The model adequately classified 72.4% of all cases with arrhythmia after 10 years of follow-up. In this model, participant's age above 55 years at baseline and re-intervention performed after the intervention were identified as significant predictors of arrhythmia occurrence after a 10-year follow-up, independent from gender and arterial hypertension at baseline.

Discussion

According to the guidelines for the treatment of supraventricular tachycardia, catheter-ablation of the slow pathway AV node is indicated in patients with diagnosed AVNRT – Class I, Level B-RN [6]. In our study, in a group of 64 patients who were available after 10 years of follow-up, no supraventricular tachycardia relapse was registered in a single patient. High long-term effectiveness of 90-100% of the procedure was recorded in studies by D'Este et al. [7], Clague et al. [8] and Kimman et al. [9]. The D'Este et al. [7] study also monitored a group of patients on antiarrhythmic therapy. In the last mentioned study, after three years of follow-up, AVNRT relapse was no longer registered.

In our group during the follow-up period, seven patients died (five due to malignancies, one due to pulmonary embolism and one due to acute abdomen).

During and immediately after the catheter-ablation, AV block was registered in 3 (3.3%) patients and after 10 years in six (10.5%) patients. In 1 patient from this group third-degree AV block was registered, which required the implantation of the pacemaker, immediately after the ablation. The other five patients had first-degre AV block, and were recommended for further monitoring. In a multicenter study with 880 patients [5] complete AV block was registered in 4.7% of patients in total (slow and fast pathway ablation), or in 2.0% of patients who underwent slow pathway ablation. In a recent study by Jensen [10] the rate of late AV block appearance was 0.57%. Our results largely coincide with the results of the published studies so far.

The use of anti-arrhythmic therapy was significantly reduced during the follow-up period. The average number of anti-arrhythmic drugs used to prevent supraventricular tachycardia before ablation was 2.1 while this number was reduced to 0.5 in the period after the ablation and after the ten-year follow-up. More than a half of all patients did not have the need for medication in the meantime. Most patients used beta-blockers. A similar result was also found in the Brachmann et al. [11] study where about 58% of patients were exempted from antiarrhythmic drugs.

In some previously published papers, it has been shown that radio frequency catheter-ablation of slow pathway may be responsible for the emergence of substrate for new arrhythmias. In 30% of patients who underwent slow pathway ablation, new arrhythmias were reported: half of them had atrial fibrillation or atrial flutter, while at the second half were registered atrial premature beats or sinus tachycardia [8]. The assumption for the onset of atrial flutter is that the slow pathway is anatomically close to zone of slow conduction in the right atrium [12, 13]. In the study of Schernthaner et al. [14], 35% of patients in the follow-up period had arrhythmias that required the use of anti-arrhythmic drugs. It is possible that AVNRT is a substrate for the occurrence of atrial fibrillation in a group of younger patients [15], and in these patients, slow pathway ablation would be sufficient to prevent atrial fibrillation.

In our patient group, 17 (29.8%) of them had documented arrhythmia in the monitoring period. From this group, 10 patients had sinus tachycardia, two patients had extrasystoles, and five patients had atrial fibrillation or atrial flutter. A small number of patients had short palpitations not documented by an electrocardiogram. Most of these patients used beta-blockers during the monitoring period.

Using the univariate logistic regression model, our study showed that independent predictors of the onset of these arrhythmias over the course of 10 years follow-up were patients'age above 55 years at baseline and the need for re-intervention, independent from gender and arterial hypertension at baseline. The possible reason for the occurrence of

sinus tachycardia as the dominant arrhythmia in this group of patients is the ablation of parasympathetic fibres in the septal zone, that leads to an acceleration of the heart rate [16] while the other possible reason is the rebound phenomenon after stopping the taking of the beta-blocker in order to prepare for intervention. Nevertheless, other studies contradict this finding by showing that younger patients may be more prone to AVNRT recidivism than the older ones [17].

The limitation of this study is a relatively small sample, particularly after the follow-up, where almost a third of the original sample was lost. Although the patients were called for medical examination on the regular basis, the contact was lost due to unresponsiveness, address change, death or other circumstances. The presented results cannot therefore be easily generalized to the whole population of patients with AVNRT, but they might serve as an indicator of a long-term prediction of adverse outcomes of the intervention.

Conclusion

Our long-term follow-up study of patients following radio frequency catheter-ablation AVNRT showed that ablation is an effective and safe method of treating these patients. Total mortality was not associated with cardiovascular causes. The rate of recurrence of tachycardia and the complication rate are very low and most patients do not use antiarrhythmic therapy during the monitoring period. Independent predictors for the occurrence of other arrhythmias were age above 55 years and the need for re-intervention, and the most commonly reported arrhythmia during the follow-up period was sinus tachycardia.

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Analysis of duplications versus deletions in the dystrophin gene in Serbian cohort with dystrophinopathies

Uporedna analiza duplikacija i delecija u genu za distrofin u grupi bolesnika sa distrofinopatijom iz Srbije

Jasmina Maksić*, Valerija Dobričić[†], Lukas Rasulić[‡§], Nela Maksimović[‡], Marija Branković[‡], Vedrana Milić Rašić[‡‖], Vidosava Rakočević Stojanović[†‡], Ivana Novaković[‡]

University of Belgrade, *Faculty for Special Education and Rehabilitation, [‡]Faculty of Medicine, Belgrade, Serbia; Clinical Center of Serbia, [†]Neurology Clinic, [§]Clinic for Neurosurgery, Belgrade, Serbia; [‖]Clinic for Neurology and Psychiatry for Child and Youth, Belgrade, Serbia

Abstract

Background/Aim. Duchenne muscular dystrophy (DMD) and its allelic form Becker muscular dystrophy (BMD) are X-linked diseases that affect males, characterized by progressive muscle and cardiopulmonary weakness, especially in DMD as a severe form of the disease. They result from mutations in the dystrophin gene, and the most common changes are large intragenic deletions and duplications (80%). One third of patients have *de novo* mutation and 2/3 of the mothers are estimated as carriers. The aim of the study was to analyze the frequency of duplications versus deletions in the dystrophin gene in patients with dystrophinopathies, as well as to analyze the phenotypic effect of large mutations obtained and to determine the carrier status of female relatives in probands with duplications. **Methods.** We examined 22 DMD and 35 BMD unrelated patients and 6 female relatives of the probands where duplications were found. We used polymerase chain reaction (PCR) and multiplex ligation-dependent probe amplification (MLPA) methods, according to the protocol, to detect or confirm mutations in probands and female carriers. **Results**. In probands, there were 34 (59.6%) large deletions (mostly affected exons 44–60) and 6 (10.5%) large duplications in 4 DMD and 2 BMD patients. Also, duplications were found in 3 out of 4 (75%) tested mothers. The distribution of duplications was heterogeneous, affecting N-terminal and central rod domain, and included more exons, except for one DMD patient who had duplication of exon 2. An exception from the Monaco rule was present in 9.5% of DMD and 15.8% of BMD probands, i.e. in 12.5% of DMD/BMD cases. **Conclusion.** In 57 DMD/BMD probands, we found 59.6% of large deletions and 10.5% of large duplications. The most affected region of the DMD gene was the central rod domain. An exception to Monaco's rule was present in 12.5% of DMD/BMD cases. Three out of 4 examined proband's mothers were confirmed as carriers.

Key words:
gene deletion; gene duplication; genetics, medical; genetic diseases, inborn; muscular dystrophy, duchenne; women.

Apstrakt

Uvod/Cilj. Dišenova mišićna distrofija (DMD) i njegova alelna forma, Bekerova mišićna distrofija (BMD), su X-vezane nasledne bolesti od kojih obolevaju muškarci, a karakteriše ih progresivna mišićna i kardiopulmonalna slabost, posebno kod DMD kao težeg oblika bolesti. Ove bolesti nastaju kao posledica mutacija u genu za distrofin, a najčešće su prisutne intragenske delecije i duplikacije (80%). Novonastalu mutaciju ima1/3 bolesnika, a procenjeno je da su 2/3 majki nosioci. Cilj rada je bio da se analizira učestalost duplikacija u odnosu na delecije u genu za distrofin kod bolesnika sa distrofinopatijom, kao i da se ispita efekat dobijenih mutacija na fenotip kod probanda i utvrdimo status nosioca kod ženskih srodnika probanda sa duplikacijama. **Metode.** Studijom je bilo obuhvaćeno 22 DMD i 35 BMD nesrodnih bolesnika i šest ženskih srodnika probanda kod kojih su bile otkrivene duplikacije. Za otkrivanje ili potvrdu mutacije, kod probanda i ženskih nosioca, korišćene su metode: lančana reakcija polimerazom (PCR) i višestruko umnožavanje vezanih sondi (MLPA), prema datom protokolu. **Rezultati.** Kod probanda je nađeno 34 (59,6%) velikih delecija (najčešće su bili zahvaćeni egzoni 44–60) i 6 velikih duplikacija (10,5%) kod 4 DMD i 2 BMD bolesnika.

Correspondence to: Lukas Rasulić, Clinical Center of Serbia, Clinic for Neurosurgery, Belgrade, Faculty of Medicine, University of Belgrade, Višegradska 26, 11 000 Belgrade, Serbia. E-mail: lukas.rasulic@gmail.com

Takođe, duplikacije su nađene kod 3 od 4 (75%) testirane majke. Distribucija duplikacija je bila heterogena, obuhvatala je N-terminalni i štapićasti region i uključivala je veći broj egzona, osim kod jednog DMD bolesnika koji je imao duplikaciju egzona 2. Odstupanje od Monakovog pravila je bilo prisutno kod 9,5% DMD probanda, odnosno kod 15,8% BMD probanda, to jest kod 12,5% slučajeva. **Zaključak.** Kod 57 DMD/BMD probanda nađeno je 59,6% velikih delecija i 10,5% velikih duplikacija. Najčešće je bio zahvaćen štapićasti domen u DMD genu. Odstupanje od Monakovog pravila je bilo prisutno u 12,5% DMD/BMD slučajeva. Tri od četiri ispitane majke probanda su bile potvrđene kao nosioci.

Ključne reči:
geni, delecija; geni, duplikacija; genetika, medicinska; genetičke bolesti, urođene; distrofija, mišićna, dišen; žene.

Introduction

Duchene muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are dystrophinopathies that result from mutations in the gene encoding the dystrophin protein. They have X-linked recessive inherited pattern, where male family members are affected, while women are mostly the healthy carriers of the disease. DMD is the most severe form of dystrophinopathy, with an incidence of 1: 3,500 live-born males. Symptoms of the illness occur around the third year of life, progressing over time with increasingly pronounced weakness of the skeletal and cardiac muscles (cardio/pulmonary weakness) in the twentieth year of life. Its allele form, BMD, is characterized by a lower incidence, a later onset of the disease, a slower progression of symptoms and sometimes by a very mild clinical picture[1]. The gene for dystrophin protein (DMD gene) is located on the short arm of the X chromosome (Xp21.2–p21.1). With a size of 2.4 million base pairs, 79 exons and a main transcript length of 14 kb, it provides the full length of the protein (427 kDa) [2]. Four functional domains can be distinguished in the structure of dystrophin. Through the N-terminal domain, dystrophin binds to the f-actin of the cytoskeleton, and through the C-terminal domain it binds with proteins and glycoproteins of the sarcolemma, so called dystrophin associated-proteins which produce a dystrophin-glycoprotein complex [3, 4]. This complex stabilizes the sarcolemma and protects muscle fibers from damage caused by their long-term contraction [3, 5].

Due to its extreme size, the DMD gene is often subject to change. Of all the mutations in the DMD gene, in 65%–70% of cases there are intragenic deletions of one or more exons. The disposition of the detected deletions in the gene is specific, and the most commonly affected are exons 45–55 (the distal part of the gene), and exons 2–20 (the proximal part), the so-called "hot spots". Duplications are present in 5%–15% of cases, while the remaining cases are due to small mutations (less than one exon) – point mutations, small deletions, small insertions, splice sites changes [6, 7]. One-third of the mutations in the DMD gene are *de novo* mutations. It has been shown that the size and localization of the mutations are not correlated with the severity of the clinical presentation, bearing in mind that small lead to a more severe DMD phenotype, i.e. mutations of a large number of exons can result in a milder BMD phenotype. According to the Monaco rule, the effect of mutations on the phenotype depends on whether the mutation changes the reading frame of the genetic code or not [8]. The severe clinical presentation of DMD patients is the result of frameshift mutations in the dystrophin gene. These mutations (deletions or duplications) change the reading frame (out-of-frame), leading to the creation of practically undetectable amounts of shortened, nonfunctional protein. Deletions that do not change the reading frame (in-frame), result in the creation of shortened, partially functional protein, which is associated with a milder clinical presentation of BMD patients. However, about 10% of DMD/BMD patients deviate from this rule [9].

Grouping deletions in predilected areas in the DMD gene facilitates their detection. Multiplex polymerase chain reaction (PCR) is a technique that identifies 98% of all deletions in the DMD gene by analyzing 19 exons [10, 11]. The disadvantage of this method is that it does not detect duplications in the DMD gene of the proband, nor mutations in female carriers. For this purpose, Southern blotting, quantitative PCR (qPCR) and multiplex amplification and probe hybridization (MAPH) methods were used, but have proved complicated for routine application in practice [12]. In recent years, the principal method for detecting deletions and duplications in the DMD gene is multiplex ligation-dependent probe amplification (MLPA). The MLPA method enables the analysis of all 79 exons in the dystrophin gene and the detection of deletions which are not in predilected areas as well as duplications in the DMD gene; it is particularly important in determining the carrier status of female members [13–15]. However, point mutations cannot be detected by these methods.

The aim of study was to detect or confirm mutations in DMD/BMD probands, analyze the mutations obtained, and in patients with proven duplications, to determine the carrier status of the female members in the family.

Methods

The study group consisted of a total of 63 respondents, 57 unrelated DMD/BMD patients (22 DMD and 35 BMD) and 6 female relatives. The study was conducted at the Neurology Clinic, Clinical Center of Serbia, Belgrade and at the Institute of Human Genetics, Faculty of Medicine, University of Belgrade. Patients were selected according to the clinical parameters for DMD/BMD [the onset of the disease, the clinical presentation, electromyography (EMG) findings, hyper creatine phosphokinase (CPK)]. The genomic DNA was extracted from the peripheral blood samples of the subjects according to standard salting-out procedure [16]. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade.

The multiplex PCR method was applied to one group of patients. For the analysis of 26 exons of the DMD gene, three sets of primers were used – A, B, and C. Set A covered exons: 4, 8, 12, 17, 19, 44, 45, 46, 48, 51; Set B covered exons: Pm, 3, 6, 13, 43, 47, 50, 52, 60; and Set C covered exons: Pb, 16, 32, 34, 41, 42, 49 [17]. The analysis was performed according to the DMD/BMD multiplex PCR protocol [18].

The MLPA method was applied to newly diagnosed patients who did not previously have PCR, as well as to those later diagnosed to have. In patients with no deletions in the DMD gene detected by the PCR method, the MLPA was used to detect either deletions in other areas of the gene, or gene duplication. In patients where a deletion was detected using the PCR method, the MLPA was applied in order to more precisely define the mutation rates. In the probands with duplications found, the MLPA method was also used to examine the female members of the families. Two complementary SALSA MLPA kits, P034 and P035 (MRC Holland, Netherlands) were used to detect duplications and deletions in the DMD gene, according to the given protocol [13]. The analysis was carried out using ABI Thermal CyclerVerity and ABI 3500 Genetic Analyzer apparatus, and soft data processing was carried out using Coffalyser. Net. To predict the effect of duplication on the phenotype, we used the Reading-frame checker, version 1.9, which is available at www.dmd.nl [19].

For statistical analysis, frequencies, percentages, means and standard deviations (SD) were used as descriptive statistics and the χ^2 test for interrelation between variables. Analyses were performed in SPSS Statistics, version 20.

Results

The total sample consisted of 63 respondents, 57 unrelated DMD/BMD patients and 6 female relatives of probands where duplications were found. First, 57 unrelated patients were examined, 22 (38.6%) DMD and 35 (61.4%) BMD. In 9 patients, only the PCR method was applied, in 28 only the MLPA method, and in 20 patients, both methods were applied.

At the time of first neurological evaluation and the first genetic analysis, the average age of DMD/BMD probands was 17.24 ± 12.39 years, where the youngest patient was 1 year, and the oldest one 49 years old. Among DMD probands, the average age was 7.71 ± 5.29 years, whereby the youngest patient was 1 year, and the oldest one 21 years old. In BMD patients the average age was 23.90 ± 11.57 years, the youngest patient was 3 years, and the oldest one 49 years old.

The PCR method was used in a total of 29 DMD/BMD patients. In 18 (62.1%) patients, deletions of one or more exons were found, while in 11 (37.9%) patients, no deletions were found in the dystrophin gene.

The MLPA method was used in a total of 48 patients. In 25 (52.1%) patients, large deletions were found, in 6 (12.5%), large duplications were found, while in 17 (35.4%) patients, no deletions and duplications were found.

Analysis of the overall sample of patients, regardless of the method applied, showed that in 34 (59.6%) respondents deletions were found, in 6 (10.5%) duplications were found, while in 17 (29.8%) respondents no deletions or duplications were found.

Both methods, the PCR and MLPA, were applied to a total of 20 patients. Among nine patients, deletions were found using the PCR method, while with the MLPA method, the same deletion rate was found in 5 (55.6%) patients, and a higher deletion rate was found in 4 (44.4%) patients. Among the remaining 11 patients, no deletions were found using the PCR method, while using the MLPA method, 5 (45.5%) patients were found to have deletions in another area, duplications were found in 4 (36.4%) patients, and in 2, no deletions or duplications were found (Table 1).

We analyzed the age in which the first genetic analysis was done in DMD/BMD probands with deletion, duplication, and with no deletion or duplication, respectively. In the group with deletions, the average age was 16.31 ± 12.16 years, in the group with duplications 11.00 ± 5.18 years, and in probands with no deletion or duplication the average age was 22.38 ± 3.88 years.

We also analyzed the correlation between the mutations found and the phenotypes (Table 2). In patients with a diagnosis of DMD, the mutations found were significantly higher (77.3% deletion and 18.2% duplication) than in patients with BMD. In 45.7% of the patients with BMD, no mutations were found (neither deletions nor duplications), while in patients with DMD this percentage was 4.5%.

The results of the chi-square test showed that there was a statistically significant difference [χ^2 (1) = 11.54, p = 0.003] in the frequency of the mutations regarding established phenotypes.

Table 1
Findings in probands using the polymerase chain reaction (PCR) and multiplex ligation-dependent probe ampiflication (MLPA) methods

PCR	MLPA					Total
	confirmed deletion	confirmed deletion and found larger	deletion at another location	duplication	no deletion or duplication	
Patients with deletions, n (%)	5 (55.6)	4 (44.4)	0 (0.0)	0 (0.0)	0 (0.0)	9 (100.0)
Patients with no deletions, n (%)	0 (0.0)	0 (0.0)	5 (45.5)	4 (36.4)	2 (18.2)	11 (100.0)
Total patients, n (%)	5 (25.0)	4 (20.0)	5 (25.0)	4 (20.0)	2 (10.0)	20 (100.0)

Table 2

Frequency of the mutations in relation to the proband's phenotype

Proband's phenotype	Mutations			Total	*p*
	deletion	duplication	no deletion or duplication		
DMD, n (%)	17 (77.3)	4 (18.2)	1 (4.5)	22 (100.0)	
BMD, n (%)	17 (48.6)	2 (5.7)	16 (45.7)	35 (100.0)	< 0.05
Total, n (%)	34 (59.6)	6 (10.5)	17 (29.8)	57 (100.0)	

DMD – Duchenne muscular dystrophy; BMD – Becker muscular dystrophy.

Table 3

Phenotype by Reading frame checker crosstabulation

Proband's phenotype	Mutations		Total
	in-frame	out of-frame	
DMD, n (%)	2 (9.5)	(90.5)	21 (100)
BMD, n (%)	16 (84.2)	3 (15.8)	19 (100)
Total, n (%)	18 (45.0)	22 (55.0)	40 (100)

DMD – Duchenne muscular dystrophy; BMD – Becker muscular dystrophy.

Using the Reading-frame checker (version 1.9) we analyzed the large mutations obtained as well as their correlation to the probands' phenotype (Table 3). Among DMD probands, 2 (9.5%) of them had in-frame mutations (del 3–15; del 33.34), and 19 (90.5%) had out-of-frame mutations. In BMD probands, 16 (84.2%) had in-frame mutations, and 3 (15.8%) had out-of-frame mutations (del 44–48; del 44–49; dupl. 18–27).

Deletions were found in 17 DMD and 17 BMD probands. In 27 (79.4%) DMD/BMD probands more exons were affected and were mostly localized in the distal part of DMD gene (exons 44–60), in 6 DMD and 12 BMD patients (66.7%). Among BMD probands, the most common was deletion of exons 45–48 (5 times) and deletions of exons 45–47 and 45–49 (3 times each), while in DMD patients affected exons were more heterogeneous. The largest deletions covered 31 exons in one BMD patient (exons 12–43). Deletions of one exon were present in 7 (20.6%) patients – exons 1, 44, 48, 50 and 59, of which 5 patients (71.4%) had the DMD phenotype and both patients with the BMD phenotype had single deletion of exon 48. In 13 DMD and all BMD probands affected exons covered the central rod domain of the DMD gene, while in 2 DMD probands deletion included only N-terminal domain, in one patient both of those domains, and in one DMD patient the central rod domain and C-terminus were included. An exception to Monaco's rule in patients with deletions was present in 2 DMD and 2 BMD probands.

Duplications were found in 4 DMD and 2 BMD patients. In 16.7% and 83.3% of probands, duplications of one exon and more exons were found, respectively. The distribution of duplications was heterogeneous, affecting N-terminal and central rod domain. Among the probands with the Duchenne phenotype, 1 (25%) had a duplication of exon 2, and in 3 (75%) of them, the duplication affected more exons, as well as in both BMD probands. Duplications of more exons are shown in Table 4. In 4 probands with the Duchenne phenotype, all of them had frameshift mutations, while in probands with the Becker phenotype, one of them had in-frame mutations and one out-of-frame mutations.

Also, for each DMD proband with duplication, the carrier status of the mother, and in one trial, of two sisters, was analyzed using the MLPA method. In two BMD probands, female relatives were not examined. According to the data, in half of the female relatives, duplications were found. Duplications were found in 3 (75%) mothers, while in one (25%) mother, no duplications were found. In addition, duplications found in 3 mothers were the same as in sons of the probands. In the family where three female members were examined – the mother and two sisters, duplication was found in the mother, but not in sisters (Table 4).

Table 4

Duplications in probands and female relatives

Respondents	Affected exons						Total
	2	8–16	18–27	21–42 / 45–48	31–44	52–62	
Probands							
DMD	1	1		1		1	4
BMD			1		1		2
Female relatives							
mother	1	0		1		1	3
sisters	0						0

DMD – Duchenne muscular dystrophy; BMD – Becker muscular dystrophy.
1 – duplication found; 0 – duplication not found.

Discussion

As the largest detected gene in the human genome, the DMD gene is susceptible to changes. Diagnostic genetic testing is performed on symptomatic patients and in making a prenatal genetic diagnosis, as well as in order to determinate the carrier status of female. In our sample of 57 probands (22 DMD and 35 BMD), the average age at the time of first neurological evaluation and the first genetic analysis was 17.24 years. Among DMD probands, the youngest patient was 1 year, and the oldest one 21 years old. In BMD probands, the youngest patient was 3 years and the oldest one 49 years old. The data obtained are in line with the fact that the symptoms of DMD begin around third year of life (sometimes earlier), and the loss of mobility is present up to 12 years of age [1]. On the other hand symptoms of BMD begin around 10 years of age and in most patients the symptoms are present up to 20 years, the course of the disease is slower, and even in severe forms of the disease the loss of mobility is not present before the age of 16. We also found that in DMD/BMD patients with duplication, the mean age was lower than in the group with deletion and in the group with no mutations detected.

The multiplex PCR is a method that detects deletions in predilected areas in the dystrophin gene. In our sample, using this method, deletions were detected in 62.1% of the patients. With the use of the MLPA method, all deletions found using the PCR method were confirmed. Also, in 20% of the cases, extended deletions were found, in 25% of the cases deletions were detected at another location (outside of the "hot spots"), and in 4 (36.4%) respondents duplications were detected. So far, these findings confirm the conclusions of a large number of authors on the effectiveness of the MLPA method [13–15, 20, 21].

To date, the largest study including 7,149 respondents carried out by Bladen et al. [7], found major mutations in 80% of cases, out of which 86% were deletions (one exon or more) and 14% duplications (one exon or more). The remaining 20% constitute small mutations (less than one exon). In our sample of 57 unrelated patients, 22 DMD and 35 BMD, analysis of the results obtained using both methods (PCR and MLPA) showed that major mutations, deletions and duplications, were detected in 40 (70.2%) respondents (21 DMD and 19 BMD), while no mutations were detected in 17 (29.8%) respondents. The large deletions found in 25 of the patients were most represented and located in the distal part of the gene, exons 44–60, out of which 14 (63.3%) had the BMD phenotype. Deletions in BMD patients included exons 44–49, the typical localization for BMD of the moderate course, often with variability in the clinical picture [22–25]. The largest deletion found covered 31 exons in one BMD patient (exons 12–43). It is known that large deletions, which are limited to the rod-domain, predominantly result in the BMD phenotype [26]. Deletions of one exon were present in 7 (20.6%) patients – exons 1, 44, 48, 50, and 59, out of which 5 (71.4%) patients had the DMD phenotype. Both patients with the BMD phenotype had single deletions of exon 48, that generally causes a very mild form of the disease [27].

Out of 17 (29.8%) respondents with no deletions or duplications, the Becker phenotype was detected in 16 of them. Possible reasons for this are either point mutations, which could not be detected using the applied methods, or another kind of myopathy. As BMD shows a wide spectrum in the clinical presentation, from borderline DMD to very mild myopathy, this phenotype can have similarities with other types of muscular dystrophy or metabolic myopathies. Further examination of three patients using the Next Generation Sequencing method, showed that mutations of the CAPN3 gene (complex heterozygous) were present in two patients, while in one patient the findings in the muscle dystrophy gene panel were negative.

Comparing to deletions, duplications are much less common in the DMD gene, and they are present in 5%–10% of DMD patients and in 5%–19% of patients with BMD [28]. However, most authors using the MLPA method find no duplication in more than 10–14% of DMD/BMD patients [7, 29]. According to molecular analyses, while deletions are mainly due to unequal crossing-over during oogenesis, duplications are more often due to an event during spermatogenesis (grandpaternal germline). Basically, duplication can be caused by the same mechanism as deletion, during homologous or non-homologous recombination, or by insertion, although analysis of breakpoints has shown that it is more likely that they occurr due to the synthesis-dependent linking of nonhomologous areas [30]. Also, duplications are more often represented in families with increased risk of recurrence. Because it is an X-linked recessive disease, female family members are mainly heterozygous carriers of the disease. In sporadic cases of DMD, it is estimated that 2/3 mothers are carriers of the mutation, in 5%–10% there is gonadal mosaicism, while in 25%–30% of cases there is a new mutation [31]. In 5%–8% of cases, women can be manifesting carriers of the mutations. Also, the mother's risk of being a carrier is greater for duplication than for deletion.

In our total sample, there were 63 respondents, 57 DMD/BMD unrelated patients and 6 female relatives from 4 DMD probands families with duplications (4 mothers and 2 sisters of the probands). A total of 9 (14.3%) duplications were found, out of which 6 (10.5%) duplications were found in 4 DMD and 2 BMD patients, and 3 in DMD mothers. Thus, in 75% of the cases, mothers were confirmed as carriers of the mutation, while in one (25%) mother there was no mutation. Also, in the mothers of carriers, the same mutations were found in the affected sons. No duplications were found in the two sisters. Apart from being less frequent, the distribution of duplications in the DMD gene itself is very different, and most often localized in the vicinity of the 5'end of the DMD gene. The most frequent duplication of an exon is the duplication of exon 2 [30]. When it comes to the duplication of a greater number of exons, according to the TREAT-NMD DMD Global database, the most described duplications in literature are those of exons 3–7, 8–9, 8–11, 8–12, 5–7, 56–62 [7]. According to Takeshima et al. [29], the most present large duplications are those of exons 3–7, and the largest, of exons 3–43.

In our sample, in one DMD patient and his mother, a duplication of single exon 2 was found, while no mutations were found in his sisters. Exon 2 is part of the gene region encompassing exons 1-8 that encode the N-terminal Actin Binding Domain-1 containing three actin-binding sites through which the protein dystrophin, binds to the cytoskeletal actin [32]. In-frame mutations in this part essentially disturb the stability of dystrophic coupling and decrease affinity for binding with F-actin, resulting in a more severe clinical picture [33]. It is estimated that about 7% of DMD patients have mutations in this domain, and the most commonly affected are exons 2–7, with the most common being exon 2 [34, 35]. In BMD patients, the presence of mutations in this domain leads to a lower level of dystrophin and also results in a more severe clinical picture. In our patients, out-of frame duplication of exon 2 was associated with the DMD phenotype.

In 3 DMD probands and two mothers, as in both BMD patients, there were duplications of a greater number of exons (77.8%). The distribution of these duplications was very different, and they all were localized in the central rod domain. In two BMD patients, there were duplications of exons 18–27 and 31–44, respectively. In one DMD patient and in another DMD patient and his mother carrier, there were duplications of exons 8–16 and 52–62, respectively; in one DMD patient and his mother, the largest duplications encompassing exons 21–42 and 45–48 were found.

The central rod domain (coded by exons 9–65) contains 24 spectrin-like repeats and four proline-rich hinges providing flexibility to the protein. Near the central part of the domain, there is the second actin-binding domain (ABD)-2, which together with ABD-1 builds a strong lateral connection with actin filaments on the one hand, while on the other hand, through a link with the cysteine-rich domain is connected to the C-terminus region [36]. It is believed that this domain contains entities that are of different functional significance, so the localization of the in-frame change has a different effect on the phenotype. In addition, it is known that major mutations in this domain, if they do not disturb the reading frame and have preserved the N-terminal and C-terminal regions, generally result in the BMD phenotype. In our patients, two duplications were associated with the BMD phenotype, and included the proximal and central part of the rod-domain. According to Beggs et al. [37], this localization results in the BMD phenotype of mild progression. In 3 patients, duplications were associated with the DMD phenotype, each with a different localization in the domain, and all were out of the scope of reading the genetic code, which led to the creation of very small amounts of shortened, non-functional protein.

According to Monaco et al. [8], out-of-frame mutation correlates with a severe clinical presentation in DMD patients, while the in-frame mutation results in a milder form of the disease, i.e. the BMD phenotype. Recent studies suggest that duplications, which are more commonly present in BMD, result in exceptions from the Monaco rule in over 30% of cases [28, 29]. By analyzing the detected mutations using the Reading-frame checker (version 1.9) [19], we found that there were exceptions from the Monaco rule in 9.5% of DMD probands, and in 15.8% of BMD probands. In DMD probands, 2 of them had in-frame mutations. One DMD proband had in-frame deletion of exons 3–15, that disturbs the 5' binding site in the gene which causes DMD, with a typical onset in 3rd or 4th exon, and extending into the rod domain [38, 39]. The other DMD proband had deletion of exons 33 and 34. Deletion of these exons, as an in-frame mutation, is mainly described in BMD patients, but smaller deletions, while only deletions of exon 33 or exon 34 lead to DMD [40, 41]. In our case del 33,34 led to DMD phenotypes in a boy of 2 years of age. Baumbach et al. [42] reported that deletion of exons containing HindIII fragments could result in either the DMD or the intermediate DMD/BMD phenotype.

Among BMD probands, 3 of them had out-of frame mutations (del 44–48; del 44–49; dupl 18–28). According to literature, the reason for this contradiction, is the appearance of an alternative splicing which, by the exon-skipping mechanism, leads to the reestablishment of the reading frame and the creation of shortened, but functional dystrophin, and the BMD phenotype [28, 43]. Apparently, the association of genotype with phenotype, apart from the size, location and state of the reading frame, has other complex impacts that can alter the phenotype of patients.

Conclusion

In 57 unrelated probands, 34 (59.6%) deletions of one or more exons (the most commonly affected exons 44–60) covering the central rod domain, and 6 (10.5%) duplications affecting N-terminal and central rod domain were found. Distribution of duplications was heterogeneous and included more exons, except for one DMD patient who had duplication of exon 2. In DMD probands, the mean age at the time of the first genetic analysis was 7.71 years, and in BMD probands it was 23.90 years. An exception from the Monaco rule was present in 9.5% of DMD probands, and in 15.8% of BMD probands. Also, duplications were found in 3 out of 4 (75%) tested mothers who were confirmed as carriers.

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Skeletal changes in patients with mandibular prognathism after mandibular set back and bimaxillary surgery – A comparative cephalometric study

Skeletne promene kod bolesnika sa mandibularnim prognatizmom nakon mandibularne i bimaksilarne hirurgije – komparativna rendgenkraniometrijska studija

Vladimir Sinobad*†, Ljiljana Strajnić‡, Tamara Sinobad§

University of Belgrade, *Faculty of Dental Medicine, †Clinic for Maxillofacial Surgery, Belgrade, Serbia; ‡Clinic for Dentistry of Vojvodina, Novi Sad, Serbia; §Zepter Dental Polyclinic, Belgrade, Serbia

Abstract

Bacground/Aim. Recently, maxillary and bimaxillary surgery gained the primacy in the surgical correction of class III deformities. The aim of this investigation was to compare the changes in the skeletal relationships in patients with mandibular prognathism after bimaxillary surgery. **Methods.** The study included 70 subjects divided into three groups. Twenty class III patients of the experimental group 1 underwent bilateral sagittal ramus osteotomy and twenty patients of the experimental group 2 were subjected to bimaxillary surgery. The control group consisted of 30 subjects with skeletal class I and physiological occlusion. Cephalometric research was conducted on 110 lateral cephalometric radiographs made in subjects of the experimental groups 1 and 2 before and after surgery and in subjects of the control group. Using the computer program "Dr. Ceph", 30 linear and angular skeletal variables were analyzed on each radiograph. **Results.** Bimaxillary osteotomies changed most of variables that characterize the mandibular prognathism. The changes in the sagittal plane included the significant increase of sella-nasion to the A point (SNA) angle (by 4º on the average) and the A point to B point (ANB) angle (6°), and significant reduction in angles sella-nasion to the B point (SNB) (3º), gonial angle (ArGoMe) (8°), gonial angle inferior (NGoMe) (6.2º), and Björks sum (7°). The vertical relationships were normalized by significant reduction in overall anterior face height N-Me (by 5 mm on the average), the lower anterior face height ANS-Me (4 mm), significant increase in the total posterior face height S-Go (2.5–3 mm), lower posterior face height PNS-Go (4 mm), and significant reduction of the basal and mandibular plane angles. **Conclusion.** Compared to the isolated mandibular operations, bimaxillary surgery changes more efficiently the sagittal and vertical skeletal relations in patients with class III deformities and harmonizes more successfully the entire skeletal facial profile.

Key words:
malocclusion, angle class III; oral surgical procedures; cephalometry; maxilla; mandible; treatment outcome.

Apstrakt

Uvod/Cilj. Maksilarna i bimaksilarna hirurgija dobila je nedavno primat u hirurškim korekcijama deformiteta klase III. Cilj ovog istraživanja je bio da se uporede promene u skeletnim odnosima kod bolesnika sa mandibularnim prognatizmom posle bimaksilarne operacije. **Metode.** Ispitivanjem je obuhvaćeno 70 ispitanika podeljenih u tri grupe. Dvadeset ispitanika klase III eksperimentalne grupe 1 podvrgnuto je bilateralnoj sagitalnoj ramus osteotomiji, a dvadeset ispitanika eksperimentalne grupe 2 podvrgnuto je bimaksilarnoj operaciji. Kontrolnu grupu činilo je 30 ispitanika sa skeletnom klasom I i fiziološkom okluzijom. Rendgen-kraniometrijsko istraživanje obavljeno je na 110 bočnih telerendgen snimaka urađenih kod ispitanika u eksperimentalnim grupama 1 i 2 pre i posle operacije i ispitanika kontrolne grupe. Koristeći kompjuterski program „Dr.Ceph", na svakom telerendgenu analizirano je 30 linearnih i ugaonih skeletnih varijabli. **Rezultati.** Bimaksilarna osteotomija promenila je većinu varijabli koje karakterišu mandibularni prognatizam. Promene u sagitalnoj ravni uključuju značajan porast ugla maksilarnog prognatizma (SNA) (od 4º u proseku) i ugla gagitalnog odnosa tela gornje i donje vilice (ANB) ugla (6°), značajno smanjenje uglova

Correspondence to: Ljiljana Strajnić, Clinic for Dentistry of Vojvodina, Hajduk Veljkova 12, 21 000 Novi Sad, Serbia.
E-mail: ljiljana.strajnic@mf.uns.ac.rs

ugao mandibularnog prognatizma (SNB) (3º), ganialnog ugla (ArGoMe) (8°), donjeg gonialnog ugla (NGoMe) (6,2º), i Bjorkovog poligona (7°). Vertikalni odnosi su normalizovani značajnim smanjenjem ukupne prednje visine lica N-Me (od 5 mm u proseku), prednje donje visine lica (ANB-Me) (4 mm), povećanjem ukupne zadnje visine lica M-Go (2,5–3 mm), zadnje donje visine lice PNS-Go (4 mm), i značajnim smanjenjem mandibularnih uglova. **Zaključak.** U poređenju sa izolovanom mandibularnom operacijom bimaksilarna hirurgija menja efikasnije sagitalne i vertikalne skeletne odnose kod bolesnik sa deformitetima klase III i uspešnije harmonizuje ceo skeletni profil lica.

Ključne reči:
malokluzija, klase III; hirurgija, oralna, procedure; kefalometrija; maksila; mandibula; lečenje, ishod.

Introduction

Literature data indicate that severe forms of dentofacial deformities occur in 0.5% of people in the general population. The fact is, however, that of all patients requiring orthognathic surgery 28–34% are those with mandibular prognathism [1].

The treatment modalities in patients with class III deformities have been altered and perfected over the time. It turned out that efforts of classical orthodontic therapy in the childhood and adolescence were often insufficient to achieve optimal functional and aesthetic results in these patients [2]. The decision to apply a surgical treatment depends on many factors which include primarily phenotypic characteristics of the present deformity, age of the patient, and then, various psychological and social moments.

Investigation of phenotypic characteristics of class III deformities have revealed the great variety of underlying skeletal and dental patterns that are mainly related to different ethnical groups. In the majority of cases, class III deformities are the combination of maxillary retrognathia, mandibular prognathism and varying degrees of vertical dyscrepances [3–5].

The results of these researches significantly changed approaches and modalities in correcting class III deformities. Until the 80s of the last century, isolated mandibular operations were commonly used in surgical correction of mandibular prognathism, because the opinion prevailed that increased mandible was the primary cause of deformity. Beginning with Obwegeser who introduced the bilateral sagittal split ramus osteotomy (BSSRO) in the early sixties of the last century, this surgical technique has been until today successfully used in correcting class II and III deformities. The procedure and the numerous advantages of this method are detailed in scientific literature sources [6–8]. The fact is, however, that this operation does not provide the best results for all patients with class III deformities. Numerous studies indicate that most of the skeletal dimensions in these patients even after surgery remain typical of mandibular prognathism [9–11].

Extensive research of craniofacial morphology in patients with class III deformities and improvement of surgical techniques resulted in new trends in their surgical correction [1, 12–14]. In the recent years maxillary and bimaxillary surgery gained primacy in the surgical correction of class III deformities and the adequate orthodontic preparation became a necessary overture to a successful surgical correction [14–18].

The aim of this investigation was to compare the changes in the skeletal relationships in patients with mandibular prognathism after BSSRO with changes in these relations after bimaxillary surgery, in order to objectively examine the results of each of these operative techniques and accurately define the indication area for each of them.

Methods

The Ethical Review Board of our Faculty of Dental Medicine had approved this study.

The sample of the present study comprised 70 subjects divided into three groups: two experimental groups and the one control group. Each experimental group (1 and 2) consisted of 20 patients, mean age 19.8 ± 5.3 years, who were admitted at the Department of Maxillofacial Surgery, Faculty of Dental Medicine in Belgrade, for surgical correction of mandibular prognathism in the period from 2003–2013. The control group consisted of 30 young persons, mean age 21.5 ± 3.5 years, with skeletal class I and physiologic occlusion.

For the purposes of cephalometric research, totally 110 lateral cephalometric radiographs were made and divided into five groups (the groups A1, B1, A2, B2 and C).

The group A1 consisted of 20 lateral cephalometric radiographs derived from the patients of the experimental group 1 [patients underwent bilateral sagittal ramus osteotomy according to Obwegeser and Dal Pont (BSSRO)] before surgery and before orthodontic preparation. Diagnosis of mandibular prognathism in this group was based on the analysis of linear and angular skeletal parameters – the basic indicators of prognathism [anterior total facial height (N-Me) = 122.7 ± 7.78 mm; anterior lower facial height (ANS-Me) = 71.1 ± 6.13 mm; length of mandibular body (Go-Me) = 77.2 ± 7.01 mm; posterior total facial height (PNS-A) = 43,2 ± 4,00 mm; posterior total facial height (S-Go) = 78.4 ± 7.3 mm; anterioposterior position of the maxillar relative to the anterior cranial base (SNA) = 81.2 ± 4.36º; anterioposterior position of the mandible relative to the anterior cranial base (SNB) = 85.9 ± 5.60º; relationsip of the maxilla and mandible in the sagital plane (ANB) = -4.7 ± 2.50º; gonial angle by Bjö rk (ArGoMe) = 132.7 ± 7.91º; Björks sum = 385.9 ± 6.60º)] [11]. The Group B1 consisted of 20 lateral cephalometric radiographs derived from the same patients of the experimental group 1, 6 months to a year after bilateral sagittal ramus osteotomy.

The group A2 (consisted of 20 lateral cephalometric radiographs derived from the patients of the experimental

group 2 (patients operated on by bimaxillary approach that involved Lefort I osteotomy of the maxilla and bilateral sagittal ramus osteotomy of the mandible) before surgery and before orthodontic preparation. Diagnosis of mandibular prognathism in this group was based on the analysis of linear and angular skeletal parameters – the basic indicators of prognathism (N-Me = 124.0 ± 6.89 mm; ANS-Me = 71.0 ± 6.45 mm; Go-Me = 77.6 ± 6.53 mm; PNS-A = 43.6 ± 3.56 mm; S-Go = 76.6 ± 5.20 mm; SNA = 79.2 ± 4.66º; SNB = 84.0 ± 4.38º; ANB = -4.7 ± 3.04º; ArGoMe = 135.5 ± 10.85º; Björks sum = 398.8 ± 9.91º) [18].

The group B2 consisted of 20 lateral cephalometric radiographs derived from the same patients of the experimental group 2, 6 months to a year after bimaxillary surgery.

The group C consisted of 30 lateral cephalometric radiographs made in subjects of the control group. This collection was selected from the files of our dental school (archive of the author).

Lateral cephalometric radiographs were made in the Plan-Meca Radiological Center and the Center for the Head and Neck Radiology at the Facultyl of Dental Medicine in Belgrade with a special apparatus „Orthoceph“ (*Siemens*, Bensheim, Germany). The recordings were made by standard techniques at a voltage of 65 to 80 kV and strength of 20 mA, and the exposure was from 1 to 1.5 sec. Recordings were performed on the X-ray films 18 x 24 cm. All radiographs were scanned and transformed into digital form.

The choice of operative technique

All subjects of the experimental groups were referred to presurgical orthodontic therapy for a period of one and a half year, and then subjected to surgical correction.

Analysis of linear and angular skeletal parameters in the experimental groups A1 and A2 indicated that mandibular prognathism in both experimental groups showed similar chephalometric parameters. The fact is, however, that some specificities were observed in the experimental group A2. The analyses of linear skeletal parameters showed that the total anterior face height and lower anterior face height were greater in the group A2 than in the group A1. The posterior face height was lower in the experimental group A2 than in the group A1.

The analyses of angular sceletal parameters showed lower mean values of SNA angle in the experimental group A2 than in the group A1, and mean values of Björks sum where greater in the experimental group A2 then in the group A1 [18].

The previous research indicated that in the experimental group A2 before surgery, there were significantly more subjects with SNA angle values below the biometrick norm (40%), and significantly less subjects with SNA values within the biometric norm (25%). As SNA angle is one of the indicators of sagittal maxillary position, it could be concluded that 40% of subjects in the experimental group A2 had a pronounced maxillary retrusion.

According to these values, the largest number of subjects in the experimental group A2 had an underdeveloped maxilla associated with the pronounced mandible (over 50%) or normally developed mandible (25%). Increased vertical facial dimension was found in 85% of subjects [18].

The results of preliminary cephalometric research in the experimental group A1 were decisive for the selection of surgical techniques in this group. As the deformity in patients of the experimental group A2 was mainly due to deficient maxilla, normally developed or pronounced mandible, with differently expressed increased vertical face parameters of viscerocranium, a successive bimaxillary approach was used in surgical correction.

The subjects in the experimental group 1 underwent bilateral sagittal ramus osteotomy according to Obwegeser and Dal Pont (BSSRO). A wire fixation was used to fix the bone fragments. After surgery, a combination of solid and elastic intermaxillary immobilization was applied for a period of 6–8 weeks.

The surgical procedure in subjects of the experimental group 2 was performed by a successive bimaxillary approach that involved Lefort I osteotomy of the maxilla and bilateral sagittal ramus osteotomy of the mandible. The rigid fixation (mini titanium plates and screws) were used to fix the bone fragments. A combination of solid and elastic intermaxillary immobilization was applied for a period of 6–8 weeks after surgery.

Cephalometric analysis

All lateral cephalograms, made in the experimental groups 1 and 2 before and after surgery as well as in the control group were subjected to cephalometric analysis. For this purpose a special computer program "Dr. Ceph" (FYI Technologies, GA, USA, last revised edition - version 9.7.) was used (Figure 1).

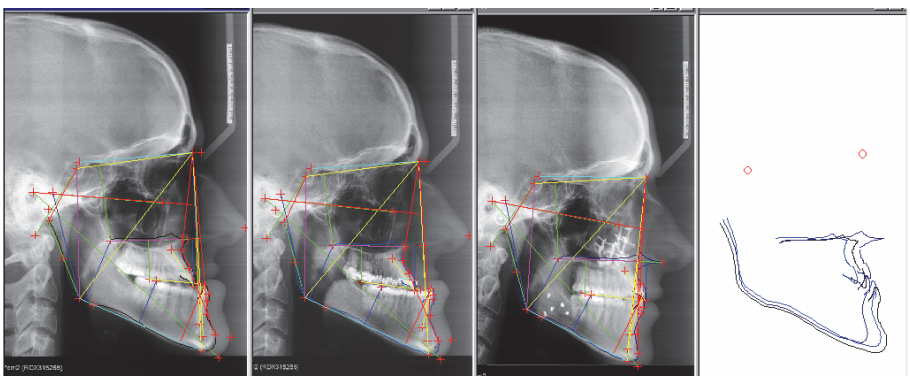

Fig. 1 – Cephalometric analysis of parameters by the „Dr.Ceph“ computer software.

Thanks to the possibilities of the computer software "Dr Ceph", on each cephalogram (the groups: A1, B1, A2, B2 and C) the values of 30 linear (Figure 2) and angular (Figure 3) skeletal variables and proportions of certain linear parameters were recorded and evaluated.

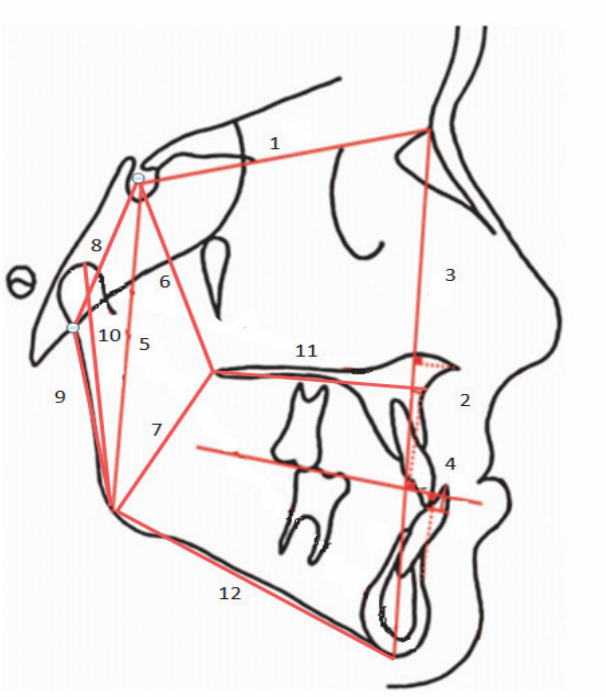

Fig. 2 – Examined linear variables:

1. N-Se – length of the anterior cranial base;
2. N-Me – anterior total facial height;
3. N-ANS – anterior upper facial height;
4. ANS-Me – anterior lower facial height;
5. S-Go – posterior total facial height;
6. S-PNS – posterior upper facial height;
7. PNS-Go – posterior lower facial height;
8. S-Ar – length of the posterior cranial base;
9. Ar-Go – length of the ramus;
10. Co-Go – height of the ramus;
11. PNS-A – length of the maxillary body;
12. Go-Me – length of the mandibular body.

Numerical values of the examined skeletal variables were subjected to statistical analysis and compared. To verify the changes in skeletal relationships due to surgical correction, the values of selected skeletal variables derived from subjects of the experimental groups 1 and 2 were compared before and 6 month after surgery. The results of this part of investigation are presented in previous studies [11, 18].

In this paper the mean postoperative values of investigated skeletal variables were compared between the experimental groups 1 and 2 and the control group in order to evaluate the success of applied surgical technique in correcting the mandibular prognathism.

In adition, the quantitative differences in the values of examined variables before and after surgery were evaluated in groups operated by different surgical techniques.

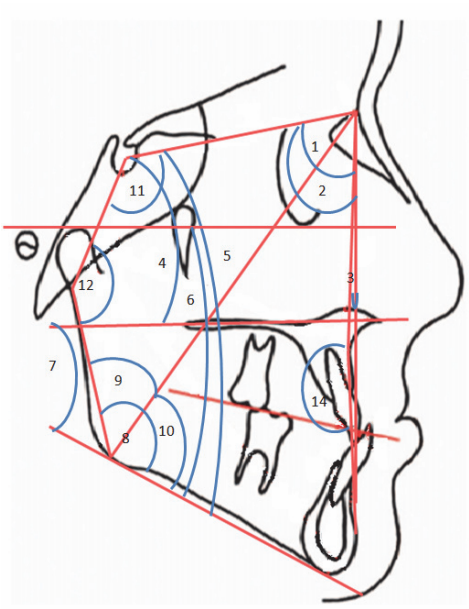

Fig. 3 – Examined angular variables:

1. SNA – anteroposterior position of the maxilla relative to the anterior cranial base;
2. SNB – anteroposterior position of the mandible relative to the anterior cranial base;
3. ANB – relationship of the maxilla and mandible in the sagittal plane;
4. N-S/PP – inclination of the maxilla to the anterior cranial base;
5. N-S/MP – inclination of the mandible to the anterior cranial base;
6. FH/MP – the relationship between the Frankfurt plane and mandibular plane;
7. PP/MP – the relationship between the basic jaw planes;
8. ArGoMe – gonial angle by Björk;
9. ArGoN – upper part of the gonial angle;
10. NGoMe – lower part of the gonial angle;
11. NSAr – angle of the saddle by Björk;
12. SArGo – articular angle by Björk;
13. Björk sum – the sum of the angles NSAr, SArGo and ArGoMe;
14 NAPg – angle of facial skeletal convexity.

Results

Comparison of mean values of linear skeletal variables in patients treated with BSSRO with the mean values of these variables in patients operated by bimaxillary osteotomies revealed the significant differences in postoperative values of 8 linear skeletal variables: S-Go, PNS-Go, S-Ar, Ar-Go, Co-Go, S-Go/N-Me, PNS-A and Go-Me (Table 1).

The anterior total facial height N-Me, and the anterior lower facial height ANS-Me were significantly reduced by both operative techniques compared to the situation before surgery, although the reduction was greater after bimaxillary surgery (Table 2). Unlike BSSRO which had no impact on dimensions of the posterior facial height, bimaxillary surgery increased significantly the posterior total facial height (S-Go) (d = 2.67±3.52 mm) and the posterior lower facial height (PNS-Go) (d = 4.1 ± 1.39 mm) (Table 2).

Table 1

Comparison of mean values of linear skeletal variables and proportions of certain linear parameters after BSSRO (the experimental group 1) and after bimaxillary surgery (the experimental group 2) with the values in the control group

Variables	Control group mean ± SD	Experimental group 1 mean ± SD	Experimental group 2 mean ± SD	ANOVA-test *p*	
N-Se	63.7 ± 6.3	65.0 ± 4.5	66.8 ± 4.7	0.236	ns
N-Me	114.9 ± 8.5	119.7 ± 10.3	118.9 ± 7.8	0.132	ns
N-ANS	50.3 ± 4.6	51.3 ± 3.9	52.1 ± 5.1	0.452	ns
ANS-Me	64.5 ± 5.8	68.4 ± 7.7	66.7 ± 6.5	0.102	ns
S-Go	78.5 ± 5.9	77.3 ± 6.6[a]	79.3 ± 7.1[b]	0.046	< 0.05
S-PNS	44.0 ± 3.4	45.8 ± 4.4	44.7 ± 4.1	0.255	ns
PNS-Go	44.4 ± 4.18	41.1 ± 4.45[a]	42.8 ± 5.87[b]	0.036	< 0.05
S-Ar	36.1 ± 3.6	33.1 ± 3.7[a]	31.2 ± 5.1[a]	0.029	< 0.05
Ar-Go	46.4 ± 4.7	52.9 ± 5.2[aaa]	48.2 ± 4.7[bb]	0.0004	< 0.001
Co-Go	57.9 ± 5.0	60.9 ± 4.8	62.0 ± 5.9[a]	0.018	< 0.05
S-Go/N-Me	0.685 ± 0.043	0.652 ± 0.073[a]	0.700 ± 0.060[b]	0.021	< 0.05
N-ANS/ANS-Me	0.779 ± 0.071	0.755 ± 0.075	0.775 ± 0.095	0.356	ns
N-ANS/N-Me	0.438 ± 0.025	0.429 ± 0.024	0.436 ± 0.031	0.245	ns
ANS-Me/N-Me	0.562 ± 0.025	0.572 ± 0.024	0.564 ± 0.031	0.212	ns
PNS-A	44.5 ± 3.4	42.7 ± 4.0	46.7 ± 4.0[bb]	0.006	<0.01
Go-Me	70.2 ± 5.5	72.1 ± 7.0	74.7 ± 6.3[a]	0.047	<0.05

BSSRO – bilateral sagittal ramus osteotomy according to Obwegeser and Dal Pont; N-Se – length of the Anterior Cranial base; N-Me – anterior total facial height; N-ANSL anterior upper facial height; ANS-Me – anterior lower facial height; S-Go – posterior total facial height; S-PNS – posterior upper facial height; PNS-Go – posterior lower facial height; S-Ar – lenght of the posterior cranial base; AV-GO – lenght of the ramus; Co-Go – height of the ramus; S-Go/N-Me – posterior total lower faciheight/anterior total facial height; N-ANS/ANS-Me – anterior upper facial height/anterior lower facial height; N-ANS/N-Me – anterior upper facial height/anterior botal facial height; ANS-Me/N-Me – anterior lower facial height/anterior total facial height; PNS-A – lenght of the maxillary body; Go-Me – lenght of the mandibular body. – analysis of variance.

[a, aa, aaa] – $p < 0.05, 0.01, 0.001$ – significant difference in relation to the control group; [b, bb, bbb] – $p < 0.05, 0.01, 0.001$ – significant difference in relation to the experimental group 1 (BSSRO).

Table 2

Differences in the values of linear skeletal variables before and after surgery in groups operated on by various surgical techniques

Variables	dA1-B1 ± SD (BSSRO)	dA2-B2 ± SD (Bimaxillary surgery)	*t* - test *p*	
N-S	-1.24 ± 3.43	0.03 ± 0.08	0.224	ns
N-Me	-3.01 ± 6.75	-5.07 ± 6.58	0.358	ns
N-ANS	-0.28 ± 3.12	-0.88 ± 3.25	0.258	ns
ANS-Me	-2.67 ± 5.06	-4.32 ± 6.09	0.125	ns
S-Go	-0.90 ± 4.62	2.67 ± 3.52	0.009	**
S-PNS	-0.49 ± 2.44	-0.21 ± 2.57	0.244	ns
S-Ar	0.05 ± 3.13	0.77 ± 1.51	0.315	ns
Ar-Go	-1.56 ± 4.80	0.03 ± 6.10	0.533	ns
Co-Go	-1.49 ± 4.19	0.19 ± 6.25	0.474	ns
SGo/N-Me	0.009 ± 0.043	0.033 ± 0.044	0.033	*
N-ANS/ANS-Me	0.023 ± 0.056	0.017 ± 0.082	0.369	ns
N-ANS/N-Me	0.005 ± 0.019	0.007 ± 0.027	0.327	ns
ANS-Me/N-Me	-0.005 ± 0.019	-0.007 ± 0.027	0.444	ns
PNS-A	-0.51 ± 1.92	3.09 ± 3.17	0.0003	***
Go-Me	-5.06 ± 4.98	-2.92 ± 3.61	0.221	ns
PNS-Go	1.2 ± 0.16	4.1 ± 1.39	0.0002	***

BSSRO – bilateral sagittal ramus osteotomy according to Obwegeser and Dal Pont; 1 – the experimental group 1 (BSSRO); 2 – the experimental group 2 (bimaxillary surgery);

A – before surgery; B – 6 months after surgery; d – differences between experimental groups; N-S – lenght of anterior cranial base; N-Me – anterior total facial height; N-ANS – anterior upper facial height; ANS-Me– anterior lower facial height; S-Go – posterior total facial height; S-PNS – posterior upper facial height; S-Ar – lenght of the posterior cranial base; Ar-Go – lenght of the ramus; Co-Go – height of the ramus; SGo/N-Me – posteror total facial height/anterior total facial height; N-ANS/ANS-Me – anterior upper facial height/anterior lower facial height; N-ANS/N-Me – anterior upper facial ehight/anterior total facial height; ANS-Me/N-Me – anterior lower facial height/anterior total facial height; PNS-A – length of the maxillary body; Go-Me – length of the mandibular body; PNS-Go – posterior lower facial height.

significance levels: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns – not significant.

The relationship between posterior and anterior total facial heights after bimaxillary surgery changed in favor of posterior facial height, which led to harmonization of facial dimensions in operated patients. After bimaxillary surgery the values of these linear variables were very similar to the values of the same variables in the control group.

Length of the posterior cranial base (S-Ar) was slightly increased by bimaxillary surgery, but even after the surgery the value of this variable was significantly lower than that value in the control group.

Length and height of the mandibular ramus (Ar-Go) and (Co-Go) did not change significantly after operative techniques used, although the mean value of the ramus lenght Ar-Go was much closer to its value in the control group after bimaxillary surgery (Table 1).

After LeFort I maxillary advancement, the effective length of the maxilla PNS-A was significantly increased when compared to the value before the operation. The length and the position of the maxilla did not change after BSSRO. On the contrary, the length of the mandibular body Go-Me was much more reduced by BSSRO, because the disharmony in jaw relations using this operative technique was compensated only by shortening the mandibular body and by set back of its proximal segment (Table 1). By analyzing the differences in the values of these variables before and after surgical procedures, it was obvious that maxilla was moved forward by Lefort I osteotomy by an average of 3.09 ± 3.17 mm, while the mandible within the same operation was moved distally to 2.92 ± 3.61 mm. On the contrary, with BSSRO, mandible was shortened by an average of -5.06 ± 4.98 mm, which was supposed to be a disadvantge of this operative procedure (Table 2).

Comparison of the mean values of angular skeletal variables in patients treated by BSSRO with the mean values of these variables in patients operated by bimaxillary osteotomies indicated that there were significant differences among 9 angular skeletal variables: SNB, ANB, NS/MP, FH/MP, PP/MP, ArGoMe, NGoMe, SArGo and NAPg (Table 3).

It turned out that bimaxillary surgery, compared to isolated operations on the mandible, changed far more efficiently the values of mentioned angular variables, and made them closer to their values in the control group. The amount of these changes was far more illustrative in the Table 4, which presents the differences in the values of examined angular variables after BSSRO and after bimaxillary surgery. After bimaxillary surgery, the angle SNA increased by an average of 4.5º, while BSSRO failed to change it. On the contrary, the angle SNB was much more reduced by BSSRO than by bimaxillary surgery, for the simple reason that with the first operative technique mandible was shortened and moved back to more than 5 mm, which consequently led to distal shift of the point B.

The angle ANB was more significantly normalized by bimaxillary surgery, where the difference between the pre- and postoperative values amounted to around 6º. This was a direct result of an increase in the SNA angle after Lefort I maxillary advancement and reduction of the SNB angle by bilateral sagittal ramus osteotomy in the same operative procedure.

Table 3

Comparison of mean values of angular skeletal variables after BSSRO (the experimental group 1) and after bimaxillary surgery (the experimental group 2) with the values in the control group

Variables	Control group mean ± SD	Experimental group 1 mean ± SD	Experimental group 2 mean ± SD	ANOVA test *p*	
SNA	81.4 ± 3.4	81.6 ± 4.1	83.8 ± 5.6	0.360	ns
SNB	79.3 ± 3.1	81.6 ± 4.3[a]	82.8 ± 4.7[aa]	0.003	< 0.01
ANB	2.22 ± 1.31	-0.03 ± 1.11[aaa]	1.42 ± 1.23[bb]	0.0004	< 0.001
NS/PP	8.3 ± 3.5	7.2 ± 3.5	9.2 ± 5.6	0.255	ns
NS/MP	30.7 ± 5.6	35.0 ± 8.1[a]	33.4 ± 7.2	0.033	< 0.05
FH/MP	23.3 ± 5.6	27.3 ± 7.4[a]	24.3 ± 6.4	0.028	< 0.05
PP/MP	22.9 ± 5.6	28.0 ± 8.7[a]	23.5 ± 8.8[b]	0.022	< 0.05
ArGoMe	123.1 ± 5.9	130.5 ± 10.0[aa]	127.5 ± 7.4	0.005	< 0.01
ArGoN	50.0 ± 3.2	51.4 ± 5.7	50.8 ± 5.6	0.169	ns
NGoMe	73.1 ± 4.6	79.1 ± 6.5[aaa]	76.7 ± 4.5	0.0006	< 0.001
NSAr	123.5 ± 6.7	120.3 ± 6,8	125.3 ± 8.5	0.211	ns
SArGo	144.3 ± 6.3	144.0 ± 8.4	139.3 ± 10.6[a]	0.023	< 0.05
Björk sum	390.9 ± 5.3	394.7 ± 8.4	392.1 ± 6.0	0.364	ns
NAPg	176.8±1.86	174.0 ± 4.2[a]	170.8 ± 6.4[aa,b]	0.007	<0.01

BSSRO – bilateral sagittal ramus osteotomy according to Obwegeser and Data Pont; SNA – anteroposterior position of the maxilla relative to the anterior cranial base; SNB – anteropsterior position of the mandible relative to the anterior cromal base; ANB – relationship of the maxilla and mandible in the sagital plane; NS/PP – inslination of the masilla to the anterior cranial base; NS/MP – inclination of the madible to the anterior chronial base; FH/MP – the ralationship between the Frankfurt plane and mandibular plane; PP/MP – the selationship between the basic jaw planes; ArGoMe – gonial angle by Björk; ArGoN – upper fast of the gonial angle; NGoMe – lower past of the gonial angle; NSAr – angle of the saddle by Björk; SArGo – articular angle by Björk; NAPg – angle of facial seeletal convexity.

[a, aa, aaa] – $p < 0.05, 0.01, 0.001$ – significant difference in relation to the control group; [b, bb, bbb] – $p < 0.05, 0.01, 0.001$ – significant difference in relation to the experimental group 1 (BSSRO).

Table 4
Differences in the values of angular skeletal variables before and after surgery in groups operated by various surgical techniques

Variables	dA1-B1 ± SD (BSSRO)	dA1-B1 ± SD (Bimax. sur.)	*t*-test	*p*
SNA	0.2 ± 1.3	4.5 ± 3.2	0.00003	***
SNB	-4.4 ± 2.5	-1.3 ± 3.0	0.00042	***
ANB	4.7 ± 2.0	-3.4 ± 3.5	0.00002	***
NS/PP	0.5 ± 2.0	0.4 ± 5.0	0.766	ns
NS/MP	0.1 ± 6.3	-3.8 ± 6.9	0.033	*
FH/MP	-1.8 ± 8.2	-4.7 ± 6.5	0.322	ns
PP/MP	-0.2 ± 6.0	-4.7 ± 7.4	0.024	*
ArGoMe	2.2 ± 10.3	-8.0 ± 6.7	0.012	*
ArGoN	-0.9 ± 5.2	-0.5 ± 8.6	0.288	ns
NGoMe	-1.3 ± 6.9	-5.7 ± 5.5	0.011	*
NSAr	1.3 ± 5.4	0.2 ± 6.1	0.711	ns
SArGo	0.8 ± 8.9	0.9 ± 10.4	0.622	ns
Björk sum	-0.2 ± 6.1	-6.7 ± 9.2	0.039	*
NAPg	-6.1 ± 4.8	-1.3 ± 10.0	0.005	**

BSSRO – bilateral sagittal ramus osteotomy according to Obwegeser and Data Pont; the experimental group 1 (BSSRO); 2 – the experimental group 2 (bimaxillary surgery); A – before surgery; B – 6 months after surgery; d – differences between experimantal groups; SNA – anteroposterior position of the maxilla relative to the anterior cranial base; SNB – anteropsterior position of the mandible relative to the anterior cromal base; ANB – relationship of the maxilla and mandible in the sagital plane; NS/PP – inslination of the masilla to the anterior cranial base; NS/MP – inclination of the madible to the anterior chronial base; FH/MP – the ralationship between the Frankfurt plane and mandibular plane; PP/MP – the selationship between the basic jaw planes; ArGoMe – gonial angle by Björk; ArGoN – upper fast of the gonial angle; NGoMe – lower past of the gonial angle; NSAr – angle of the saddle by Bjö rk; SArGo – articular angle by Björk; NAPg – angle of facial seeletal convexity.
significance levels: *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns – not significant.

Bimaxillary surgery reduced also more efficiently angular values NS/MP, FH/MP, PP/MP, ArGoMe, NGoMe and Björk sum, what can be seen by comparing the differences between pre- and postoperative values of these angles after each operative technique (Table 4). After bimaxillary surgery, these angular values were much closer to their values in the control group, while after BSSRO their values remained characteristic for mandibular prognathism (Table 3).

Comparison of differences in pre- and postoperative values of these angles revealed that the NS/MP angle after bimaxillary surgery decreased nearly for 4º, the relationship between the Frankfurt plane and mandibular plane（FH/MP) and the relationship between the basic jow planes (PP/MP) angles for almost 5º, the ArGoMe angle for 8º, the NGoMe for almost 6º and Björk sum by approximately 7º. After BSSRO the values of these angles remained almost unchanged (Table 4).

It is interesting that the angle of skeletal convexity NAPg was much more reduced after BSSRO than after bimaxillary surgery as a result of greater distal displacement of the proximal mandibular segment and the point PG during bilateral sagittal ramus osteotomy.

Discussion

Cephalometric studies indicate the high variaty of skeletal morphology in patients with class III deformities. In various ethnic groups, these deformities are presented in different phenotypic forms that have an obvious genetic background [3–5]. In accordance with a large number of studies, in 50–60% of patients class III deformities are the combination of maxillary retrognathia, mandibular prognathism and varying degrees of vertical dyscrepances [3–5, 19, 20].

The results of these studies have significantly changed approaches and modalities in correcting class III deformities. The isolated mandibular operations have been used for years in surgical correction of mandibular prognathism. Today, most clinicians and researchers prefer the maxillary and bimaxillary surgery. Advantages and disadvantages of these operative procedures in correcting class III deformities are still debated in the professional literature and in clinical practice.

This study was conducted in order to compare the results of bilateral sagittal ramus osteotomy (BSSRO) and the results of bimaxillary operations in patients who were operated at the Clinic for Maxillofacial Surgery at the Faculty of Dental Medicine in Belgrade. A detailed overview of the results related to these operative techniques in correcting mandibular prognathism is presented in the master's and doctoral thesis of the author [11, 18].

Comparison of outcomes of bimaxillary surgery and BSSRO in correcting the class III deformities in this study clearly speaks in favor of bimaxillary surgery. Comparative analysis of mean values of selected linear and angular skeletal variables after surgery revealed that BSSRO altered significantly only 2 linear variables (the lower anterior face height ANS-Me and the length of the mandible Go-Me) and three angular variables (SNB, ANB and the angle of facial skeletal convexity - NAPg).

It turned out, that BSSRO neither had an effect on the overall posterior face height, the length of the mandibular

ramus, the lenght of maxilla, nor on angular relationships between these cranial structures and the mandible. The values of gonial angles ArGoMe, NGoMe, the relationship of mandibular plane to the anterior cranial base SN/MP, and the values of the basal angle PP/MP are typical for mandibular prognathism after these operations.

Vukadinovic [21] in 1985, Gjorup and Athanasiu [22] in 1991, Pike and Sundheim [23] in 1997, Joss and Thuer [24] in 2008, Sinobad [11] in 2010, state similar results stressing that BSSRO could not solve the extreme vertical imbalance in facial proportions, often present in patients with class III deformities. The relationship of the mandible to the anterior cranial base, the basal angle and the relationship of the occlusal plane to the mandibular plane remain mostly unchanged after such operations.

Although the value of the ANB angle after this operation increased by an average of 4°, it still shows negative mean value (-0.03 + 1.11°), indicating that progeny jaw relationship persists in most treated patients. Similar postoperative values of this angle were found by Vukadinovic [21] in 1985. (-0.21°), Gjorup [22] in 1991. (0°), Pike and Sundheim [23] in 1997 (-0.3°), Joss and Thuer [24] in 2008 (-0.95°), Sinobad [11] in 2010 (-0.03 ± 1.11).

By comparing the mean values of examined skeletal variables in the experimental group 1 after BSSRO with the values of the same variables in the control group, significant differences in the values of most skeletal variables were observed, particularly to angles ANB, ArGoMe, NGoMe, basal angle PP/MP, as well as the relationship of mandibular plane to the anterior cranial base SN/MP, which after surgery remained typical for mandibular prognathism.

These results actually suggest that bilateral sagittal ramus osteotomy did not change essentialy the basic craniofacial skeletal assembly, typical for mandibular prognathism. Due to significant distal displacement of mandibular proximal segment (more than 5 mm) BSSRO changed significantly the jaw relationships in the sagittal plane and skeletal facial convexity. However, its impact on the vertical relationships was almost insignificant, what is in agreement with the results of similar studies [11, 21, 22, 25–27].

The fact is, however, that reduction of the lower anterior face height in treated patients, and thus the total anterior facial height, shortening of the mandible by an average of 5.7 ± 4.2 mm, an increase in ANB angle by an average of 4° and the angle of skeletal convexity NAPg by an average of 8.1° led to a significant correction of facial profile and thus the apearance of operated patients which is an undoubted success of this operation.

Unlike the BSSRO, bimaxillary surgery changed significantly 8 linear and almost all angular variables, which led to essential changes of skeletal relations and to harmonization of facial dimensions in operated patients. These operations alter in a specific way the effective lengths of the maxilla and mandible. Maxilla, and thus the middle segment of the face were moved forward on the average of 3.9 ± 3.17 mm, while the body of the mandible was shortened much less than after BSSRO, on the average of 2.9 ± 3.6 mm.

According to the results of some studies, a large amount of distal displacement and significant elongation of the last part of mandibular body after BSSRO may endanger the normal function of surrounding muscles (masseter, pterygoid. med., pterigomasseteric connection), which is a potential risk of subsequent relapse [24, 26]. The introduction of maxillary osteotomy reduces the need for large distal movement of the proximal mandibular segment and thus elongation of posterior mandibular body in the osteotomy site. This also reduces the need for large rotation of the proximal mandibular segment in order to compensate open bite in the frontal area, and optimise the lower anterior face height [16–18, 22, 25–28].

Specificity of bimaxillary operations was a significant increase in the total posterior and lower posterior face heights (on average by 2.67 ± 3.52 mm and 3.9 ± 1.3 mm, respectively) and length of the posterior cranial base S-Ar (on average by 0.77 ± 1.51 mm), which normalized the relationship between overall anterior and posterior face heights in operated patients. These dimensions remained unchanged after BSSRO.

Due to maxillary repositioning during Lefort I osteotomy, bimaxillary surgery changed significantly the angular values SNA, SNB and ANB. Judging by differences between the values of these variables after each operative procedure, the angle SNA was increased by an average of 4.5 ± 3.2º by LeFort I osteotomy, which is a specifity of this operative procedure and the SNB angle was reduced by an average of slightly more than 2º. This is in agreement with the results of Johnston et al. [26], Al Gunaid et al. [16], Al Delayme et al. [27], Sinobad [18], Aydil et al. [28], Van Sickls and Walender [29].

After isolated operations on the mandible the values of the SNA angle do not change, but changes in the values of SNB angle are far more significant, because of the greater distal displacement of the proximal mandibular segment [11, 21, 24]. The angle of facial skeletal convexity NAPg was also significantly changed after BSSRO due to greater distal displacement of the Pg point during this procedure. Similar results are found in other cephalometric studies [11, 22, 24–26].

Bimaxillary surgery reduced most of the vertical components of mandibular prognathism [30].

Judging by differences between the values of angles NS/MP, FH/MP, ArGoMe, ArGoN and Björk's sum after BSSRO and after bimaxillary surgery, it is obvious, that bimaxillary surgery reduced more efficiently these indicators of prognathism and made their values significantly closer to biometric standards. The SNA angle after bimaxillary osteotomy increased by 4.5 ± 3.2º, the angle NS/MP decreased by 3.8 ± 6.9º, the angle FH/MP decreased by 4.7 ± 6.5º, the ArGoMe decreased by 8.0 ± 6.7º, the NGoMe by 5.7 ± 5.5º and Björk's sum by 6.7 ± 9.2º. On the contrary, these angular values were almost unchanged after BSSRO.

However, bimaxillary operations did not eliminate all skeletal indicators of mandibular prognathism. The values of angles SNB and ANB after surgery were still significantly different from the biometric values. This is confirmed by Johnston et al. [26] who note that the values of SNB and ANB

angles after bimaxillary surgery are significantly improved, but even after treatment, in 54% of patients the ANB angle values are still below the ideal, while 52% of patients still have the great values of the SNB angle.

Compared to many positive effects of bimaxillary surgery these are certainly nonsignificant disadvantages, but in any case, it should point to the need of much greater attention in the course of orthodontic preparation of these patients for surgical intervention and the orthodontist obligation to harmonize the occlusal relationships in the postoperative period.

Conclusion

Bimaxillary surgery changed more significantly the sagittal and vertical jaw relationships as well as relation of the jaws to the anterior cranial base compared to the isolated operations on the mandible. Most of linear and angular skeletal dimension, which had been deformed before surgery, after surgery were much closer to, or even the same as biometric standards.

Bimaxillary operations acted simultaneously on the middle and lower facial segment and therefore harmonized more successfully the facial dimensions and entire skeletal facial profile. The special benefits of these operations were the significant increase in the posterior facial height, posterior cranial base and thesaddle angle NSAr, as well as significant reduction of the value of Björks sum.

Le Fort I maxillary advancement had a particularly good effect in patients where deformity was caused by retrognathia and maxillary dysplasia. Anterior displacement of the maxilla surgically is moderate, reduced to a distance of about 3–3.5 mm. Distal displacepment of proximal mandibular segment was reduced to amounts of 3 mm on average, what is an advantage of bimaxillary surgery.

The isolated mandibular operations could not solve the extreme vertical imbalance in facial proportions, often present in patients with class III deformities. The relationship of the mandible to the anterior cranial base, the basal angle and the relationship of the occlusal plane to the mandibular plane remained mostly unchanged after such operations.

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Serum IgG antibodies against *Helicobacter pylori* low molecular weight antigens 50kDa, 30kDa and Urease A 26 kDa, along with vacuolating cytotoxin A are associated with the outcome of the infection

Serumska IgG antitela protiv *Helicobacter pylori* antigena male molekulske mase 50kDa, 30kDa i ureaza A 26kDa, uporedo sa vakuolizirajućim citotoksinom A povezana su sa ishodom infekcije

Nebojša Manojlović*†, Ivana Tufegdžić†‡, Elizabeta Ristanović†§, Dubravko Bokonjić†‖

Military Medical Academy, *Clinic for Gastroenterology and Hepatology, ‡Institute for Pathology, §Institute for Microbiology, ‖National Poison Control Center, Belgrade, Serbia; University of Defence, †Faculty of Medicine of Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. We designed and conducted this study due to the fact that results of the previous studies about seroreactivity to low-molecular-weight *Helicobacter pylori* antigens, cytotoxin-associated gene A (CagA), vacuolating cytotoxin A (VacA) in patients with gastric cancer and peptic ulcer were conflicting. **Methods.** The Western blot test was performed in 123 patients, 31 with gastric cancer, 31 with duodenal ulcer, 31 with gastric ulcer, 30 with gastritis and functional dyspepsia in order to determine IgG antibodies to *H. pylori* antigens (CagA, VacA, Heat shock protein 60kDa, Urease B 66 kDa, Flagellin 55kDa, 50kDa, 30 kDa, Urease A 26 kDa, 24 kDa). In this study we analyzed: seroreactivity to *H. pylori* antigens between group with functional dyspepsia and others; between grades of different histopathological parameters of inflammation of antral and corporal mucosa and between antrum-predominant gastritis and corpus-predominant gastritis + pangastritis groups. **Results.** It was shown that seropositivity to 50 kDa antigen could be used as a biomarker for functional dyspepsia, seropositivity to 30 kDa antigen for antrum-predominant gastritis and *H. pylori* colonization in the antrum, to UreaseA26 kDa antigen for pangastritis and corpus-predominant gastritis and degree of inflammation in the corpus. Seropositivity to VacA was the biomarker for gastric cancer and peptic ulcer taken together and inflammation of antral mucosa. Seropositivity to CagA was associated with more intensive inflammation of antral and corporal mucosa, Urease B66 kDa with inflammation of corpus mucosa, but neither of them with specific outcome of *H. pylori* infection and topographic distribution of gastric inflammation. **Conclusion.** Serum IgG antibodies to *H. pylori* antigens 50kDa, and VacA may represent useful biomarkers for the specific outcome of *H. pylori* infection, while serum antibodies to 30 kDa and UreaseA26 kDa antigens might be used as specific biomarkers for different topographic distribution of inflammation in gastric mucosa.

Key words:
helicobacter pylori; antigens; biomarkers; stomach ulcer; stomach neoplasms; duodenal ulcer; duodenal neoplasms.

Apstrakt

Uvod/Cilj. Do sada objavljene studije o seroreaktivnosti protiv *Helicobacter pylori* antigena male molekulske mase kao i citotoksina povezanog sa genom A (CagA), vakuolizirajućeg citoksina (VacA) kod bolesnika sa karcinomom želuca i peptičkim ulkusom pokazale su protivurečne rezultate, te smo u cilju istraživanja ove pojave dizajnirali i sproveli ovu studiju. **Metode.** Western blot test primenjen je kod 123 ispitanika, 31 sa karcinomom želuca, 31 sa ulkusom duodenuma, 31 sa ulkusom želuca, 30 sa gastritisom i funkcionalnom dispepsijom u cilju određivanja IgG antitela protiv *H. pylori* antigena (CagA, VacA, Heat shock protein 60kDa, Urease B66kDa, Flagelin55 kDa, 50kDa, 30 kDa, Urease

Correspondence to: Nebojša Manojlović, Military Medical Academy, Clinic for Gastroenterology and Hepatology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: nebojsa.manojlovic1@gmail.com

A26 kDa, 24 kDa). U ovoj studiji analizirali smo: razlike u seroreaktivnosti na *H. pylori* antigene između grupe sa funkcionalnom dispepsijom i ostalih grupa; između gradusa različitih patohistoloških parametara inflamacije antralne i korpusne mukoze i između antrum predominantnog gastritisa i korpus predominantnog pangastritisa. **Rezultati.** Seropozitivnost protiv 50 kDa antigena pokazala se kao biomarker za funkcionalnu dispepsiju, seropozitivnost protiv 30 kDa antigena bila je biomarker za antrum predominantni gastritis i gradus kolonizacije *H. pylori* u antrumu, protiv Urease A26 kDa antigena za pangastritis i korpus predominantni gastritis i stepen inflamacije u korpusu. Seropozitivnost protiv VacA bila je biomarker za karcinom želuca i peptički ulkus, kada se razmatraju kao jedinstvena grupa, i za inflamaciju antralne mukoze. Seropozitivnost protiv CagA bila je povezana sa intenzivnijom inflamacijom antralne i korpusne mukoze, Urease B66kDa antigena sa inflamacijom korpusne mukoze, ali ne i sa specifičnim ishodom *H. pylori* infekcije i topografskom distribucijom inflamacije želuca. **Zaključak.** Serumska IgG antitela protiv *H. pylori* antigena 50 kDa i VacA mogu predstavljati korisne biomarkere za specifični ishod *H. pylori* infekcije, dok bi antitela protiv 30 kDa i Urease A26 kDa antigena mogla biti specifični biomarkeri za različitu topografsku distrubuciju inflamacije želudačne mukoze.

Ključne reči:
helicobacter pylori; antigeni; biološki pokazatelji; želudac, ulkus; želudac, neoplazme; duodenum, ulkus; duodenum, neoplazme.

Introduction

Helicobacter pylori affects about 50% of the world population [1] and most of them do not develop symptoms and do not have the serious outcome of *H. pylori* infection.

Gastric cancer develops in 1–1.5% of infected people, and about 65–80% of patients with gastric cancer are infected with *H. pylori* [2, 3]. Peptic ulcer, both gastric and duodenal, develops in 10–20% of infected people. Patients with duodenal ulcer are infected with *H. pylori* in 90–100% of cases, and patients with gastric ulcer in 60–100% of cases [4, 5]. About one-quarter of population suffer from dyspepsia, and the majority of them have functional dyspepsia. Patients with functional dyspepsia are infected with *H. pylori* in about 50% of cases. Approximately 25% of the Western population suffer from dyspeptic symptoms each year. Seventy percent of them do not have organic cause and symptoms are related to so-called functional dyspepsia [6, 7].

H. pylori infection outcome is very different and depends on 3 groups of factors: virulence factors of *H. pylori*, host factors, and environmental factors [8].

H. pylori virulence factors could influence the ability of these bacteria to colonize, persist and/or induce severe disorders. Therefore, the status of certain virulence factors might be a potential biomarker to predict consequences of their carriers [9].

The extensive investigations of Cytotoxin associated with gene A (CagA) and Vacuolizing cytotoxin A (VacA) in development of different *H. pylori* associated diseases have been done. CagA has been extensively investigated and designated as an important oncoprotein that induce malignant neoplasm in mammals [10]. CagA producing strains are reported to be associated with severe clinical outcomes, especially in Western countries [11]. On the other hand, meta-analyses performed to estimate the value of serum CagA antibodies as a serum marker for gastric cancer in East Asian countries showed opposite results [12]. Meta-analysis regarding serum VacA antibodies and risk for gastric cancer and peptic ulcer presented significant association [9].

Investigation of antibodies to low molecular weight antigens as *H. pylori* virulence factors showed interesting, but opposite results, too. Serum antibodies against low molecular-weight-antigens as 19.5kDa [13–22], 26.5kDa [13–16, 20–23], 30kDa [13–21, 23], 35kDa [13–19, 21, 24] and 60kDa [13–15, 20, 25, 26] were associated with serious outcome of *H. pylori* infection in some studies, but the results were conflicting, too. Less extensive investigations of serum antibodies with conflicting results were performed including 37 kDa [17–19] and 45 kDa [14], 54kDa [24], Hsp60 [25, 26] antigens. One study was done for serum antibodies against 46kDa [24], 48kDa [24], 50kDa [27], 53kDa [22], 57kDa [20], 67kDa [27] antigens. Two studies investigating serum antibodies against 54kDa antigen [24, 28] failed to show associated with the serious outcome of infection.

We conducted cross-sectional study in order to investigate the value of seropositivity to low molecular weight antigens, along with CagA and VacA as biomarkers for detection of gastric cancer, and duodenal and gastric ulcer.

Methods

The study was conducted and performed during 2009 in the Clinic for Gastroenterology and Hepatology, the Institute of Pathology and the Institute of Microbiology of the Military Medical Academy (MMA) in Belgrade, Serbia. We selected and enrolled patients with dyspeptic symptoms, different underlying disease [gastric cancer (GCA), duodenal ulcer (DU), gastric ulcer (GU) and gastritis], and actual *H. pylori* infection confirmed by histopathological examination and the anti-*Helicobacter pylori* IgG positive Vira Blot.

We took a medical history from all patients and performed a physical examination, abdominal ultrasound (US) or computed tomography (CT), esophagogastroduodenoscopy (EGDS), complete blood count (CBC), liver and renal chemistry. Inclusion criteria were: presence of dyspepsia symptoms; previously untreated patients due to *H. pylori* infection; patients without proton pump inhibitors and H2 blockers in the last two weeks; absence of malignancy except for gastric cancer; absence of any immunological disorder; informed consent of the patient for EGDS and biopsy; blood sample for analyses; participation in the study; endoscopic and histopathological diagnosis of one of the following diseases: gastric cancer, duodenal ulcer, gastric

ulcer, gastritis; confirmed histopathological diagnosis of *H. pylori* infection; Western blot (ViraBlot) IgG positive for *H. pylori* infection.

EGDS was performed in all our patients in the Endoscopy Section using Olympus (GIFQ165, SN: 2207997, Olympus corporation, Tokyo) forward viewing EGD under local application of xylocaine spray. A minimum four gastric mucosal tissue biopsies (2 each from the antrum and corpus) and additional biopsies from any endoscopically visible lesion were taken. All patients were examined for findings that indicated endoscopic gastritis, such as erythema, hyperemia, atrophy, and mucosal nodularity according to the criteria of the Houston-updated Sydney grading system, and for gastric tumor, duodenal and gastric ulcer [29].

All the obtained biopsies were collected, placed on filter paper, fixed in 10% neutral formalin, and sent for preparation of formalin-fixed, paraffin-embedded tissue blocks. Three-micrometer-thick sections were prepared. One set of tissue sections was stained with hematoxylin and eosin (H&E) and the other with Giemsa stain for histopathological examination including detection of *H. pylori* in the gastric mucosa. The biopsies were evaluated for the intensity of mononuclear inflammatory cellular infiltrates, inflammatory activity (neutrophilic infiltrations), glandular atrophy, metaplasia and *H. pylori* colonization [30]. Additionally, the cases were graded according to the Houston-updated Sydney system [29], which was graded according to the intensity of mononuclear inflammatory cellular infiltrates within the lamina propria: absent inflammation (Grade 0), mild inflammation (Grade 1), moderate inflammation (Grade 2), and severe inflammation (Grade 3) (Table 1). Grading was done for activity, atrophy, intestinal metaplasia and degree of *H. pylori* colonization, also. Additional immunohistochemistry staining was performed in case of the tumor.

The blood samples were obtained from all of them and frozen at -20°C. Using the Western blot detection system (ViraBlot), IgG anti VacA 87 kDa, CagA 136kDa, Urease B 66 kDa (UreB 66), Heat shock protein 60 kDa (Hsp60), Flagellin 55kDa (Fla 55), 50 kDa, 30 kDa, Urease A 26 kDa (UreA 26) and 24 kDa *H. pylori* antigens were identified. *H. pylori* antigens of ViraBlot represent a combination of German patient isolates of highly antigenic *Helicobacter* strains. Bands for diagnosis of *H. pylori* infection were divided into highly specific as CagA 136kDa, VacA 87kDa, 30kDa, UreA 26kDa, 24kDa and less specific as Hsp 60kDa and 50kDa.

Diagnosis of GCA was established in 31 patients, DU in 31 patients, and GU in 31 patients, whilst in 30 patients gastritis with functional dyspepsia (FD) was diagnosed.

According to manufacturer guideline for use, the test was considered negative if there were no bands or there were nonspecific bands such as UreB 66 kDa, Hsp 66 kDa, Fla 55 kDa, 50 kDa. The test was possibly positive if there was one clear specific band of 30kDa, UreaA 26 kDa, 24 kDa. Test was positive if there was at least one band of following two specific CagA 136 kDa or VacA 87 kDa or at least one clear band of 30kDa, Urea A 26, 24 kDa or one clear band of 30 kDa, UreA 26 kDa, 24 kDa and one clear band of Hsp 60 kDa, 50 kDa.

All patients included in our cross sectional study were classified and analyzed in several ways.

The first, according to baseline diagnosis, patients were divided in four groups: GCA, DU, GU, and FD.

The second, all parameters of gastric and corpus inflammation according to Houston-updated Sydney classification: inflammation, activity, atrophy, and intestinal metaplasia and *H. pylori* colonization on the basis of seroreactivity to *H. pylori* antigens in ViraBlot [29].

The third, classification was made on the basis of predominantly located inflammation irrespective of baseline diagnosis: antrum-predominant gastritis and pangastritis along with corpus-predominant gastritis. Because of a small number of patients with corpus-predominant gastritis (only 4 participants) we made one group with pangastritis (45 participants) and corpus-predominant gastritis.

The fourth, two groups were divided on the basis of the presence of GCA and peptic ulcers as one group and FD as the other group.

Statistical analysis

Complete statistical data analysis was done with the statistical software package, SPSS Statistics 18.

Most of the variables were presented as frequency of certain categories, so *t*-test of proportion or cross-tabulation analysis [odds ratio (OR), 95% confidence intervals (CI)] were done for calculation of statistical significance of differences between groups.

In case of continuous data, variables were presented as median, minimal and maximal values (range).

All the analyses were estimated at minimal $p < 0.05$ level of statistical significance.

Table 1

Demographic and clinical characteristics of the patients

Groups	Gender (n)		Age (years)	
	male	female	median	range
GCA (n = 31)	10	21	65.0	40–85
DU (n = 31)	13	18	54.0	21–87
GU (n = 31)	12	19	67.5	34–81
FD (n = 30)	13	17	63.5	21–80
Total (n = 123)	48	75	63.0	21–87

GCA – gastric cancer; DU – duodenal ulcer; GU – gastric ulcer; FD – functional dyspepsia.

Results

Four groups of patients with GCA, DU, GU and upper FD were comparable regarding sex and age (Table 1).

The initial analysis was performed in four groups comparing antibody to all separate antigens of ViraBlod test. The frequency of serum antibody positivity to CagA was not different among the four groups. The immunoreactivity to VacA was found to be less frequent in the group of patients with FD (32%) as compared with other groups, what was statistically insignificant. The immunoreactivity to UreB66, Hsp60 and Fla 55 antigens was high in all groups of patients without any differences among them. Immunoreactivity to 24 kDa antigen was generally less frequent than other antigens, but there were no differences amonggroups. The immunoreactivity to 50 kDa antigen occurred significantly more frequently in the group of patients with FD than in the other groups ($p = 0.02$ for GCA and DU; $p = 0.01$ for GU), (Table 2).

The seroreactivity to 30 kDa antigen was observed to be significantly more frequent in patients with upper FD than in those with GCA ($p = 0.01$), and more frequent than in GCA group without reaching statistically significant difference (Table 2).

CagA seroreactivity was associated with more intensive lymphocyte infiltration of the antral and corporal gastric mucosa ($p = 0.034$; $p = 0.016$) (Table 3). UreB66 antigen seroreactivity was associated with more intensive lymphocyte infiltration of the corpus mucosa ($p = 0.04$) (Table 4).

VacA seroreactivity was associated with more intensive lymphocytic infiltration of the antral mucosa ($p = 0.014$), and there was a trend towards more intensive lymphocyte infiltration of corporal mucosa ($p = 0.061$) (Table 3). Seroreactivity to 30 kDa antigen was associated with more intensive colonization of *H. pylori* in the antral mucosa ($p = 0.01$), and seroreactivity to UreA 26kDa antigen was associated with more intensive lymphocyte infiltration of the corpus mucosa ($p = 0.005$) (Table 4).

Serum antibodies to all *H. pylori* antigens in ViraBlot test were analyzed in patients with antrum-predominant gastritis v.s. pangastritis and corpus-predominant gastritis. A significant difference was found only in antibodies to 30kDa antigen which was more frequent in the group with antrum-predominant gastritis ($p = 0.025$), (Table 4), and to UreA 26 kDa antigen which was more frequent in pangastritis and corpus-predominant gastritis ($p = 0.01$) (Table 3).

Table 2

Seroreactivity against *H. pylori* antigens in four groups of patients

WB IgG	Groups, n (%)					SP	FD v.s. others		
	GCA 31 (100)	DU 31 (100)	GU 31 (100)	FD 30 (100)	Total 123 (100)		GCA	DU	GU
CagA	30 (97)	28 (90)	29 (93)	26 (84)	113 (92)	*p* OR CI	ns	ns	ns
VacA	18 (58)	18 (58)	17 (55)	10 (32)	63 (51)	*p* OR CI	ns	ns	ns
UreB66	28 (90)	29 (93)	23 (74)	26 (84)	106 (86)	*p* OR CI	ns	ns	ns
Hsp60	30 (97)	30 (97)	25 (81)	28 (93)	113 (92)	*p* OR CI	ns	ns	ns
Fla55	30 (97)	29 (93)	29 (93)	27 (90)	116 (94)	*p* OR CI	ns	ns	ns
50 kDA	15 (48)	15 (48)	14 (45)	23 (77)	67 (54)	*p* OR CI	0.02 0.29 0.1–0.9	0.02 0.29 0.1–0.9	0.01 0.23 0.09–0.8
30 kDA	17 (55)	24 (77)	14 (45)	23 (77)	78 (63)	p OR CI	ns	ns	0.01 0.25 0.08–0.7
26 kDA	26 (84)	27 (87)	23 (74)	27 (90)	103 (85)	*p* OR CI	ns	ns	ns
24 kDA	14 (45)	13 (42)	15 (48)	12 (40)	54 (44)	*p* OR CI	ns	ns	ns

WBIgG – Western blot immunoglobulin G; GCA – gastric cancer; CagA – Cytotoxin-associated with gene A; VacA – Vaculoating cytotoxin A; UreB – urease B 66 kDA; Hsp60 – Heat shock protein 60 kDa; Fla55 – Flagellin 55 kDa; GCA – gastric cancer; DU – duodenal ulcer; GU – gastric ulcer; FD – functional dyspepsia.
SP – statistical parameters (*p* – probability; OR – odds ratio; CI – 95% confidence intervals; ns – not significant).

Table 3

Seroreactivity to *H. pylori* antigens and grade of inflammation (INF), activity (ACT), atrophy (ATR), intestinal metaplasia (IM), *H. pylori* (HP) colonization in the antrum (A) and corpus (C)

WB IgG*	INF-A	ACT-A	ATR-A	IM-A	HP-A	INF-C	ACT-C	ATR-C	IM-C	HP-C
	WB IgG + *vs* WB IgG- (probability)									
CagA	0.034	ns	ns	ns	ns	0.016	ns	ns	ns	ns
VacA	0.014	ns	ns	ns	ns	0.061	ns	ns	ns	ns
UreB66	ns	ns	ns	ns	ns	0.04	ns	ns	ns	ns
Hsp60	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fla55	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
50kDa	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
30kDa	ns	ns	ns	ns	0.01	ns	ns	ns	ns	ns
26kDa	ns	ns	ns	ns	ns	0.005	ns	ns	ns	ns
24kDa	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

For abbreviations see under Table 2.

Table 4

Seroreactivity to different *H. pylori* antigens in patients with different topographic distribution of gastritis

WB IgG	APG, n (%) (n = 74)	CPG and PG, n (%) (n = 49)	Total, n (%) (n = 123)	*p*	OR	95% CI
CagA	67 (90)	46 (94)	113 (92)	ns	na	na
VacA	34 (46)	28 (57)	62 (50)	ns	na	na
Ure B66	62 (84)	44 (90)	102 (86)	ns	na	na
Hsp60	68 (92)	44 (90)	112 (91)	ns	na	na
Fla55	72 (97)	46 (94)	118 (96)	ns	na	na
50kDa	44 (59)	23 (45)	67 (54)	ns	na	na
30kDa	51 (65)	23 (47)	84 (68)	0.025	0.39	0.19–0.84
26kDa	57 (77)	46 (94)	103 (84)	0.01	na	na
24KDa	36 (49)	20 (41)	56 (45)	ns	na	na

APG – antrum-predominant gastritis; CPG – corpus-predominant gastritis; PG – pangastritis.
***p* – probability; OR – odds ratio; CI – confidence intervals; na – not available.**
For other abbreviations see under Table 2.

Table 5

Seroreactivity against different *H. pylori* antigens in groups of patients with gastric cancer (GCA) and peptic ulcers (PU), and gastritis (G) and functional dyspepsia (FD)

WB IgG	GCA & PU, n (%) (n = 93)	G & FD, n (%) (n = 30)	Total, n (%) (n = 123)	*p*	OR	95% CI
CagA	87 (93)	26 (87)	113 (92)	ns	na	na
VacA	53 (57)	10 (32)	63 (51)	0.024	2.33	0.9–5.5
UreB66	80 (86)	26 (87)	106 (86)	ns	na	na
Hsp60	85 (91)	28 (93)	113 (92)	ns	na	na
Fla55	89 (96)	27 (90)	116 (94)	ns	na	na
50kDa	44 (47)	23 (77)	67 (54)	0.009	0.27	0.11–0.7
30kDa	55 (59)	23 (77)	78 (63)	0.08	0.44	0.11–1.13
26kDa	76 (82)	27 (90)	103 (85)	ns	na	na
24kDa	42 (45)	12 (40)	56 (45)	ns	na	na

For abbreviations see under Tables 2 and 3.

Serum antibodies to all *H. pylori* antigens in ViraBlot test were analyzed in the group of patients with GCA and both peptic ulcers v.s. the group with gastritis and FD. A significant difference was found only in antibodies to VacA which appeared more frequent in the GCA & peptic ulcer groups ($p = 0.024$, OR = 2.3), and seroreactivity to 50kDa antigen was more frequent in the gastritis-FD group ($p = 0.009$; OR = 0.27) (Table 5).

Seroreactivity to 30 kDa antigen was more frequent in the gastritis-FD and there is a trend towards significance ($p = 0.08$; OR = 0.44).

Discussion

Our report is the first one regarding seroreactivity to different *H. pylori* antigens present in the ViraBlot in the Serbian population except for CagA and VacA. Our analysis of seroreactivity to different *H. pylori* antigens in four groups (GCA, DU, GU, gastritis with FD) showed significant difference only with 50kDa and 30 kDa antigens. The frequency of antibodies to all other *H. pylori* antigens in the ViraBlod test was not different among baseline groups.

Serum antibodies to 50 kDa antigen were significantly more frequent in gastritis with FD than in groups with GCA, DU and GU. Seroreactivity to 50 kDa antigen was not associated with the grade of any parameter of gastric inflammation in the antral and corporal gastric mucosa, and was more frequent in antrum-predominant gastritis, but without reaching statistical significance. There are scarce literature data regarding immunoreactivity to 50kDa antigen. Seroreactivity to 50kDa antigen was significantly more frequent in infected persons with *H. pylori* than in noninfected ones [31], in *H. pylori* line Hpu24 in GCA than in GU ulcer, but it was not the case with line NCT11 and Hcp29 [32]. In a study from Turkey, serum antibodies to 50 kDa antigen were not different between GCA patients and patients without cancer [27]. 50kDa antigen was not highly specific for *H. pylori*, but in all our analyses it appeared as a biomarker for *H. pylori* gastritis in FD, but this association could not be explained on the basis of features of the inflammatory process in the gastric mucosa.

Seroreactivity to 30 kDa antigen was significantly more frequent in patients with FD than in GU ($p = 0.01$). 30 kDa seroreactivity was equally frequent in patients with DU as in FD, and less frequent in GCA, but without statistical significance. On the other hand, it was associated with grade of *H. pylori* colonization in the antral mucosa, and antrum-predominant gastritis, which could explain the association with DU and FD, where we could expect the antral predominant type of gastric inflammation and intensive *H. pylori* colonization in the antral mucosa.

Outer membrane protein (OMP)-30kDa antigen is specific for *H. pylori* infection Immunoreactivity to 30 kDa antigen is significantly more frequent in infected subjects with *H. pylori* than in noninfected ones [31]. A presence of serum antibodies to 30kDa OMP was investigated in 10 studies [13–21, 23]. In 4 studies, seroreactivity to 30 kDa antigen showed significant association with specific outcome of *H. pylori* infection [14, 20–23]. In Croatian population, it was associated with higher degree of antrum and corpus inflammation in the stomach [20]. In Australian population, it was associated with healthy blood donors [14], and in Lithuanian population with GCA [23]. In Thai population not actually infected with *H. pylori* it was associated with GCA [21]. In 6 studies serum antibodies to 30 kDa antigen showed no association with specific outcome of *H. pylori* infection [13, 15–19]. Four studies with Thai population did not find association of antibodies to 30 kDa antigen with GCA, DU, GU, mucosa-associated lymphoid tissue (MALT) lymphoma, and FD [13, 16, 18, 19]. In a study from Iran, there was no difference between GCA and FD [17], and in a study from France there were no differences among GU, DU, gastric erosions, MALT lymphoma and FD [15].

Serum antibody to VacA was associated with GCA and peptic ulcer after meta-analysis [9]. Our results are concordant with the previous result from Serbia and Montenegro [33] where VacA was associated with peptic ulcer, and from Croatia where serum antibodies to VacA were associated with DU [20]. Results of our study confirmed association of seroreactivity to VacA with serious infection outcome, but statistical significance was reached only when the groups with GCA and peptic ulcer were joined and tested v.s. gastritis and FD.

In our study, seroreactivity to UreA26 kDa antigen was associated with the intensity of lymphocyte infiltration in the corpus mucosa, and it was more frequent in pangastritis and corpus-predominant gastritis. Association UreA26kDa antigen seroreactivity with gastric inflammation could be related to the severe outcome of infection, but there was no association between GCA and peptic ulcer. Seroreactivity to 26 kDa antigen was previously investigated in 6 studies apart from ours [13–16, 20, 27]. In three studies, two from Thai population, it was associated with alone analyzed GCA [13], and simultaneously with CagA [16], and in one from Turkey with noncancer patients [27]. Three studies were not find association of seroreactivity to UreA26 kDa with the specific outcome of the infection [14, 15, 20]. There was no such association in Croatia, among GCA, DU and GU patients [20], in France among GU and DU patients, gastric erosions, MALT lymphoma and FD patients [15], and in mixed Australian-Chinese population among those with GCA, DU, healthy blood donors and FD patients [14, 31–35].

Our study highlighted that serum antibody to CagA was almost ubiquitous, and there were no differences among GCA, DU, GU and upper FD, between the group of patients with antrum-predominant gastritis and that with pangastritis and corpus-predominant gastritis, as well as between the group with peptic ulcer and GCA and the group with gastritis with upper FD. Our results confirmed previously published data about antibodies to CagA, to DU and gastritis, adding the data about GU and GCA in Serbian population [33]. It was associated with more intensive lymphocyte infiltration of the antrum and corpus gastric mucosa. Similar results regarding antibodies to CagA gastric cancer, peptic ulcer and inflammation of the gastric mucosa were found in Croatian population who are from the same geographic area [20].

Seroreactivity to UreB66 antigen was associated with more intensive lymphocyte infiltration of the corpus mucosa, but neither with specific topographic distribution of gastritis nor with specific outcome of infection regarding four baseline groups or GCA and peptic ulcer groups *vs.* FD. There are scarce literature data about seroreactivity to UreB66 kDa. It was investigated only in two studies. In the first Turkish study, results showed association with GCA [27], and the second Croatian study [20] showed no association with GCA and both peptic ulcer and parameters of inflammation of the gastric mucosa. Results from our study are more close to Croatian ones (two neighboring population), showing association with more intensive inflammation of the corporal mucosa, but with no significant difference in corpus-predominant gastritis and also with no significant difference between GCA and FD.

There are no other data about seroreactivity to Fla55 antigen and 24kDa antigen, and in our study we showed that both antigens were equally distributed among four investigated groups.

Seroraectivity to Hsp60 antigen in our study was present in the majority of investigated participants in groups

made on the base of different criteria, without any significant difference. Hsp60 kDa seroreactivity was more frequent in individuals infected with *H. pylori* than in noneinfected ones [31]. Other authors found association with the grade of inflammation particularly in the antral mucosa, and *H. pylori* colonization in the antrum and corpus in Estonian population [25], and with gastric atrophy in British population [26], but not with the specific outcome of infection considering GCA, gastric MALT lymphoma, both peptic ulcers, non ulcer dyspepsia and asymptomatic carriers in Thai [13], France [24], Australian and Chinese population [14].

Limitations of our study represents a relatively small number of patients, and German ViraBlot with *H. pylori* strains of German patients (not Serbian patients).

Conclusion

A presence of serum VacA antibodies was significantly associated with increased risks of peptic ulcer disease, GU and DU compared with gastritis and FD controls. The significant association was also found between serum VacA antibodies and GCA risk. Serum VacA antibodies might be a potential biomarker for the prediction of peptic ulcer disease and GCA risks. Serum antibodies to *H. pylori* antigen 50 kDa might be a potential biomarker for FD while serum antibodies to *H. pylori* antigens 30kDa and 26 kDa might be biomarkers for specific topographic distribution of inflammation in the gastric mucosa. Further investigation of serorectivity to selected *H. pylori* antigens 26kDa, 30kDa, 50kDa and VacA separately and simultaneously as biomarkers for the specific outcome of *H. pylori* infection should be justified.

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Family history of disease and risk of glioma occurrence: Results of the case-control study

Porodična istorija bolesti i rizik od nastanka glioma: rezultati studije slučaj-kontrola

Ana Azanjac Arsić*, Gordana Tončev*, Svetlana Miletić Drakulić*, Katarina Vesić*, Dejan Aleksić*, Gorica Marić†, Tatjana Pekmezović†

University of Kragujevac, Faculty of Medical Sciences, *Department of Neurology, Kragujevac, Serbia; University of Belgrade, Faculty of Medicine, †Institute of Epidemiology, Belgrade, Serbia

Abstract

Background/Aim. Malignant gliomas represent a heterogenaus group of tumors. They occur in all age groups, predominatly in males in older age. The purpose of this case-control study was to examine the association between risk for developing glioma and family history of diseases. **Methods.** The case-control study included 100 pathologically confirmed cases of glioma at the Clinical Centre Kragujevac, Serbia, between 2015 and 2016, and 200 age- and sex-matched controls without glioma and other malignant diseases in personal and family history at the same institution. After signing the informed consent all the patients filled out an epidemiological questionnaire. Multivariate logistic regression analysies was used in statistical data processing. **Results.** Malignant diseases in family history were more common in the study group than in the control group [odds ratio (OR) = 1.821, 95% confidence interval (CI) 1.004–3.305; p = 0.049]. The most common malignant tumor in the study group were cancer of the uterus (7%) and colon cancer (6%), while in the control group the most common cancer were lung cancer (6%) and cancer of the uterus (7%). Diabetes mellitus in family history was more common among control individuals than among glioma patients (OR = 0.520, 95% CI = 0.271–0.995; p = 0.048). Also, our results showed that cardiovascular diseases in family history were more common in the control group than among patients of the study group (OR = 0.557, 95% CI = 0.325–0.953; p = 0.033). **Conclusion.** In this case-control study, we observed a statistically significant relation between family history of malignant diseases and glioma. Also, we found statistically significant inverse relation between family history of cardiovascular diseases and diabetes and glioma.

Key words:
glioma, family history of disease; medical history taking; epidemiology; neoplasms.

Apstrakt

Uvod/Cilj. Maligni gliomi predstavljaju heterogenu grupu tumora koji se javljaju većinom kod starijih muškaraca. Cilj ove studije bio je da ispita vezu između porodične istorije bolesti i rizika od nastanka glioma. **Metode.** Studija slučaj- kontrola uključila je 100 ispitanika sa patohistološki potvrđenim gliomom u Kliničkom centru Kragujevac, Srbija, između 2015. i 2016. godine i 200 ispitanika kontrolne grupe, uparenih po polu i uzrastu, koji nisu imali istoriju glioma i drugih malignih bolesti u ličnoj i porodičnoj anamnezi. Nakon potpisivanja informisanog pristanka, svi bolesnici su popunili epidemiološki upitnik. Za obradu statističkih podataka korišćena je multivarijantna logistička regresija. **Rezultati.** Maligne bolesti su bile češće u studijskoj, nego u kontrolnoj grupi [*odds ratio* (OR) = 1,821, 95% *confidence interval* (CI) = 1,004–3,305; p = 0,049]. Najčešći maligni tumori u studijskoj grupi su bili karcinom materice (7%) i karcinom debelog creva (6%), dok su u kontrolnoj grupi najčešći karcinomi bili karcinom pluća (6%) i karcinom materice (7%). Šećerna bolest u porodičnoj anamnezi je bila češća kod bolesnika kontrolne grupe nego kod bolesnika sa gliomom (OR = 0,520, 95% CI = 0,271–0,995; p = 0,048). Takođe, naši rezultati su pokazali da su kardiovaskularne bolesti u porodičnoj anamnezi bile češće u kontrolnoj grupi nego u studijskoj grupi (OR = 0,557, 95% CI = 0,325–0,953; p = 0,033). **Zaključak.** U ovoj studiji slučaj-kontrola, pronašli smo statistički značajnu vezu između porodične istorije malignih bolesti i glioma. Takođe, pronašli smo statistički značajnu inverznu vezu između porodične istorije kardiovaskularnih bolesti i šećerne bolesti i glioma.

Ključne reči:
glioma, porodična istorija bolesti; istorija bolesti, uzimanje; epidemiologija; neoplazme.

Correspondence to: Ana Azanjac Arsić, University of Kragujevac, Faculty of Medical Sciences, Department of Neurology, Rudnička 13/14 34000 Kragujevac, Serbia. E-mail: ana.azanjac@yahoo.com

Introduction

Malignant gliomas are the most frequently diagnosed brain tumors in adults [1]. They represent a heterogeneous group of tumors which have histological similarity to glia, such as astrocytes and oligodendrocytes [2]. Gliomas occur in all age groups with predominance of adults over 45 years [3]. The etiology of glioma occurrence is yet unknown [4]. Well-established risk factors for glioma development include older age, male gender, Caucasian race/ethnicity and rare genetic syndromes [5–7].

Genetic predisposition and ionizing radiation affect only a small proportion of the total population, which provide little opportunity for prevention [5]. Some hereditary tumor syndromes are associated with glioma such as Li-Fraumeni, neurofibromatosis (types 1 and 2), tuberous sclerosis, nevoid basal cell carcinoma syndrome, familiar polyposis and von HippelLindau, but the nature of these associations is still unclear [6].

The influence of diseases on family history in the glioma's etiology is uncertain. Genetic factors are poorly understood. A number of studies conducted so far reported that positive family history of malignant diseases increased risk of glioma occurrence [8–15].

Based on all above mentioned, we aimed to investigate the relation among glioma and family history of diseases.

Methods

Study design

The study group consisted of 100 patients (59 males and 41 females), mean age 59.19 ± 10.03 years, with histopathologically verified diagnosis of glioma according to the World Health Organization (WHO) criteria [International Classification of Diseases (ICD)-O-3]. Out of the 100 patients, 78 (78%) had glioblastoma multiforme, 9 (9%) astrocytoma anaplasticum, 5 (5%) oligodendroglioma gradus II, 3 (3%) oligoastrocytima gradus III, 2 (2%) oligoastrocytoma gradus II and 1 (1%) patients had oligodendroglioma gradus III, 1 (1%) oligodendroglioma anaplasticum gradus III and 1 (1%) ksantoastrocytoma gradus II. The study was realized according to the Declaration of Helsinki and approved by the Ethics Committee of Clinical Centre Kragujevac.

The criteria for inclusion into the study were: patients with confirmed diagnosis of primary, previously untreated glioma. Exclusion criteria were previously diagnosed and treated glioma and glioma recidivans. The patients were treated surgically, followed by radio- and chemotherapy at the Center for Oncology, Clinical Center Kragujevac. Data were collected from April 1, 2015 to May 30, 2016. The control group included 200 patients matched by gender and age (109 males, mean age 59.32 ± 10.71 years, and 91 females, mean age 58.09 ± 9.12 years) to glioma cases. Controls were individuals admitted to the same institutions for nonmalignant conditions within the same period of time as the cases. Also, control individuals were without glioma and other malignant diseases in personal and family history. All participants signed informed consent.

Data collection

Data was collected by means of questionnaire consisting of sixth parts.The first part of the questionnaire included demographic characteristic of the patients. The second part of the questionnaire included family history of certain diseases (cardiovascular disease, diabetes mellitus, other chronic diseases and family history of malignant diseases including brain tumors). The third part of the questionnaire referred to the personal history of diseases including reproductive risk factors. The fourth part dealt with information on the risks of exposure to environmental risk factors, the fifth included data on habits (smoking, drinking coffee, tea and alcohol consumption) and the sixth part referred to information about nutrition.

Statistical analysis

The initial portion of the statistical analysis included descriptive statistics. For the comparison of qualitative variables between the patients with glioma and the control individuals, chi-square (χ^2) test was used. Categorical variables were presented as frequencies and percentages. Multivariate logistic regression analysis with odds ratio (OR) and 95% confidence intervals (CI) were performed in order to determine the effects of family history of diseases (including malignant diseases, cardiovascular diseases and diabetes mellitus) on the dependent variable (glioma). *P* value of less than 0.05 was considered statistically significant. Statistical analysis was performed using the SPSS software version 19.0.

Results

Demographic characteristics are provided in Table 1. A total of 100 patients (59 males and 41 females) and 200 control individuals (109 males and 91 females) participated in the study. The mean age of patients was (mean ± standard deviation) 59.19 ± 10.03 years and for controls 58.76 ± 10.01 years ($p = 0.729$). Control individuals were living at the hometown for a shorter time period ($p = 0.035$) and more often changed their place of residence than patients with glioma ($p = 0.007$). Patients with glioma had a higher body weight ($p = 0.002$) and higher body mass index ($p < 0.0005$) than control individuals. In an analysis comparing patients of the study group with control individuals, a significant association ($p < 0.005$) od the disease was observed in relation to the blood group AB.

We observed a statistically significant relation between family history of malignant diseases and glioma (OR = 1.821; 95% CI = 1.004–3.305, $p = 0.049$). In the study group, malignant tumors in familiy history were observed in 31 (31%) patients with glioma and in 38 (19%) patients of the control group.

Table 1

Demographic characteristics of patients with glioma (the study group) and patients in the control group

Variable	Study group (n = 100)	Control group (n = 200)	*p*
Age (years), mean ± SD			
male	59.76 ± 10.76	59.32 ± 10.71	0.779
female	58.36 ± 8.95	58.09 ± 9.12	0.876
All patients	59.19 ± 10.03	58.76 ± 10.01	0.729
Sex, n (%)			
male	59 (59)	109 (54.5)	0.537
female	41 (41)	91 (45.5)	0.537
Years spent in hometown, mean ± SD	47.39 ± 20.94	41.69 ± 23.45	0.035
Change of the place residence, n (%)	8 (8)	42 (21)	0.007
Birth weight (kg), mean ± SD	3.14 ± 0.57	3.63 ± 3.01	0.108
Body weight (kg), mean ± SD	82.88 ± 12.64	77.23 ± 17.04	0.002
Body height (cm), mean ± SD	172.84 ± 7.62	173.09 ± 9,65	0.813
BMI (kg/m^2), mean ± SD	27.57 ± 5.01	25.72 ± 3.30	< 0.0005
Blood groups, n (%)			< 0.0005
AB	31 (31)	21 (10.5)	
A	24 (24)	63 (31.5)	0.224
B	10 (10)	28 (14.0)	0.442
O	31 (31)	60 (30)	0.921
Rh factor (-), n (%)	8 (8)	25 (12.5)	0.921

BMI – body mass index; SD – standard deviation.

Table 2

Family history as a risk factor for glioma according to multivariate conditional logistic regression analysis

Variable	Study group (n = 100) n (%)	Control group (n = 200) n (%)	OR (95% CI)	*p*
Brain tumors	10 (10)	0 (0)	0.000 (0.00-)	0.999
Epilepsia	5 (5)	4 (2)	0.385(0.09–1.67)	0.203
Migraine	6 (6)	13 (6.5)	0.999 (0.32-3.08)	0.998
Neurological diseases	24 (24)	14 (7)	0.641 (0.29–1.42)	0.267
Malignant tumors	38 (38)	31 (15.5)	1.821 (1.00–3.30)	0.049
Diabetes	17 (17)	59 (29.5)	0.520 (0.27–0.99)	0.048
Cardiovascular diseases	31 (31)	102 (51)	0.557 (0.32–0.95)	0.033
Autoimmune diseases	0 (0)	1 (0.5)	5175 (0.000-)	0.999
Chronic diseases	7 (7)	22 (11)	1.687 (0.671-4.238)	0.266
Genetic syndromes	0 (0)	1 (0.5)	2999 (0.000 -)	1.000

OR – odds ratio; CI – confidence interval.

However, statistically significant inverse relation was observed between family history of cardiovascular diseases (OR = 0.557; 95% CI = 0.325–0.953, $p = 0.033$) and diabetes (OR = 0.520; 95% CI = 0.271–0.995, $p = 0.048$) and glioma (Table 2). A positive family history of cardiovascular diseases was observed in 31 (31%) patients with glioma, while in the control group 102 (51%) patients had a cardiovascular diseases in the family. Diabetes mellitus in family history was registered in 17 (17%) glioma patients, while in the control group 59 (29.5%) patients had positive family history of diabetes.

Patient with glioma and controls did not differ with respect to epilepsy, migraine, other neurological diseases (stroke, dementia, Parkinson's disease, amyotrophic lateral sclerosis – ALS, vertigo, multiple sclerosis), autoimmune diseases, chronic diseases and genetic syndromes (Table 2). The most common malignant tumors in the study group were cancer of the uterus (7%) and colon cancer (6%), while in the control group the most common cancer were lung cancer (6%) and cancer of the uterus (7%) (Table 3).

Table 3

Family history of cancer by site in the study group (patients with glioma) and the control group

Localization	Study group (n = 100) n (%)	Control group (n = 200) n (%)
Uterus	7 (7)	6 (3)
Kidney	2 (2)	2 (1)
Leukaemia	1 (1)	0 (0)
Breast	5 (5)	4 (2)
Prostate	4 (4)	4 (2)
Colon	6 (6)	5 (2.5)
Mouth	1 (1)	0 (0)
Larynx	2 (2)	1 (3.2)
Lung	0 (0)	12 (6)

Discussion

Our case-control study of 100 cases of histopathologically confirmed glioma demonstrate an association between glioma and family history of malignant diseases, diabetes

and cardiovascular diseases. Malignant diseases in family history were more common in the study group than among patients in the control group (OR = 1.821, 95% CI = 1.004–3.305; $p = 0.049$). Several studies with similar methodology reported that positive family history of tumors of the central nervous system may be associated with increased risk of glioma. Results of the study conducted by Hill et al. [8] suggested that patients with positive family history of stomach cancer, prostate cancer and Hodgkin's disease had increased risk for glioma development (2-fold), while patients with family history of colon cancer had 1.4-fold risk. Positive family history of colon or prostate cancer increased risk of glioma in individuals aged 18–49 years, while positive family history of stomach cancer or Hodgkin's disease increased risk of glioma in older individuals (≥ 50 years). Risk of astrocytoma is elevated among individuals with positive family history of breast cancer. Also, Hill et al. [8] reported that individuals with two or more relatives with malignant disease had increased risk for glioma development compared with individuals without cancer in family history. Results of Utah Population Database suggested that individuals who had first degree relatives with tumors of central nervous system had elevated risk for glioma, especially women. Moreover, men with positive history of prostate cancer had elevated risk of glioma suggested by results of several study [9–11]. Also, results of these studies suggest that glioma risk was elevated in individuals with positive family history of Hodgkin's disease.

Wrensch et al. [12]observed that positive family history of breast cancer may increase the risk of glioma. Lunch et al. [13] have reported that individuals with brain tumor had 2 or more relatives with breast cancer in 34 families. In one study, lung and breast cancer occurred more often in family members of cases than controls [14]. Results of one study suggest that patients with melanoma had increased risk for glioma [15]. Association of glioma and melanoma may be explained by deletion of common tumor suppressor genes (p16 and p14) [16].

Results of a study of Paunu et al. [17] suggested that families with 2 or more glioma cases had increased risk of glioma (4-fold). Malmer et al. [18] observed that individuals with positive family history of the low grade and the high grade gliomas in first degree relatives had elevated risk for development of these tumors. These results confirmed Scheurer et al [15]. However, results of one study with 416 glioma cases did not show relation between risk of glioma and family history of malignant disease [19].

A family history of malignant disease can be attributed to rare genetic mutation such as polymorphisms of XPD genes and exposure to environmental factors [20, 21].

In our study, we observed a statistically significant inverse relation between family history of diabetes and risk of glioma. Diabetes mellitus in family history was more common among control individuals than among glioma patients (OR = 0.520, 95% = CI 0.271–0.995; $p = 0.048$). To our best knowledge, this is the first study investigating the relationship between family history of diabetes and risk for glioma occurrence. Other studies investigated relationship of personal history of diabetes with risk of glioma development. A lower risk of glioma associated with diabetes was first reported in a 1965 study reporting a lower frequency of glioma among diabetics versus non-diabetics [22].The previous studies observed a statistically significant reduced risk of glioma in patients with diabetes type 2 [23–27]. The results of one study reported that increased HbA1c level is associated with decreased glioma risk. The results of an experimental study suggested that the use of metformin in patients with glioma may reduce the risk for its development [28].

The underlying biological pathways that could explain decreased risk of glioma in patients with diabetes are yet unknown. Specific biomarkers of immune function and insulin resistance could provide additional insight on biological mechanisms that may underlie the inverse association between diabetes and glioma risk [27].

Our results showed that cardiovascular diseases in family history were more common in the control group than among patients of the study group (OR = 0.557, 95% CI = 0.325–0.953; $p = 0.033$). The most common cardiovascular disease in our study was hypertension. Results of one case-control study observed that patients with glioma had higher prevalence of hypertension [29]. Also, a number of researchers concluded that use of some antihypertensive drugs might be potential explanation for such relationship [30].

We did not find statistically significant association between epilepsy, migraine, other neurological diseases, autoimmune and chronic diseases in family history and glioma in the study group. So far, no study examined relation between these diseases and risk of glioma.

This was the first study in our country to examine the relation between family history of diseases and risk of glioma. However, there are some limitations which should be mentioned. Namely, one of limitations of this study is a small number of patients with pathologically confirmed glioma in the study group. Another limitation of the study referes to recall bias, bearing in mind that the answers were collected by means of questionnaire.

Conclusion

In this case-control study, we observed a statistically significant relation between family history of malignant diseases and glioma. Also, we found statistically significant inverse relation between family history of cardiovascular diseases and diabetes and glioma.There is a need for further research on a much larger sample, in order to confirm these findings.

Disclosure

The authors report no conflict of interes.

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The effect of estroprogestagen therapy on lipid status in menopause depending on the drug administration route

Uticaj terapije estroprogestagenima na lipidni status u menopauzi zavisno od načina primene leka

Goran Čitlučanin*, Miomira Ivović[†‡], Milina Tančić-Gajić[†‡], Fadil Canović[§], Miloš Stojanović[†‡], Ljiljana Marina[†‡], Zorana Arizanović[†‡], Aleksandar Djogo[‖], Vladimir Gerginić[‡], Svetlana Vujović[‡]

*** Outpatient Department "Voždovac", Belgrade, Serbia; Clinical Center of Serbia, [†]Clinic for Endocrinology, Diabetes and Metabolic Diseases, Belgrade, Serbia; [§]Outpatient Department "Zemun", Belgrade, Serbia; [‖]Clinical Center Podgorica, Podgorica, Montenegro; University of Belgrade, [‡]Faculty of Medicine, Belgrade, Serbia**

Abstract

Background/Aim. In menopausal women lipid and lipoprotein values are important predictors of development of cardiovascular diseases (CVD). The use of estrogens reduces levels of low density lipoprotein cholesterol (LDL-C) and lipoprotein A [Lp(a)], and increases levels of triglycerides (TG) and high density lipoprotein cholesterol (HDL-C) depending on the dose and route of administration. Simultaneous administration of progesterone, depending on the type, can have different effects on lipids. The aim of the study was to examine the effect of estroprogestagen therapy on the lipid metabolism of women in menopause, depending on the administration route. **Methods.** A study was conducted as prospective clinical interventional study with controlled parallel groups. It included 64 women in menopause, divided into three groups: the group 1 (n = 22) on oral therapy with estroprogestagens, the group 2 (n = 17) on transdermal patch therapy with estroprogestagens and the group 3 (n = 25) treated with estroprogestagens given intramuscularly. The following biochemical parameters in the serum were determined: total cholesterol (TC), HDL-C, LDL-C, TG, Lp(a), apoprotein A (Apo-A), apoprotein B (Apo-B), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, testosterone, sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEA-SO_4), prolactin and thyroid-stimulating hormone (TSH), prior to administration of the menopausal hormonal therapy (MHT), as well as after sixth months and 2–5 years from the beginning of the therapy. The statistical significance of the difference in values obtained was examined independently and depending on the route of MHT application. **Results.** MHT, regardless of the administration route, led to a statistically significant continuous decrease of TC, LDL-C and Apo-B levels and the continuous increase of HDL-C and Apo-A levels. Serum levels of TC, LDL-C, HDL-C, Lp(a), Apo-A and Apo-B did not show a statistically significant differences among groups of women given MHT by different routes. It was found that the serum level of Apo-A increased significantly with the rise of estradiol, and the values of LDL and Apo-B decreased regardless of the route of the MHT application. **Conclusion.** MHT introduced in time, regardless of the route of administration, has beneficial effects on the lipid status of menopausal women and consequently might prevent numerous cardiovascular diseases that are the leading cause of mortality.

Key words:
hormone replacement therapy; cardiovascular diseases; lipids; menopause.

Apstrakt

Uvod/Cilj. Kod žena u menopauzi, vrednosti lipida i lipoproteina su značajni prediktori razvoja kardiovaskularnih bolesti (KVB). Primena estrogena smanjuje serumske nivoe LDL holesterola (LDL-C) i lipoproteina A [Lp(a)], uz povećanje novoa triglicerida (TG) i HDL holesterola (HDL-C), što zavisi od doze i puta primene leka. Istovremena primena progesterona, zavisno od vrste, može imati različite efekte na novo lipida u serumu. Cilj rada je bio ispitivanje uticaja terapije estroprogestagenima na metabolizam lipida žena u menopauzi, zavisno od puta primene leka. **Metode.** Istraživanje je sprovedeno po tipu prospektivne kliničke interventne, kontrolisane studije sa paralelnim grupama.

Correspondence to: Goran Čitlučanin, Outpatient Department "Voždovac", Ustanička 16, 11 000 Belgrade, Serbia.
E-mail: drgorancitlucanin@hotmail.com

Njime su bile obuhvaćene 64 žene u menopauzi, podeljene u tri grupe: grupa 1 (n = 22), na oralnoj terapiji estroprogestagenima, grupa 2 (n = 17) na terapiji transdermalnim flasterima i grupa 3 (n = 25) koja je estroprogestagene primala intramuskularno. U serumu se bili određivani nivoi: ukupnog holesterola (TC), LDL-C, HDL-C, TG, Lp(a), apolipoproteina A (Apo-A) i apolipoproteina B (Apo-B), folikulostimulirajućeg hormona (FSH), luteinizirajućeg hormona (LH), estradiola, progesterona, testosterona, *sex hormone-binding globulin* (SHBG), dehidroepiandrosteron sulfata (DHEA-S04), prolaktina i tiroid-stimulišućeg hormona (TSH), i to pre primene hormonske terapije u menopauzi (HTM), kao i posle šest meseca i 2–5 godina od početka terapije. Statistička značajnost razlika u doim vrednostima ispitivana je nezavisno i zavisno od puta primene HTM. **Rezultati.** HTM, bez obzira na put primene, dovela je do statistički značajnog kontinuiranog sniženja serumskih nivoa TC, LDL-C i Apo-B i kontinuiranog porasta nivoa HDL-C i Apo-A. Vrednosti TC, LDL-C, HDL-C, Lp(a), Apo-A i Apo-B nisu se statistički značajno razlikovale između grupa žena tretiranih HTM, primenjenom na različite načine. Uvrđeno je da s porastom nivoa estradiola u serumu stastistički značajno rastu i serumski novoi Apo-A, dok su serumski nivoi LDL-C i Apo-B bili sniženi bez obzira na put primene HTM. **Zaključak.** HTM, uvedena na vreme, bez obzira na put primene, ima povoljne efekte na lipidni status žena u menopauzi i posledično mogla bi sprečiti brojne kardiovaskularne bolesti koje su vodeći uzrok smrtnosti.

Ključne reči:
hormoni, supstituciona terapija; kardiovaskularne bolesti; lipidi; menopauza.

Introduction

In menopausal women, lipid and lipoprotein levels in the serum are significant predictors of atherosclerosis development and risk factors for cardiovascular diseases (CVD). Significant increase or decrease in serum levels of some lipids and lipoproteins significantly increases the risk of CVD [1–3]. In menopause, due to decrease in estradiol concentrations, there are higher concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), apolipoprotein B (Apo-B) and lipoprotein (a) [Lp(a)], and lower concentrations of high-density lipoprotein cholesterol (HDL-C) compared to values in women in reproductive period [4, 5]. During this period small, thick LDL particles dominate, prone to modifications, more precisely oxidation, glycosylation and acetylation, which all together additionally increase the risk of atherogenesis and the development of CVD [6, 7].

A study in Denmark has shown that the introduction of menopausal hormonal therapy (MHT), immediately after menopause, significantly reduces the risk of myocardial infarction or cardiac insufficiency, because TG, TC and LDL-C are growing after 6 months of the last menstrual period [8–10].

Analysis of randomized controlled trials (RCTs) from the Cochrane Base in 2015 showed that MHT, applied within 10 years of the last menstruation, reduced the risk of coronary disease [11]. Relationship between centripetal obesity and lipid status disorders, i.e. increased risk for CVD is known. The North American Menopause Society (NAMS) states that MHT can help in reducing abdominal accumulation of adipose tissue and preventing body mass gain [12].

Estrogen therapy reduces LDL-C and Lp(a), increases TG and HDL-C, depending on the drug dose and the route of its administration. Simultaneous administration of progesterone, depending on the type, may have different effects on lipids [13].

The aim of the study was to examine the influence of estroprogestagen therapy on the metabolism of lipids in menopausal women depending on the route of drug administration.

Methods

The research was carried out at the Clinic for Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Serbia in Belgrade, from 1996 to 2010, as a prospective clinical interventional controlled study with parallel groups. The study included 64 women in menopause divided into three groups: the group 1 (n = 22) – women in menopause on estroprogestagen oral therapy (2 mg of estradiol in the form of estradiol hemihydrate; 2 mg of estradiol and 1 mg of norethisterone acetate; 1 mg estradiol), the group 2 (n = 17) – menopausal women treated with transdermal estroprogestagen patch (50 μg of 17-β estradiol and 250 μg of norethidron acetate daily) and the group 3 (n = 25) – menopausal women treated with estroprogestagene (4 mg of estradiol valerate and 200 mg of prasterone) given intramuscularly.

Exclusion criteria were: bleeding from the uterus of unknown etiology in the last 2 years; previous, existing or suspected breast cancer; the malignancy of any localization in the past 5 years; endometrial thickness > 5 mm; existing liver dysfunction or liver disease; an earlier or existing thromboembolic process; application of hormone substitution therapy in the last 12 months; body mass index (BMI) > 30 kg/m^2; poor motivation.

According to the study protocol, the following patient's data were taken: age of menarche and menopause, period from last menstruation to beginning of MHT and the age when MHT began.

To assess the metabolic profile, we determined: TC (mmol/L), HDL-C (mmol/L), LDL-C (mmol/L), TG (mmol/L), Lp(a) (g/L), apolipoprotein (Apo-A) (g/L), Apo-B (g/L). Lp(a), Apo-A, and Apo-B were determined by the method of nephelometry (Nephelometer BN/100, Behring, Germany). TC, LDL-C, HDL-C and TG were determined by chromatography (Boeringher Mannheim accessories).

The hormone status was defined according to serum levels of following hormones: follicle-stimulating hormone (FSH) (IU/L), luteinizing hormone (LH) (IU/L), estradiol (E2) (pmol/L), progesterone (P) (nmol/L), testosterone (T)

(nmol/L), sex hormone-binding globulin (SHBG) (nmol/L), dehydroepiandrosterone sulfate (DHEA-S04 (μmol/L), prolactin (PRL) (mIU/L) and thyroid-stimulating hormone (TSH) (mIU/L). The radioimmunoassay (RIA) method was used to determine serum concentrations of these hormones. The reference values of these parameters are given by the appropriate analytical method. Lipid and hormonal status were assessed prior to administration of MHT (initially), after sixth month (first control) and after 2–5 years from the MHT beginning (second control). All procedures were approved by the local Ethic Committee and conducted in accordance with the Helsinki Declaration.

Results were present as mean ± standard deviation (SD). Differences between groups were assessed by two-way analysis of variance (ANOVA) with Bonferroni post hoc analysis for continuous variables. Correlations between parameters were analyzed with Parson's test. Differences were considered statistically significant at $p < 0.05$. SPSS 18.0 software was used for the statistical analysis.

Results

The study included 64 women in menopause, aged between 34 and 59 years. Respondents had their first menstruation at an average age of 14 (13.35 ± 1.53), and their last menstruation at an average age of 47 (46.72 ± 4.27). The average period, from the last menstruation to the beginning of the MHT, was 2.5 years (2.48 ± 2.47), and the MHT started in 50s (49.19 ± 4.62). No statistically significant difference was found among patient groups, depending on the route of MHT administration, for any of the mentioned parameters ($p > 0.05$), that is the groups were homogeneous.

During the monitoring of MHT administration, a statistically significant continuous decrease of TC, LDL-C and Apo-B serum levels was observed. Only after 2–5 years of the MHT, a significant reduction in TC level occurred, whereas statistically significant decrease of LDL-C and Apo-B was observed after 6th month from the MHT beginning and this tendency continued for following 2–5 years of the MHT administration. Statistically significant increase of HDL-C and Apo-A was recorded. While significant increase of HDL-C level was recorded only after 2–5 years of continuous MHT, an increase of Apo-A level was recorded after 6 months from the MHT beginning and it was maintained during following 2–5 years of monitoring. TG and Lp(a) levels had an insignificant tendency to decrease during the MHT (Table 1).

The results of hormonal analyses during the MHT, regardless of the route of administration of estroprogestagen, are shown in Table 2. During the MHT, a continuous decline in the serum levels of FSH, LH, DHEA-SO4 and progesterone was observed, and this decline was statistically significant. FSH and LH levels had statistically significant decline over 6 months from the MHT beginning and this decline continued during 2–5 years of the MHT application. A statistically significant reduction in DHEA-SO4 and progesterone levels were noted after 2–5 years of the MHT application. On the other hand, serum levels of estradiol and SHBG had a statistically significant continuous growth throughout the entire period of the MHT administration. Statistically significant increase in both estradiol and SHBG levels was recorded after 6 months from the MHT beginning and it was maintained in following 2–5 years of the MHT. Serum level of testosterone was decreased during MHT compared to the period prior to the therapy beginning, but this decrease was not maintained during 2–5 years of the MHT administration.

For TG levels, a statistically significant difference was found only after 2–5 years of the MHT administration. It was found that in women on transdermal MHT there were a statistically significantly lower TG levels comparing to those in women on oral MHT; this difference was noted only after 2–5 years of the MHT (TG: oral route 1.71 ± 0.75 mmol/L, transdermal 1.11 ± 0.57 mmol/L, intramuscular 1.39 ± 0.649 mmol/L; $p < 0.05$). Serum levels of TC, LDL-C, HDL-C, Lp(a), Apo-A and Apo-B had no statistically significant differences among groups of women given MHT by different route, in any period of monitoring (Table 3).

The results of hormonal analyses during the MHT, depending on the route of administration of estroprogestagen, are shown in Table 4. After 6 months from the MHT beginning, statistically significantly lower FSH and LH levels were found in intramuscularly treated women compared to those given MHT orally or transdermally. During 2–5 years of MHT, lowest FSH levels were noticed in women given MHT by the oral route. On the other hand, there were no differences in LH serum levels regardless of the MHT administration route.

There were statistically significantly higher levels of SHBG in women on oral MHT compared to the other two groups of women after 6 months, as well as after 2–5 years of continuous MHT.

Although TSH levels were in the normal range during the entire monitoring period, regardless of the MHT administration route, in intramuscularly treated women, compared with those treated orally, statistically significantly lower levels were recorded after 2–5 years of the treatment.

Serum levels of estradiol had constant increase in all three groups of women regardless of the MHT administration route, without statistically significant differences among them in any of the period monitored.

Also, there were no statistically significant differences in serum levels of progesterone, testosterone, prolactin and DHEA-SO4 among three groups of women given MHT by different route in any of the period monitored.

There was a positive correlation of serum levels of FSH and LH with LDL-C level, as well as positive, statistically significant correlation of serum levels of estradiol with Apo-A levels and negative with LDL-C and Apo-B levels, which suggests favorable influence of MHT on the reduction of cardiovascular risk.

Table 1

Lipid status during menopausal hormonal therapy (MHT) regardless of the route of administration

Lipids	Initially	First control	Second control	*p*
TC (mmol/L)	6.33 ± 1.30	5.89 ± 0.93	5.80 ± 0.80	< 0.01
LDL-C (mmol/L)	4.30 ± 1.10	3.87 ± 0.84	3.72 ± 0.81	< 0.01
HDL-C (mmol/L)	1.33 ± 0.40	1.39 ± 0.28	1.48 ± 0.32	< 0.05
TG (mmol/L)	1.75 ± 0.98	1.57 ± 0.069	1.43 ± 0.69	ns
Lp (a) (g/L)	0.28 ± 0.36	0.24 ± 0.32	0.22 ± 0.30	ns
Apo-A (g/L)	1.56 ± 0.28	1.64 ± 0.25	1.79 ± 0.26	< 0.001
Apo-B (g/L)	1.36 ± 0.32	1.23 ± 0.28	1.08 ± 0.27	< 0.001

ns – not statistically significant; initially – prior to MHT; first control – after sixth month of MHT; second control – after 2–5 years of MHT; TC – total cholesterol; LDL-C – low density lipoprotein cholesterol; HDL-C – high density lipoprotein cholesterol; TG – triglicerides; Lp(a) – lipoprotein a; Apo-A – apolipoprotein A; Apo-B – apolipoprotein B.

Table 2

Hormone status during menopausal hormonal therapy (MHT) regardless of the route of administration

Hormones	Initially	First control	Second control	*p*
FSH (IU/L)	74.2 ± 21.2	32.1 ± 13.9	27.6 ± 12.5	< 0.001
LH (IU/L)	32.9 ± 19.3	18.9 ± 11.45	16.3 ± 10.9	< 0.001
Estradiol (pmol/L)	12.8 ± 7.3	79.1 ± 55.2	101.3 ± 50.3	< 0.001
Progesterone (nmol/L)	3.21 ± 0.70	2.91 ± 0.75	2.77 ± 0.87	< 0.001
Testosterone (nmol/L)	1.14 ± 0.60	0.92 ± 0.46	1.10 ± 0.71	< 0.01
DHEA-SO4 (μmol/L)	2.80 ± 1.53	2.27 ± 1.13	1.90 ± 0.94	< 0.001
SHBG (nmol/L)	45.4 ± 17.0	62.0 ± 22.9	71.8 ± 31.3	< 0.001
Prolactin (mIU/L)	233.4 ± 83.8	248.7 ± 120.8	225.8 ± 84.8	ns
TSH (mIU/L)	2.23 ± 0.74	2.27 ± 0.74	2.11 ± 0.70	ns

ns – no statistical significance; initially – prior to MHT; first control – after sixth month of MHT; second control – after 2–5 years of MHT; FSH – follicle stimulating hormone; LH – luteinizing hormone; DHEA-SO4 – dehydroepiandrosterone sulfate; TSH – thyroid-stimulating hormone.

Table 3

Lipid status during menopausal hormonal therapy (MHT) depending on the route of administration

Lipids	Time of analysis	Oral	Transdermal	Intramuscular	*p*
TC (mmol/L)	Initially	6.27 ± 1.35	6.11 ± 0.93	6.54 ± 1.47	ns
	First control	5.98 ± 0.92	5.81 ± 0.83	5.85 ± 1.03	ns
	Second control	5.68 ± 0.73	5.69 ± 0.73	5.99 ± 0.88	ns
LDL-C (mmol/L)	Initially	4.26 ± 1.04	3.98 ± 0.83	4.55 ± 1.28	ns
	First control	3.92 ± 0.80	3.67 ± 0.71	3.96 ± 0.97	ns
	Second control	3.63 ± 0.66	3.57 ± 0.75	3.90 ± 0.95	ns
HDL-C (mmol/L)	Initially	1.26 ± 0.40	1.41 ± 0.35	1.33 ± 0.44	ns
	First control	1.36 ± 0.27	1.42 ± 0.30	1.41 ± 0.27	ns
	Second control	1.57 ± 0.31	1.40 ± 0.27	1.44 ± 0.36	ns
TG (mmol/L)	Initially	1.80 ± 0.68	1.73 ± 1.24	1.72 ± 1.04	ns
	First control	1.71 ± 0.63	1.43 ± 0.79	1.54 ± 0.67	ns
	Second control	1.71 ± 0.75	1.11 ± 0.57	1.39 ± 0.64	< 0.05
Lp(a) (g/L)	Initially	0.16 ± 0.20	0.31 ± 0.38	0.37 ± 0.45	ns
	First control	0.15 ± 0.18	0.22 ± 0.20	0.35 ± 0.44	ns
	Second control	0.13 ± 0.16	0.20 ± 0.19	0.33 ± 0.43	ns
Apo-A (g/L)	Initially	1.53 ± 0.32	1.59 ± 0.21	1.55 ± 0.30	ns
	First control	1.67 ± 0.25	1.64 ± 0.24	1.61 ± 0.27	ns
	Second control	1.82 ± 0.25	1.74 ± 0.24	1.80 ± 0.28	ns
Apo-B (g/L)	Initially	1.36 ± 0.34	1.38 ± 0.31	1.34 ± 0.32	ns
	First control	1.23 ± 0.31	1.19 ± 0.27	1.25 ± 0.27	ns
	Second control	1.09 ± 0.26	1.06 ± 0.33	1.08 ± 0.27	ns

ns – not statistically significant; initially – prior to MHT; first control – after sixth month of MHT; second control – after 2–5 years of MHT; TC – cholesterol; LDL – low density lipoproteins; HDL – high density lipoproteins; TG – triglycerides; Lp(a) – lipoprotein a; Apo-A – apolipoprotein A; Apo-B – apolipoprotein B.

Table 4

Hormone status during menopausal hormonal therapy (MHT) depending on the route of administration

Hormones	Time of analysis	Oral	Trandermal	Intramuscular	*p*
FSH (IU/L)	Initially	73.1 ± 26.0	78.5 ± 20.4	72.2 ± 17.1	ns
	First control	33.1 ± 16.3	38.3 ± 8.4	27.1 ± 13.1	< 0.05
	Second control	23.1 ± 11.3	33.9 ± 14.6	27.4 ± 10.5	< 0.05
LH (IU/L)	Initially	35.2 ± 28.6	33.6 ± 10.7	30.2 ± 13.3	ns
	First control	18.1 ± 11.8	24.8 ± 12.1	15.7 ± 9.3	< 0.05
	Second control	13.9 ± 8.9	18.4 ± 10.3	16.9 ± 12.9	ns
Estradiol (pmol/L)	Initially	15,8 ± 8.3	11.6 ± 6.2	11.1 ± 6.5	ns
	First control	94.8 ± 85.6	70.1 ± 20.9	71.5 ± 30.8	ns
	Second control	114.1 ± 68.2	96.7 ± 42.7	93.1 ± 33.4	ns
Progesterone (nmol/L)	Initially	3.35 ± 0.88	3.15 ± 0.54	3.10 ± 0.59	ns
	First control	3.00 ± 0.90	2.95 ± 0.64	2.80 ± 0.69	ns
	Second control	2.65 ± 0.91	2.53 ± 0.78	3.05 ± 0.84	ns
Testosterone (nmol/L)	Initially	1.11 ± 0.40	1.27 ± 0.70	1.09 ± 0.69	ns
	First control	0.88 ± 0.40	0.94 ± 0.50	0.95 ± 0.49	ns
	Second control	0.99 ± 0.47	1.05 ± 0.60	1.24 ± 0.94	ns
DHEA-SO4(μmo/L)	Initially	3.31 ± 1.72	2.80 ± 1.72	2.36 ± 1.08	ns
	First control	2.52 ± 1.38	2.24 ± 1.01	2.08 ± 0.97	ns
	Second control	1.93 ± 1.10	1.97 ± 0.90	1.83 ± 0.86	ns
SHBG (nmol/L)	Initially	43.8 ± 18.2	46.6 ± 15.3	46.0 ± 17.6	ns
	First control	71.5 ± 24.1	52.8 ± 18.2	59.9 ± 22.3	< 0.05
	Second control	97.0 ± 31.0	52.2 ± 20.9	62.9 ± 22.3	< 0.001
Prolactin (mIU/L)	Initially	210.0 ± 93.3	228.2 ± 91.1	257.6 ± 64.3	ns
	First control	225.1 ± 98.7	260.1 ± 117.8	260.7 ± 140.9	ns
	Second control	223.0 ± 84.6	204.5 ± 73.1	242.3 ± 91.9	ns
TSH (mIU/L)	Initially	2.40 ± 0.77	2.18 ± 0.77	2.13 ± 0.70	ns
	First control	2.47 ± 0.75	2.30 ± 0.69	2.09 ± 0.75	ns
	Second control	2.43 ± 0.42	2.09 ± 0.53	1.88 ± 0.87	< 0.05

Ns – not statistically significant; initially – prior to MHT; first control – after sixth month of MHT; second control – after 2–5 years of MHT; FSH – follicle-stimulating hormone; LH – luteinizing hormone; DHEA-SO4 – dehydroepiandrosterone sulfate; SHBG – sex hormone-binding globulin; TSH – thyroid-stimulating hormone.

Discussion

In the reproductive period the serum estradiol concentration is in the range from 40 to 400 pg/mL while in menopause it is decreased to 5–20 pg/mL. The main source of estrogen in menopause is the peripheral conversion of androstenedione from the adrenal glands to estrone, owing to the activity of the aromatase complex in the fat tissue, muscles, skin and liver which does not provide sufficient estrogen, as we demonstrated in our study. Estradiol deficiency leads to a number of symptoms and signs [14], the redistribution of fat deposits and the increase in visceral deposits [15]. Menopause leads to the development of central obesity, the atherogenic lipid profile increase and increase in the prevalence of metabolic syndrome (MS) regardless of the age and other factors [16].

A significant number of papers have been published so far, suggesting that hormone therapy in the menopause reduces the risk of coronary artery disease in healthy mopausal women [17, 18].

Omodei et al. [19] reported that estradiol valerate in a dose of 2 mg, administered during the period of 1–21 days, and ciproterone acetate, given in a dose of 1 mg during the period of 12–21 days, followed by a 7-day pause, after 6 months of the therapy, have resulted in decrease of TC, LDL-C and Apo-B levels, followed by a slight increase in the levels of TG, producing cardioprotective effects.

The effect of transdermal estrogen therapy (50 μg of estradiol), in combination with progestogen (sequential administration of 5 mg medroxyprogesterone acetate – MPA). on the lipid status was examined. There was a decrease in the serum levels of TC, and LDL-C. A higher dose of estradiol (100 μg) led to an increase in HDL-C level, especially HDL2 fraction, while HDL3 one decreased [20].

An analysis of 248 studies published in the period from 1974 to 2000 gave data for 42 different MHT regimens. Regimens including only estrogens increased only HDL-C, and lowered LDL-C and TC. Oral estrogens increased the serum levels of TC. Transdermal estradiol-17-beta lowered TC levels. Progestagens had a small effect on the estrogen-induced reduction of LDL-C and TC. The estrogen-induced increase in HDL-C and TG levels was followed by different effects of progestagens depending on their type [21]. Metabolic effects of progestin added were correlated with a dose, a relative androgenic potential of hormone preparation, and a dose of estrogen. C-21 derivatives of hydroxyprogesterone (e.g., medroxyprogesterone and medrogestone) are less metabolically active than 19-nortestoterone derivatives (e.g., norethindrone and levonorgestrel) [22].

In our study, MHT (estrogen/progestagen combination), regardless of the route of administration, led to the continuous decrease of TC, LDL-C and Apo-B levels in the serum. Statistically significant reduction of TC levels were recorded only after 2–5 years from the MHT beginning, while statistically significant reduction of LDL-C and Apo-B levels was evident already after 6 months of MHT administration and it was maintained in the following 2–5 years of the therapy. The serum levels of TG had a nonsignificant tendency to decline during the period of observation, which was in accordance with results of other studies.

An increase of only 0.26 mmol/L of HDL-C leads to 42–50% of reduction in the risk for coronary disease [23]. In our study, a significant increase in HDL was observed only after 2–5 years of MHT administration. A statistically significant increase in Apo-A level was observed after 6 months of MHT administration and it was maintained during 2–5 years of the therapy. The results of our study also showed that oral administration of estroprogestagen therapy over 2–5 years led to a significant increase in HDL-C level (from 1.26 ± 0.40 mmol/L to 1.57 ± 0.31 mmol/L) while transdermal and intramuscular MHT had nonsignificant influence on the serum level of HDL-C.

Several studies showed a significant increase in Lp(a) level in menopause as well as an increased risk for CVD, which did not depend on LDL-C level [7]. Soma et al. [24] found a significant reduction in Lp(a) level in women in menopause who used 1.25 mg of conjugated equine estrogen (CEE) per day, in combination with 10 mg MPA for 10 days in a month. This result becomes more important when we highlight the fact that in some women, reduction of Lp(a) levels by the use of pharmacological agents or diet has not occurred. Bukowska et al. [25] found that after 3 months of MHT, Lp (a) level in the serum did not significantly deviate from baseline, regardless of the route of administration. MHT generally has lowered Lp(a) levels as presented in 41 studies including 20 different drug formulations [21].

In this study, a nonsignificant decrease of Lp(a) serum level was found. During MHT, independently of the route of administration, serum levels of estradiol were significantly increased whereas FSH and LH levels were significantly reduced. A significant reduction in testosterone levels was observed after 6 months and progesterone levels after 2–5 years of the MHT administration.

After oral administration, concentrations of estradiol in portal circulation have been 4–5 times higher than concentrations in systemic circulation [26]. This finding explains why estrogens are more presented in hepatocytes than in cells of other organs. Because of that, orally administered estrogens have more effects on the liver when compared to their effects after parenteral administration. Treatment with estrogens may increase the hepatic production of triglycerides, very low density lipoproteins (VLDL) and Apo-B100 secretion. Walsh et al. [26] found that 2 mg per day of micronized estradiol administrated orally increases the production of Apo-B in large VLDL particles to a much greater extent than in smaller VLDL particles.

Faith et al. [27] compared the effect of oral (2 mg/day) and transdermal (50 mcg/day, 7-day patch) estrogen substitution therapy (EST) on the lipid profile within 12 weeks in women at 49 ± 6 years of age. While TC level did not change, TG level was increased from 1.39 to 1.61 mmol/L after oral EST. An increase in HDL-C level after oral administration of EST was more significant than after transdermally administered EST. Changes in LDL levels were also significant: LDL levels were decreased after oral administration of EST, compared to a nonsignificant decrease after transdermal administration. Taking into consideration these changes in LDL levels depending on the route of EST administration as well as the fact that a physician is familiar with patient's lipid profile, a physician can initiate personalized EST.

Some studies suggest that transdermal estrogen substitution compared to the oral one has a significant influence on the rise in HDL serum level. Thus, Camilli et al. [28] have found statistically significantly higher HDL levels during transdermal therapy. Nanda et al. [29] found lower serum levels of HDL in hysterectomized women (less than 40 mg/dL in 87% women). A significant decrease in TC and LDL-C, as well as a significant increase in HDL-C levels were observed after EST (both oral and transdermal); the response to oral therapy was relatively faster. After 3 and 6 months, the number of cases with HDL-C level above 40 mg/dL, from initial 13% increased to 63% during MHT administered orally, while during transdermal MHT that increase was from 30% to 60%. Serum levels of TG decreased significantly when transdermal EST was administrated whereas their elevation was noticed when EST was given by oral route. EST, either oral or transdermal, has a beneficial effect on the serum lipid profile of women in the menopause. The oral route had higher impact on an increase in serum levels of HDL-C, while the transdermal route was better for decrease of the serum TG levels. Therefore, the transdermal route should be a therapeutic choice for women with elevated serum TG levels. Such findings, referring to TG, are in accordance with our results, which showed that longer transdermal MHT administration (over 2–5 years) helped to achieve statistically significantly lower TG levels when compared to the oral route of MHT administration. It should be noted that such results we obtained by estroprogestagen MHT while in the above mentioned study EST was administrated.

Natural progesterone does not significantly affect plasma lipoprotein levels. Synthetic progestins, especially those with evident androgenic activity (e.g., norethindrone, levonorgestrel), may have significant metabolic effects, especially when TG level reduction is concerned, although the mechanism of this effect is not quite clear [22].

Oral progestogene leads to concentrations of the hormone 10–15 times higher than those obtained after its intramuscular administration[30]. A study by Hirvonen et al. [31] found a 20% reduction in HDL-C in women who were on norethindrone and norgestrel therapy but did not find changes in women treated by MPA and natural progesterone,

which confirms the hypothesis that progestagens do not erase the effects of estrogen on lipid metabolism.

A meta-analysis of 24 selected studies showed that MHT significantly reduced Lp(a) concentrations compared to placebo. Oral administration of estrogen led to a greater reduction of Lp(a) compared to the transdermal one. There was no difference between continuous and cyclic MHT treatment, conventional therapy and low dose estrogen therapy, mono estrogen therapy and combined estrogen/progestagen therapy [32].

Meschia et al. [33] compared the effects of oral and transdermal administration of EST on the lipid status. Lp(a) level decreased after 3 months of EST administered either transdermally or orally (12% and 22%, respectively). There was no further decline after 3 months. Total cholesterol and LDL-C levels decreased significantly after 3 months of the therapy administered by both routes. There was no difference in these effects on Lp(a), LDL and TC levels depending on the route of administration. The concentrations of HDL-C and TG were increased only in the group on therapy with oral estrogens. The lowering effect was quickly achieved because the maximal effect was observed after 3 months of the therapy.

There are data indicating that transdermal patches with estrogen are safer and potentially more effective than oral estrogen therapy [34].

Our study showed that oral, transdermal and intramuscular route of estroprogestagen MHT did not differ significantly in the effects on TC, LDL-C, HDL-c, Lp(a), Apo-A and Apo-B levels in the serum after 6 months and 2–5 years from the MHT beginning. A significant increase in Apo-A and a significant decrease in Apo-B levels were observed after more than 6 months of the MHT beginning regardless of the route of administration.

Estradiol level in the serum was continuously increasing by all MHT regimens used, and no statistically significant difference was found in parameters of the lipid status among different routes of the MHT administration in the observation period. It was also confirmed that an increase of estradiol levels in the serum statistically significantly correlated with an increase of Apo-A levels and decrease of LDL-C and Apo-B levels, regardless of the MHT route of administration.

Conclusion

Estroprogestagen therapy in menopause, introduced on time, regardless of the route of administration, has beneficial effects on the lipid status of menopausal women and consequently might prevent numerous cardiovascular diseases that are the leading cause of mortality.

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Immunohistochemical expression of androgen receptors in prostate carcinoma and benign prostatic hyperplasia

Imunohistohemijska ekspresija androgenih receptora kod karcinoma prostate i benigne hiperplazije prostate

Sandra Trivunić Dajko*†, Jovo Bogdanović‡§, Aleksandra Fejsa Levakov†‖, Mirjana Živojinov*†, Matilda Djolai†‖

University of Novi Sad, Faculty of Medicine, *Department of Pathology, ‡Department of surgery, ‖Department of Histology and Embriology, Novi Sad, Serbia; Clinical Centre of Vojvodina, †Centre for Pathology and Histology, §Clinic of Urology, Novi Sad, Serbia

Abstract

Background/Aim. Prostate carcinoma (PCa) and its parent organ are influenced by hormones, which is used for therapeutic purposes. Through androgen receptors (AR) androgens influence cell growth and function, proliferation, differentiation, apoptosis, lipid metabolism and secretory activity of the prostate, as well as development and progression of PCa. An antiandrogen therapy is carried out in patients with metastatic PCa, in order to block effects of androgens. By conducting immunohistochemical analysis of androgen receptors in the PCa tissue, we can assume how the tumour will react to an administered antiandrogen therapy, both in androgen-positive and androgen-negative, resistant tumours. Knowledge of the presence of AR in the tumour tissue may serve as a prognostic indicator in histopathological analysis. The aim of this study was to evaluate the expression of AR in patients with benign prostatic hyperplasia (BPH) and in those with PCa, before therapy. **Methods.** Immunohistochemical analysis was carried out by using anti-human AR monoclonal antibody AR441 (DAKO), and presence and intensity of AR were semi-quantitatively evaluated in 195 patients, 165 with BPH and 30 with PCa. Material for analysis was obtained by needle biopsy or transurethral resection of the prostate (TURP). **Results.** All secretory cells in patients with BPH were intensively androgen positive, while in patients with PCa they were mostly moderately to highly positive, but with foci of negativity. The observed negative correlation between AR and Gleason score and the International Society of Urologic Pathology (ISUP) grade group of PCa was not statistically significant. **Conclusion.** Study results indicate that PCa, before therapy, is androgen-dependent, with a high level of AR expression.

Key words:
prostate neoplasms; receptors, androgen; prostatic hyperplasia; immunohistochemistry.

Apstrakt

Uvod/Cilj. Karcinom prostate (PCa), kao i njegov ishodišni organ se nalaze pod uticajem hormona, što je iskorišćeno u terapijske svrhe. Androgeni preko androgenih receptora (AR) utiču na ćelijski rast i funkciju, proliferaciju, diferencijaciju, apoptozu, lipidni metabolizam i sekretornu aktivnost prostate, ali i na razvoj i progresiju PCa. Antiandrogena terapija se sprovodi kod bolesnika sa metastaskim PCa, upravo sa ciljem da blokira dejstvo androgena. Imunohistohemijskom analizom AR u tkivu prostate sa karcinomom, možemo da pretpostavimo kako će tumor reagovati na datu antiandrogenu terapiju, bilo da se radi o androgen pozitivnim ili androgen negativnim, rezistentnim tumorima. Saznanja o zastupljenosti AR u tkivu tumora mogla bi poslužiti kao prognostički indikator u patohistološkoj analizi. Cilj rada je bio evaluacija ekspresije AR kod bolesnika sa benignom hiperplazijom (BHP) i kod bolesnika sa PCa, pre sprovedene terapije. **Metode.** Imunohistohemijska analiza je sprovedena uz upotrebu *anti-human* AR *monoclonal antibody* AR441 (DAKO), uz semikvantitativnu procenu prisustva i inteziteta AR kod 195 bolesnika, 165 sa BHP i 30 sa PCa. Materijal je dobijen iglenom biopsijom ili transuretralnom resekcijom prostate (TURP). **Rezultati.** Sve sekretorne ćelije kod bolesnika sa BHP su bile intezivno androgen pozitivne, dok su kod bolesnika sa PCa, mahom bile umereno do izrazito pozitivne, ali sa fokusima negativnosti. Uočena je negativna korelacija AR sa Gleason skorom i *International Society of Urologic Pathology* (ISUP) gradus grupom PCa, koja nije bila statistički značajna. **Zaključak.** Rezultati studije su pokazali da je PCa, pre sprovedene terapije, androgen zavisan sa visokim stepenom ekspresije AR.

Ključne reči:
prostata, neoplazme; receptori, androidni; prostata, hiperplazija; imunohistohemija.

Correspondence to: Sandra Trivunić Dajko, University of Novi Sad, Faculty of Medicine, Department of Pathology, Hajduk Veljkova 3, 21 000 Novi Sad, Serbia. E-mail: sandra.trivunic-dajko@mf.uns.ac.rs

Introduction

So far, there have been no findings whether accessory sex glands, such as prostate, secrete hormones, but it was proved that they are under the influence of hormones [1, 2]. Through androgen receptors (AR) in prostate tissue testicular androgens regulate vital aspects of the gland, such as: cell growth and function, proliferation, differentiation, apoptosis, lipid metabolism and secretory activity. Primary hormonal mediator of benign prostatic hyperplasia (BPH) is 5α-dihydrotestosterone (DHT). This androgen is the main intracellular metabolite of testosterone, and it is produced focally in stromal cells from the circulating testosterone, under the influence of the enzyme 5-reductase. DHT influences stromal cells autocrinally, and epithelial cells paracrinally, increasing their mitotic activity due to binding to receptors in these cells. Mitotic effect of DHT is about ten times stronger than the same effect of testosterone. In addition to DHT, other factors can also influence the mitotic activity in the prostate, such as the concentration of estradiol. The effect of estradiol is based on the increase in the number of nuclear receptors for DHT in prostate cells [3].

Benign prostatic hyperplasia is the most common disease of this gland in men, and PCa is one of the most diagnosed malignancies and the second leading cause of death among men in industrialised countries. The development and progression of PCa, as well as its parent tissue, depend on testosterone and dihydrotestosterone. Back in 1941, Huggins and Hodges [4] stated the assumption that PCa is under hormonal influence of androgens.

Modern approach to PCa therapy is carried out according to the indications for each stage of the disease separately (monitoring, curative treatment and hormonal therapy) [5, 6]. The endocrine, hormone therapy is used to cure metastatic carcinoma. It acts adjuvantly with a goal to inhibit stimulatory actions of androgens on PCa cells. This can also be achieved by surgical or pharmacological castration. Administration of gonadotropin-releasing hormone (LH-RH) agonists and/or antiandrogen leads to a pharmacological blockade [7].

Prostate carcinoma therapy is preceded by its diagnostics, wherein a pathologist has the final decision. The gold standard for the histopathological diagnosis of PCa is prostate biopsy, as well as the analysis of the prostatic tissue after transurethral resection of the prostate (TURP) and prostatectomy.

Using immunohistochemical determination of AR in patients with PCa we wish to morphologically substantiate the claims that the majority of tumours is androgen-dependent from the beginning, and that the initial antiandrogen therapy is purposeful. Over time, therapies create clones of androgen resistant cells, which leads to the resistance of the tumour to the androgen blockade, which prospectively, could morphologically and immunohistochemically be proven by the analysis of the material gained by TURP or prostate biopsy, of course only in patients who did not undergo prostatectomy. This claim has also been presented by many other authors [8–10].

Histopathological analysis after immunohistochemical staining (IHC) has revealed that AR are intranuclearly located, and their determination could prospectively serve as a prognostic indicator for patients with metastatic PCa [11–14].

Methods

The study was prospective and retrospective, and was carried out in the Centre for Pathology and Histology of the Clinical Centre of Vojvodina in Novi Sad, Republic of Serbia, wherein the materials of 195 male patients were histopathologically analyzed, after being obtained by transrectal needle biopsies of the prostate tissue and TURP at the Clinic for Urology. The materials were fixed in 4% formalin, and then they were embedded in paraffin blocks, cut and stained in a standard way, with hematoxylin-eosin (HE), and analyzed immunohistochemically for androgen receptor antibodies (DAKO).

Using histological analysis patients were divided into two groups: an experimental group with histopathologically diagnosed PCa (30 patients) and the control group with histopathologically diagnosed BPH (165 patients).

After immunohistochemical staining, AR of secretory cells were semi-quantitatively evaluated. Negatively stained nuclei were marked with a zero (0), and positively stained nuclei with a plus (+). The intensity of nuclei staining was also evaluated as follows: light staining (+), moderate staining (++), and pronounced nuclei staining (+++).

In addition to the immunohistochemical analysis of AR, age and prostate specific antigen (PSA) levels were analyzed in both groups of patients, with additional analysis of the Gleason score and the International Society of Urological Pathology (ISUP) grade group in patients with PCa.

Results

Mean age of all 195 patients in both groups was 69.26 ± 0.46 years (median 69 years), with the oldest being 89, and the youngest 51 years old. Mean age of patients with PCa was 68.97 ± 1.44 years, with the oldest being 81, and the youngest 51 years old. Most patients were in the seventh decade of life. Mean age of patients with BPH was 69.3 ± 0.4 years, with the oldest being 89, and the youngest 53 years old. Most patients were in the sixth decade of life. Difference in patients' age comparing the experimental and the control group was not wss not statistically significant.

Mean serum PSA level in all 195 patients was 15.97 ± 1.58 ng/mL, the lowest measured value being 1.62 ng/mL, the highest 115 ng/mL, and the most frequent one was 11.15 ng/mL. Mean value of PSA levels in PCa patients was 21.64 ± 4.5 ng/mL. The lowest measured value was 5.8 ng/mL, and the highest one 115 ng/mL. Mean value of PSA levels in BPH patients was 14.16 ± 1.46 ng/mL. The lowest measured value was 1.62 ng/mL, and the highest one 110 ng/mL. There was no statistically significant difference between PSA levels in the two study groups.

Table 1 shows the distribution of patients according to the semi-quantitatively estimated values of AR in PCa, compared to the number of patients. Using the semi-quantitative analysis of androgen receptors, tumours were given 2.7 ± 0.1 pluses on average, with the minimum of two, maximum of three, and most frequently three pluses. Most carcinoma patients (21 people or 70%) have the value of AR (+++), and the remaining patients (9 people or 30%) the value of AR (++). There were no patients whose tumour AR were semi-quantitatively graded with (+) or zero (Figures 1a–f).

Table 1
Distribution of patients with prostate carcinoma (PCa) according to the semi-quantitatively determined representation of androgen receptors (AR) in the PCa tissue

Semi-quantitative evaluation of androgen receptors	Patients, n (%)
0	0 (0)
+	0 (0)
++	9 (30)
+++	21 (70)
Total	30 (100)

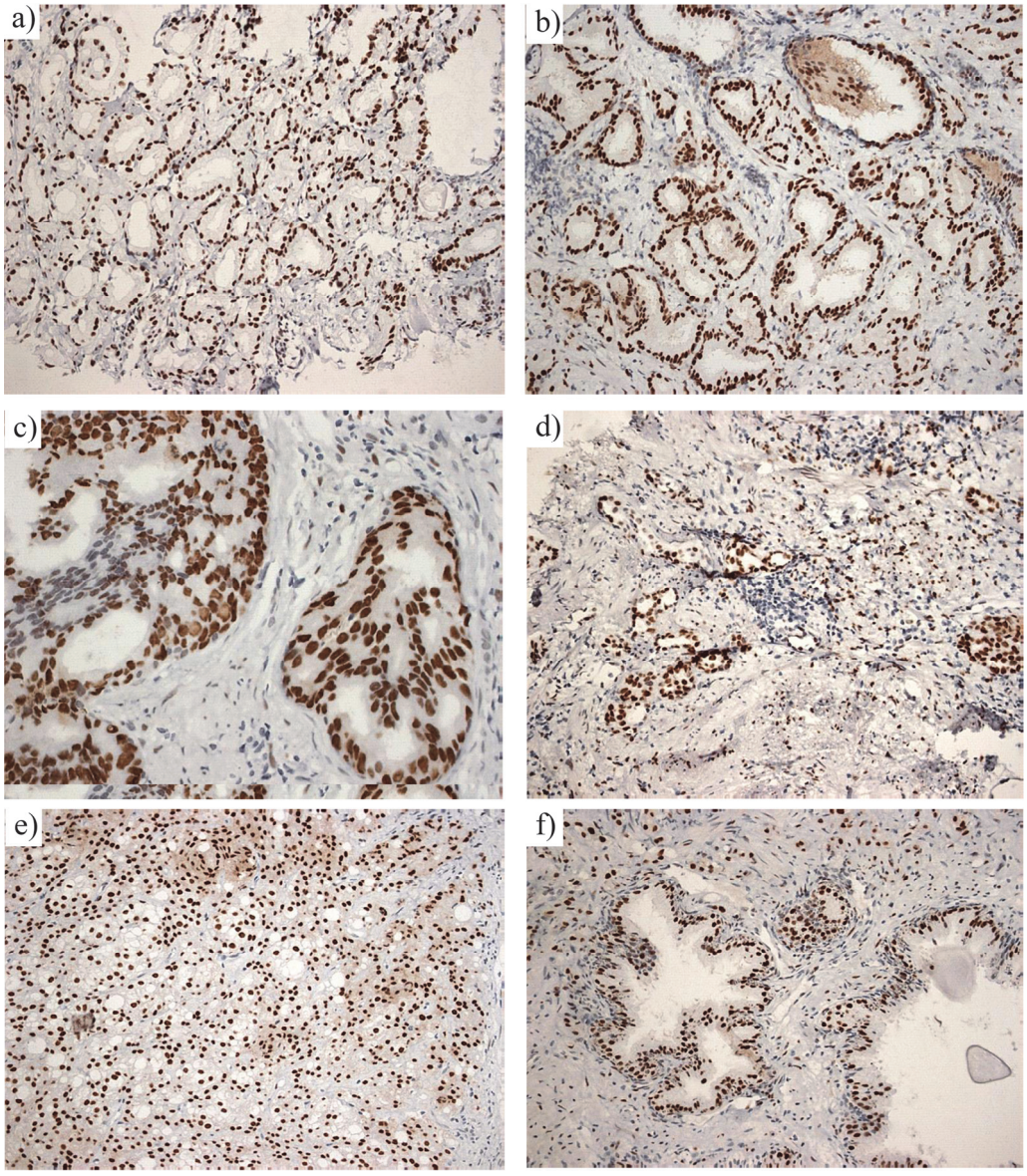

Fig. 1 – Immunohistochemical staining for androgen receptors in the prostate tissue: a) Androgen positive nuclei of prostate adenocarcinoma tumour cells (+++), the Gleason grade 2 (×20); b) Androgen positive nuclei of prostate adenocarcinoma tumour cells, the Gleason grade 3 (+++) and androgen negative nuclei of *PIN basal cell layer* (×20); c) Androgen positive and androgen negative nuclei of prostate adenocarcinoma tumour cells, the Gleason grade 3 (++), (×40); d) Androgen positive and androgen negative nuclei of prostate adenocarcinoma tumour cells, the Gleason grade 4 (++) (×20); e) Androgen positive nuclei of prostate adenocarcinoma tumour cells, the Gleason grade 5 (+++) (×20); f) Androgen negative nuclei of basal cell layer and androgen positive nuclei of secretory cells of the prostate gland with benign prostatic hyperplasia (×20).

By comparing the semi-quantitatively estimated AR with the Gleason score and the ISUP grade group it could be noticed that with the increase of the Gleason score, tumour dedifferentiation, the number of nuclei with positive AR in carcinoma tissue decreased. The correlation existed, it was negative, but slight (-0.125). By comparing the semi-quantitatively estimated AR and the ISUP grade group it could also be noticed that with an increase in histological grade, tumour dedifferentiation, the number of AR+ nuclei in carcinoma decreased. The correlation existed, it was negative, but slight (-0.16). Intensity of nuclei staining for AR was identical in all tumours being (+++).

The semi-quantitative evaluation of AR in patients with BPH (the control group) showed that all nuclei of glandular epithelium secretory cells were AR positive (+++). Basal cell nuclei were AR negative (0) (Figure 1f).

Discussion

Benign prostatic hyperplasia is the most common prostate gland disease [15], as evidenced by our study with 30 (15.38%) individuals having carcinoma, and 165 (84.62%) having BPH.

Mean age of people with carcinoma in our study was 68.97 ± 1.43 years, with the youngest being 51, and the oldest 81 years old, and most patients (14 patients or 46.66%) were in the seventh decade of life. Our results are consistent with the literature, and they indicate that PCa is a disease of men older than 50 years and that only 1% of these tumours are diagnosed in people under 50, and that their incidence reach the peak around the age of 75 [15].

All 30 patients of the experimental group (with PCa) had a histomorphological diagnosis of acinar adenocarcinoma, with no other types of PCa detected, as was expected, considering it accounts for over 90% of all histological types of PCa [16, 17].

The semi-quantitaive evaluation of AR in carcinoma patients evaluated tumours with 2.7 ± 0.1 pluses on average, with the minimum of (++) and maximum of (+++), most often (+++). More than two thirds of patients with carcinoma (21 people or 70%) were evaluated as AR (+++), and about one third (9 people or 30%) as AR (++). Among the patients there were no those whose AR were semi-quantitatively evaluated with (+) or (0). The results obtained are in accordance with literature data that the majority of prostate adenocarcinomas has positive AR [18–20].

Results of correlation of semi-quantitatively evaluated AR with the Gleason score were negative, with negligible correlation coefficient (-0.125). Approximate values were obtained by correlating the ISUP grade group and semi-quantitatively evaluated AR (-0.16). It can be argued that in certain number of carcinoma the increase in the Gleason score and the grade group causes the decrease in the number of nuclei with positive AR. The more dedifferentiated tumour, the more likely it will have androgen-resistant cells. However, it should not be left out that certain tumours of the same grade had differently evaluated AR, meaning that only morphology (hematoxylin-eosin staining) fails to show the precise extent and intensity of nuclei positive for AR. Our results correlate with the results of other authors, who claim that carcinomas with low scores do not have a significantly higher content of AR than those with high Gleason score. On the other side, there are the authors who claim otherwise, but one cannot exclude studies that have not determined the existence of correlation between the Gleason score and AR representation in the PCa tissue [21–30].

Conclusion

All analysed tumours were androgen sensitive (+++ or ++).

Prostate carcinomas of the same Gleason score or ISUP grade group had different degree of AR presence, from which follows that based only on histomorphological appearance of carcinoma, its ISUP grade group or the Gleason score on HE staining, the extent and intensity of nuclei positive for AR cannot be precisely determined.

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Combined spinal-epidural anesthesia in a patient with spinal muscular atrophy type II undergoing a cesarean section: A case report

Kombinovana spinalna-epiduralna anestezija za carski rez kod porodilje sa spinalnom mišićnom atrofijom tip II

Slavica Krušić*, Branka Nikolić*†, Rastko Maglić*†

***Obstetrics and Gynecology Clinic "Narodni front" Belgrade, Serbia;**
University of Belgrade, †Faculty of Medicine, Belgrade, Serbia

Abstract

Introduction. Anesthetic management of a patient with spinal muscular atrophy type II, who underwent elective cesarean section with neuraxial anesthesia is presented in this case report. **Case report.** A 33-year old woman with first pregnancy and no previous birth, at 39 weeks gestational age was scheduled for a cesarean section due to placenta *previa*. She had a history of spinal muscular atrophy type II, that confined her to a wheelchair, and a surgical history that included corrective surgery for kyphoscoliosis. The patient had predictors for a difficult intubation (limited mouth opening and reduced neck extension) so the decision was made to attempt the needle-through-needle combined spinal-epidural technique for surgical anesthesia. Harrington rods and scar tissue complicated placement of the combined spinal-epidural anesthesia, however successful placement was achieved. **Conclusion.** Spinal muscular atrophy in pregnancy is rare and represents big challenge for an anesthesiologist due to respiratory dysfunction, anticipated difficult intubation, severe kyphoscoliosis and limitations of the use neuromuscular blocking agents. The potential risks need to be considered when administering anesthesia in patients with spinal muscular atrophy undergoing a cesarean section.

Key words:
labor; cesarean section; myotonic disorders; anesthesia, epidural; anesthesia, spinal.

Apstrakt

Uvod. U ovom slučaju prikazano je vođenje anestezije kod porodilje sa spinalnom mišićnom atrofijom tip II, kod koje je urađen elektivan carski rez u neuroaksijalnoj anesteziji. **Prikaz slučaja.** Žena, stara 33 godine, kojoj je ovo bila prva trudnoća, u 39-oj nedelji gestacije bila je planirana za carski rez zbog placente previje. U anamnezi je imala spinalnu mišićnu atrofiju tip II, korektivnu operaciju kifoskolioze, i bila je vezana za invalidska kolica. Odluka da se radi u kombinovanoj spinalno-epiduralnoj anesteziji donešena je zbog prisustva prediktora za otežanu intubaciju (ograničeno otvaranje usta, ograničena pokretljivost vratne kičme). Haringtonove šipke i ožiljno tkivo komplikovali su primenu kombinovane spinalno-epiduralne anestezije, ali je anestezija ipak uspešno primenjena. **Zaključak.** Spinalna mišićna atrofija u trudnoći veoma je retka i predstavlja veliki izazov za anesteziologa zbog respiratorne disfunkcije, očekivane otežane intubacije, teške kifoskolioze i ograničenja u primeni neuromišićnih relaksanata. U radu su prikazani potencijalni rizici koje treba uzeti u obzir prilikom primene anestezije za carski rez kod porodilja sa spinalnom mišićnom atrofijom.

Ključne reči:
porođaj; carski rez; distrofija, miotonička; anestezija, epiduralna; anestezija, spinalna.

Introduction

Spinal muscular atrophy (SMA) was first described by Austrian and German neurologists, Werding and Hoffman in the 19th century [1]. SMA is a neuromuscular disease that is accompanied by degeneration of alpha motor neurons of the spinal cord, resulting in progressive proximal muscle weakness and paralysis [1]. Disruption of the survival motor neuron 1 gene on chromosome V causes SMA in about 95% of the patients [1]. The incidence of SMA is 1 : 6,000 to 1 : 10,000 live births [2]. There are four types of SMA dependent on age of onset and severity of clinical features (Table 1) [3]. Diagnosis is confirmed using molecular genetic analysis, electromyography and muscle biopsy. There is yet no cure for the condition [4]. Pregnancy in women with SMA is rare and management can be challenging for obstetric and anesthesiology teams involved [5].

Correspondence to: Slavica Krušić, Obstetrics and Gynecology Clinic "Narodni front" Belgrade, Kraljice Natalije 72/15, 11 000 Beograd, Serbia. E-mail: vicesla@gmail.com

Table 1

Classification and clinical characteristics of spinal muscular atrophy [3]

Types	Age of onset	Clinical features	Average survival
Acute infantile form (Werding–Hoffman disease) – type I	< 6 months	Severe muscle weakness, hypotonia, bulbar dysfunction, spinal deformities, respiratory failure	Bad (< 2 years)
Chronic infantile form (intermediate) – type II	6–18 months	Moderate muscular weakness, susceptibility to respiratory infections, spinal deformities, supportive sit but never stand	Middle (10–40 years)
Chronic juvenile form (mild, Kugelberg–Welander disease) – type III	> 18 months	Mild to moderate muscular weakness, mild restrictive lung disease, may or may not have spinal deformities, walk during adulthood	Normal life span
IV (adult)	> 30 years	Mild muscular weakness, mild weakness in arms and legs, walk unaided	Normal life span

There is no description in literature on the effects of SMA on uterine musculature, but since the uterus is autonomically innervated, it is anticipated to have a normal contraction strength and pattern result in a vaginal delivery 6. However, labor may not be effective and majority of patients with SMA are delivered via cesarean section 7, 8. Hereby we presented a case of an anesthetic management of a patient with SMA type II who underwent elective cesarean section with neuraxial anesthesia.

Case report

A 33-year-old woman, G1-parity-P0, at 39 weeks of gestational age was scheduled for a cesarean section due to central placenta *previa*. She had a history of SMA type II, the diagnosis which was based on electromyographic reports and clinical progression of symptoms. Her symptoms included muscle weakness that began when she was 11 months old, when she presented as a "limp baby". The patient's motor development was slow and she started to walk when she was 3 years old, always aided. At the age of 5–6 years, she was diagnosed with kyphoscoliosis and this was presumed to be a result of the disease progression involving muscle weakness of the trunk and extremities. When she was 9 years old, she underwent surgical correction of kyphoscoliosis with insertion of Harrington rods. She remained wheelchair-bound following the surgery due to muscle weakness. The patient had never undergone a muscle biopsy or had any genetic testing performed and there was no family history of neuromuscular disease. During her pregnancy she did not describe any deterioration in her symptoms or signs of SMA.

Preoperative biochemical, hematologic and blood gas analysis were within normal limits. Her body mass index was 25 kg/m^2 (weight 68 kg, height 1.65 m). The patient had predictors for a difficult intubation, which included: Mallampati class 4 (Figure 1); reduced mouth opening to 10 mm; thyromental distance < 6 cm; and reduced neck flexion (Figure 2). She did not describe any bulbar muscle weakness, however, she was prone to respiratory infections prior to pregnancy (the patient was a smoker). The patient was the American Society of Anesthesiologists (ASA) class 3. The decision was made to proceed with a scheduled cesarean section and the needle-through-needle combined spinal-epidural (CSE) anesthesia technique was chosen as the safest and most appropriate mode of anesthesia due to predictors of difficult intubation. Preoperative vital signs were within normal limits (oxygen saturation 98% on air, respiratory rate 15/min, heart rate 88/min, blood pressure 125/70 mm Hg).

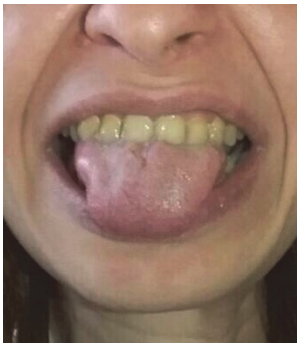

Fig. 1 – Predictors of a difficult airway with our patient: Mallampati class 4.

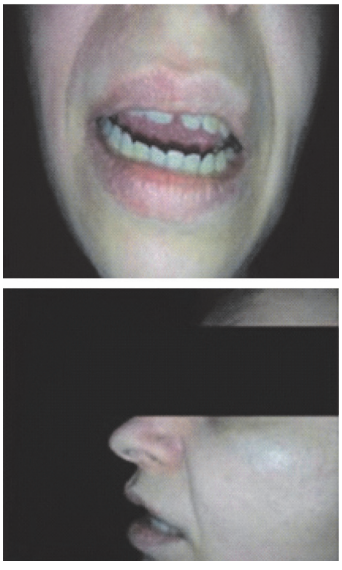

Fig. 2 – Predictors of a difficult airway with our patient: reduced mouth opening.

Preoperatively, two large-bore peripheral intravenous (IV) cannulas (16 G) were placed and the patient received ranitidine 50 mg i.v., ondansetron 4 mg i.v., and dexa-

methasone 4 mg i.v. A CSE procedure was performed in the sitting position at the L3/4 interspace using a loss of resistance technique with saline [Perican® 18G Tuohy needle, Pencan® 27 G spinal needle, Perifix® 20 G nylon epidural catheter, Espocan® docking system (B. Braun Medical Inc. Melsungen, Germany)]. Placement of the CSE took three attempts due to presence of scar tissue and Harrington rods (Figure 3). Depth of the epidural space from skin was 4 cm. Intrathecal drug administration consisted of 0.5% isobaric bupivacaine 12 mg and fentanyl 25 μg. Sensory block height to dermatomal level T4 was confirmed bilaterally prior to commencement of surgery. The patient was positioned supine with left uterine displacement. The patient's cardiovascular status remained stable throughout surgery, and the surgery proceeded without any complications (duration 55 minutes). A healthy female infant was delivered: weight 2,850 g; length 47 cm; head circumference 33 cm. Apgar score at 1 minute and 5 minutes was 10/10. Intraoperative fluids consisted of Ringer's lactate solution 1,500 mL and hydroxyethyl starch 500 mL, and estimated blood loss was 500 mL. The patient was transferred to the intensive care unit postoperatively for monitoring. Postoperative analgesia included intermittent 4 mL boluses *via* the epidural catheter (0.25% bupivacaine + fentanyl 5μg/mL) and diclofenac 75 mg intramuscularly as required [if the Visual Analogue Scale (VAS) ≥ 3/10]. On the postoperative day 1 the patient received 5 × 4 mL boluses *via* the epidural catheter in addition to two doses of diclofenac. Pain control was deemed satisfactory and her VAS never exceeded 3/10. There were no reported incidences of nausea, vomiting, pruritis, or urinary retention and there was no evidence of disease progression. The patient had a delayed discharge (on the postoperative day 8) due to physiological jaundice in the neonate. At 3 years of age the child did not exhibit any symptoms or signs of the neuromuscular disease.

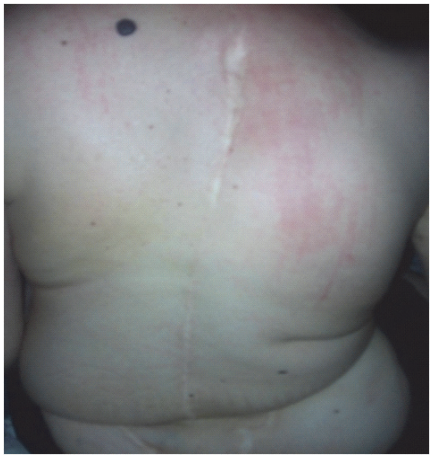

Fig 3. – Post-corrective surgery for thoracolumbar kyphoscoliosis of our patient.

Discussion

There are rare descriptions of anesthetic management of a patient with SMA type II with predictors for a difficult intubation. In this case the various anesthetic options for labor analgesia and surgical anesthesia and the risks and benefits of each technique were considered within the multidisciplinary team as well as with the patient, to optimize the safest and best outcome for the patient and her infant. There is limited literature describing anesthetic management of a pregnant women with SMA and both regional blocks and general anesthesia have been used [7]. However, Bollag et al. [6] present a literature review of 18 case reports describing anesthetic management of patients with SMA. There were 12/18 patients delivered *via* scheduled cesarean section: 7/12 patients received general anesthesia (GA), 4/7 were due to failed neuraxial anesthesia; 4/12 patients received neuraxial anesthesia, 1/4 required a secondary neuraxial technique; 1/12 patients received local anesthetic infiltration and i.v. sedation; 2/18 patients delivered *via* non-scheduled cesarean section and 4/18 patients had vaginal deliveries [6]. In case series of 12 patients with SMA, who delivered a total of 17 infants, obstetric complications were reported in 13/17 (76%) deliveries, and included: premature labor (6/17 deliveries) and preterm delivery (2/17 deliveries); prolonged labor (4/17 deliveries); and prolonged recovery postpartum (6/12 women), cesarean section (3/12). An exacerbation of muscle weakness after the second trimester was noted in 8/12 (67%) of the women, with lasting disability in 5/12 (40%) of the women [9].

There are several case reports in the literature describing successful placement of neuraxial anesthesia, which include single-shot spinals, spinal catheters and CSE anesthesia techniques [10–12]. The presented patient had limited mouth opening and reduced neck extension, so the decision was made to attempt the needle-through-needle CSE technique for surgical anesthesia. Harrington rods and scar tissue complicated placement of the CSE anesthesia, however successful placement was achieved. Neuroaxial anesthesia can be technically difficult. Epidural anesthesia may fail due to unpredictable inadequate spread of local anesthetics, particularly if there had been severe scoliosis and corrective back surgery using Harrington rods, due to scar tissue, and if you use an epidural catheter or continuous spinal catheter allows careful titration to achieve the desired dermatome level [8]. Positioning a wheelchair-bound patient and defining landmarks can be difficult, therefore it may be beneficial to use ultrasound-guidance prior to attempting placement of neuraxial anesthesia [6]. Reported doses administered for spinal anesthesia range from 7.5–14 mg hyperbaric bupivacaine, fentanyl 15–25 μg, and morphine 0.1 mg [3, 8, 11]. Other options reported for cesarean section include local anesthetic infiltration (0.5% lidocaine 100 mL) supplemented with i.v. sedation (midazolam, morphine and propofol) and occasionally with the addition of oxygen/nitrous oxide, but this technique will not provide a block as dense as with a neuraxial technique [6, 13]. Ilioinguinal, iliohypogastric and transversus abdominis plane nerve blocks have been used as options for intra- and post-operative pain control [5, 14].

General anesthesia in patients with SMA was complicated by underlying restrictive lung disease (RLD), sensitivity to nondepolarizing muscle relaxants, potential for hyperkalemia with succinylcholine and likelihood of difficult intubation [15]. It may be necessary to perform an awake fiberoptic intubation

(FOI) if the patient has predictors for a difficult airway [2, 16]. Dexmedetomidine has been administered for sedation during an awake FOI [17]. In patients with SMA there are no contra-indications to standard agents for induction of anesthesia and volatile anesthetics for maintenance of anesthesia [5]. The administration of a depolarizing muscle relaxant agent (e.g. succinylcholine) is contraindicated due to chronic denervation that can lead to rhabdomyolysis and severe hyperkalemia [2, 5, 18]. Nondepolarizing muscle relaxant (NDMR) agents can safely be administered and reversed using neostigmine [4], however, patients with SMA are sensitive to NDMR drugs and therefore a reduced dose should be administered with close monitoring followed by complete reversal [2]. Some authors recommend avoidance of neuromuscular blockade in patients with SMA, there by intubating without blockade, especially in the presence of preoperative respiratory disorders [5]. The use of sugammadex in a patient with SMA undergoing a cesarean section with GA has not been described in the literature, however it has successfully been used in patient with SMA undergoing GA for laparoscopic cholecystectomy [19]. Severe RLD is often present in patients with SMA, and with the additional stress from physiological changes of pregnancy, pulmonary function can be worsen during pregnancy but may improve post-delivery [9]. However, postoperative ventilatory support in the intensive care unit may be necessary due to respiratory weakness [6, 18]. Intra- and postoperative analgesia can be achieved using multimodal analgesia regimens, including opioids [3, 7, 20].

Based on carefully estimation of overall complex medical conditions and the anticipated difficult intubation in this case, the patient-tailored approach to delivery was made and the needle-through-needle CSE anesthesia technique was chosen.

There are limitations in the consideration of the presented clinacal experience related to anesthetic management of patients with SMA type II undergoing cesarean section, because no generalisations was possible since it was a case study. Thus, further studies and analyses of various options of anesthetic management are needed to provide the evidence of the appropriate choice for safe delivery.

Conclusion

In summary, it is highly recommended that pregnant patients with SMA as high-risk patients have a multidisciplinary team approach to plan for a safe delivery, due to complexities of these cases. There are various anesthetic options for labor analgesia and surgical anesthesia, so the risks and benefits of each technique should be discussed within the highly skilled multidisciplinary team as well as with the patient, to optimize the safest and best outcome for the patient and her infant.

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Visceral leishmaniasis in a patient with ulcerative colitis – A case report

Visceralna lajšmanioza kod bolesnice sa ulceroznim kolitisom

Goran Janković*†, Lena Martinović*†, Zorica Dakić†‡, Dragana Mijač*†, Miloš Štulić*†, Miodrag Krstić*†

Clinical Center of Serbia, *Clinic for Gastroenterology and Hepatology, Belgrade, Serbia; University of Belgrade, †Faculty of Medicine, ‡Department of Microbiology, Belgrade, Serbia

Abstract

Introduction. There is a rise of visceral leishmaniasis in immunocompromised patients due to increased availability of immunomodulatory drugs. In order to point at the occurrence of visceral leishmaniasis in patients with inflammatory bowel disease (IBD), we reported a case of female patient with a travel history to European Mediterranean countries, who was on immunosuppressive treatment due to ulcerative colitis. **Case report.** A 29-year-old female patient was admitted to hospital due to severe relapse of ulcerative colitis. Corticosteroid therapy was administered in addition to previous longterm azathioprine, with clinical response to the treatment. During the course of the disease she had recurrent high-grade fever with marked hepatosplenomegaly and pancytopenia. The diagnosis of leishmaniasis was established by positive serology tests and microscopic finding of amastigotes in bone marrow smears. The disseminated infection was responsive to treatment with liposomal amphotericin B, but therapy had to be discontinued due to urticarial rush. Subsequent therapy with antimony was administered, but it had to be stopped too due to liver toxicity. No further treatment for leishmaniasis was initiated as the clinical and laboratory data suggested that the patient had responded to the treatment. She was discharged from hospital in IBD remission and without signs of the infection. **Conclusion.** Visceral leishmaniasis should be considered in IBD patients with fever of unknown origin and relevant travel history in order to achieve favorable disease outcome.

Key words:
colitis, ulcerative; diagnosis; immunosuppressive agents; leishmaniasis; risk assessment; serology.

Apstrakt

Uvod. Visceralna lajšmanioza je u porastu kod imunokompromitovanih bolesnika zbog povećane dostupnosti imunomodulatornih lekova. Da bi ukazali na mogućnost postojanja visceralne lajšmanioze kod bolesnika sa zapaljenskom bolesti creva, prikazali smo bolesnicu sa ulceroznim kolitisom lečenu imunosupresivnom terapijom, koja je prethodno boravila u evropskim, mediteranskim zemljama. **Prikaz bolesnika.** Bolesnica, starosti 29 godina, primljena je u bolnicu sa teškim relapsom ulceroznog kolitisa. Pored dugogodišnje terapije azatioprinom primenjeno je i lečenje kortikosteroidima na koje je dobijen klinički odgovor. U toku lečenja bolesnica je bila visokofebrilna sa izraženom hepatosplenomegalijom i pancitopenijom. Dijagnoza lajšmanioze postavljena je serološkim testovima i mikroskopskim nalazom amastigota u sternalnom punktatu. Na terapiju lipozomalnim amfotericinom B dobijen je povoljan odgovor, ali je lečenje moralo biti prekinuto zbog generalizovane urtikarije. Potom je primenjeno lečenje preparatom petovalentnog antimona, ali je i ono moralo biti prekinuto zbog hepatotoksičnosti. S obzirom na to da je kod bolesnice već dobijen terapijski odgovor, dalje lečenje lajšmanioze nije primenjivano. Na otpustu iz bolnice ulcerozni kolitis je bio u remisiji i nije bilo znakova lajšmanioze. **Zaključak.** Kod bolesnika sa zapaljenjskom bolesti creva i febrilnošću nejasnog uzroka, koji su prethodno putovali u endemske krajeve, treba razmotriti i postojanje visceralne lajšmanioze u cilju postizanja povoljnog ishoda bolesti.

Ključne reči:
kolitis, ulcerativni; dijagnoza; imunosupresivi; lajšmanioza; rizik, procena; serologija.

Introduction

Leishmaniasis is an infectious disease caused by protozoan parasites of the genus Leishmania predominantly transmitted via the bite of an infected phlebotomine sand fly [1]. The most severe form is visceral leishmaniasis (VL) (kala-azar) where some of the internal organs of the body such as bone marrow, liver, spleen, etc. are affected. The

Correspondence to: Goran Janković, Clinical Center of Serbia, Clinic for Gastroenterology and Hepatology, Koste Todorovića 2, 11 000 Belgrade, Serbia. E-mail: goran.jankovic.beograd@gmail.com

global rise of VL cases is due to increasing numbers of immunosuppressed patients who have a history of travel to endemic countries [2]. Without adequate therapy severe cases of VL usually have unfavorable outcomes [1]. Thus, it is important for clinicians to be aware of this rare and potentially fatal disease [3]. Ulcerative colitis (UC) is not a rare disease [4], but reports of VL in patients with UC are scarce [5, 6]. We presented a case of a patient with inflammatory bowel disease (IBD) in whom VL occurred.

Case report

A 29-year old woman with 8-year history of UC and primary sclerosing cholangitis was admitted to the Clinic for Gastroenterology and Hepatology, Clinical Center of Serbia in Belgrade with frequent bloody stools, high-grade fever, abdominal pain, anorexia, fatigue and weight loss, that occurred for several days before hospitalization. Due to extensive, corticosteroid dependent UC she was treated with azathioprine 2 mg/kg/24 h for several years. Three months prior to hospitalization she had traveled on vacation to Montenegro sea coast and Greece (region of Athens) for 3 weeks. On physical examination she was undernourished (37 kg), Mayo score was 8, and she had a fever (38.3°C). Laboratory tests showed high C reactive protein level [44.3 mg/L (normal levels are bellow 3.0 mg/L)], high erythrocyte sedimentation rate (ESR) [78 mm/h (normal range under 20 mm/h)], mild elevation of alkaline phosphatase [167 IU/L (normal range 37–116 U/L)], and low serum albumin concentration [26 g/L (normal range 35–55 g/L)]. Immunological analyses showed elevation IgG [18.7 g/L (normal range 7–16 g/L)] and positive pANCA 1 : 256 (reference range < 1 : 40 titer). The stool culture and microscopy on enteric pathogens were negative. Urgent flexible rectosigmoidoscopy confirmed the presence of active colitis with continuously inflamed mucosa, complete loss of vascular pattern, granular appearance, friability and multiple erosions. Gastroscopy revealed mild chronic gastritis and reflux oesophagitis grade A. Chest x-ray at admission was normal. A 40 mg dose of prednisolone was administered with subsequent disease activity response. Due to recurrent fever chest X-ray was repeated after two weeks of hospitalization. The result showed round infiltrate in left hilar zone, and treatment with ceftriaxon and ciprofloxacin was administered. High fever subsided and computed tomography (CT) chest scan revealed regression of inflammation. However, after 3 days high fever recurred (39.5°C) and progressive hepatosplenomegaly (spleen 30 cm on CT scan) and pancytopenia [hemoglobin 78 g/L (normal range, 115–165 g/L), white blood count (WBC) 1.0×10^9/L (normal range 4–11 $\times 10^9$/L), platelets 40×10^{12}/L (normal range 150–450 $\times 10^9$/L)] were observed. Blood, sputum and urine cultures on several occasions were negative, as well as angiotensin-converting enyzme (ACE), hepatitis B surface antigens (HBsAg), anti-hepatitis C virus (HCV), polymerase chain reaction (PCR) Koch's bacillus (KB), skin tuberculin test and sputum KB analyses. Antibodies to Epstein-Barr virus (EBV), *Pneumocystis* pneumonia (PCP), cytomegalovirus (CMV), HIV, and *Mycoplasma pneumonia* were also negative. Ultrasound examination of the heart was normal. Ultrasound of thyroid gland showed two nodal changes in the left lobe that had benign characteristics, and thyroid hormone levels were within normal range. Doppler ultrasonography of the portal system showed no presence of thrombotic masses. Bone marrow (BM) aspiration demonstrated mildly hypercellular smears without the presence of parasites. In search for the fever etiology, leishmanial serology for determination of specific antibodies in the serum was proposed. Both the qualitative rapid dipstick rK39 test and the quantitative indirect hemagglutination assay were positive (a titer of 1 : 128). Because of positive leishmanial serology and negative reevaluation for parasites in previous bone marrow smears, BM aspiration was repeated. Direct microscopic examination of Giemsa-stained BM smears revealed only a few amastigotes of *Leishmania* spp. which were released from destroyed macrophages in the extracellular area (Figure 1).

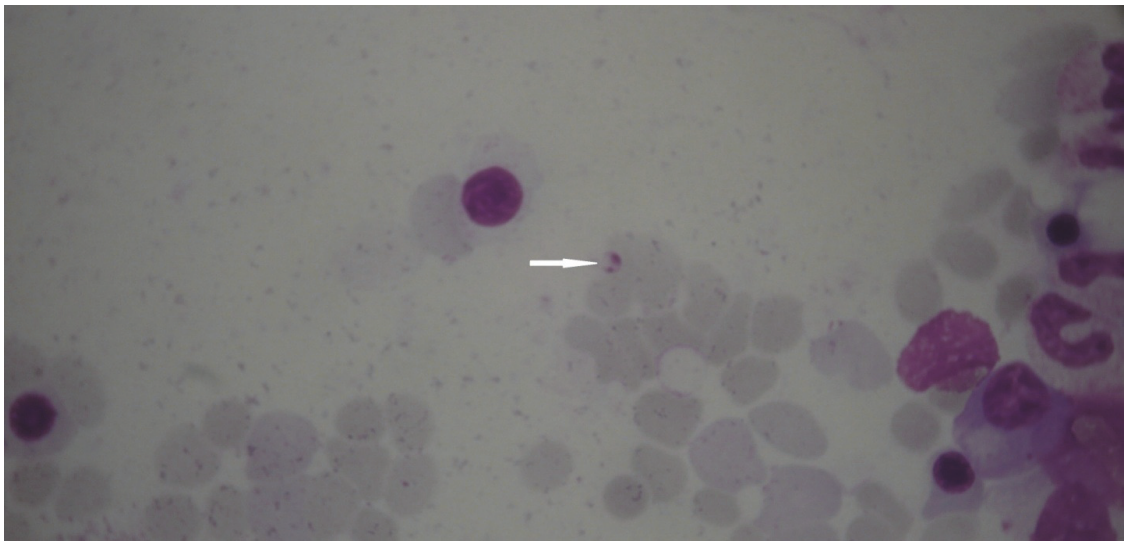

Fig. 1 – *Leishmania* sp. amastigote in the extracellular area (arrow) in Giemsa-stained bone marrow aspiration smear under oil immersion (× 1000).

Treatment with lyophilized amphotericin B in a dose 3 mg/kg was administered and fever disappeared after four days of the therapy, while inflammatory markers decreased. However, on the 5th day of the therapy urticarial rush developed so the treatment with amphotericin was discontinued. This therapy was considered too short for complete treatment, so another therapy with pentavalent antimony was started. However, on the 7th day of antimonial therapy, elevated serum transaminase levels [aspartate aminotransferase (AST) 203 IU/L (normal ranage 8–40 U/L), alanine aminotransferase (ALT) 76 IU/L (normal ranage 19–25 U/L)], as well as alkaline phosphatase [238 IU/L (normal ranage 37–116 U/L)], and gamma glutamyl transferase (γGT) (238 IU/L) were observed and the therapy was stopped. During the next several days laboratory findings continued to improve and returned to normal values. At discharge from hospital the patient was afebrile and UC was in clinical and laboratory remission, which was maintained at follow-up examinations during the following year.

Discussion

Immunosuppression is an established risk factor for VL [2, 7, 8]. The immunology and pathogenesis of leishmaniasis are complex [9]. Immunosuppressive conditions that predispose patients to VL can arise from many different causes; the exact mechanisms are not perfectly understood [8]. The rise of VL in immunocompromised patients due to increased availability of immunomodulatory and immune-ablative drugs offers new clinical challenges [10]. Patients previously treated with more than two immunosuppressive drugs are at particular risk for opportunistic infections [11]. Opportunistic infections have been increasingly reported in anti-tumor necrosis factor (TNF)-treated patients [12], but publications on Leishmania infections in patients treated with TNF inhibitors are still not frequent [11, 13, 14]. In the presented case VL occurred in an immunocompromised malnourished patient with severe relapse of autoimmune disease treated with two immunosuppressants. A combination of these factors contributed to the development of VL.

Leishmaniasis can have a number of diverse clinical variations with atypical and severe presentations in immunocompromised patients [8]. Latent infection can become clinically apparent within years to decades after exposure of people who become immunosuppressed [1]. The typical clinical symptoms are fever and splenomegaly. Leucopoenia and anemia are the most frequent hematological disorders [7]. These findings occur in a setting of complex clinical manifestations of underlying disease. In the presented case VL was suspected because of prolonged febrile state with progressing splenomegaly and pancytopenia, as well. Diagnosis of VL may be made with microscopic visualization of the parasite in infected tissue (such as bone marrow, liver, lymph node, colon mucosa or blood), with positive serological tests (DAT and k39 antibody) or with identification of *Leishmania* DNA [3, 10]. Light microscopy accurately detects *Leishmania* amastigotes in stained tissue samples even in immunocompromised patient. If the first procedure does not identify parasites but the clinical index of suspicion is high, repeated sampling is recommended [15, 16]. Serology also appears to be useful for supportive evidence for the diagnosis of VL in immunocompromised patients, but some comparative studies of different serological tests showed conflicting results [7, 17, 18]. Our case confirmed that the best diagnostic approach is the use of combination of methods, as the negative result of one test does not exclude the presence of VL.

Treatment is the prerequisite for good outcome of VL. Liposomal amphotericin B is the drug of choice for VL [1]. Pentavalent antimonials are also well established treatment for leishmaniasis, although there has been evidence of increased resistance in the recent decades [19]. Published trials showed that therapy with either antimonials or amphotericin B provided similar cure rates, but toxicity was higher with antimonials [7, 8, 20, 21]. This was confirmed by meta analysis of 17 studies in HIV infected individuals as the main difference among treatment regimens was in higher mortality rate with antimony use [18.4%, 95% confidence interval (CI) 13.3–25%)] [22]. Published cases of fatal toxicity related to antimonials include severe toxic hepatitis and pancreatitis [7], fatal arrhythmia [23], and unexpected sudden death [20]. In pediatric patients, a recent trial showed that N-methylglucamine antimoniate (n = 51) and amphotericin B deoxycholate (n = 50) had similar cure rates (94.1% vs. 94%, respectively) and serious adverse events (SAE) incidence was similar in both groups [24]. Treatment may be complicated with drug interactions between antileishmanial and other administered medications and their coinciding toxicity [21]. In the presented case both administered medications led to drug toxicity, but additional effect of their successive use had favorable outcome. Recently, an oral agent miltefosine became approved for the treatment of VL. In meta analysis of 2 trials with 523 participants (majority from India) miltefosine was as effective as amphotericin B deoxycholate in achieving VL definitive cure (relative risk 0.99, 95% CI 0.95–1.03) [25]. However, there is limited available evidence to support its use in southern Europe and Latin America or in immunocompromised patients with VL [26].

The lack of an effective vaccine or drugs to prevent infection emphasizes that prevention is crucial to break the global rise of leishmaniasis [26, 27]. Measures to prevent sand fly bites are advised for immunocompromised patients travelling to endemic areas [1, 28].

The reason for the uncommon published cases of VL in patients with UC might be due to their different geographic distribution. Leishmaniasis is native to a variety of developing countries [26]. Occasional cases of VL in Europe have been imported mostly from the Mediterranean region where the prevalence of latent infection is high [7, 10, 29]. On the contrary, IBD is more common in the industrialized world, particularly Western Europe and North America [30]. Incidence of VL is currently on the rise in nonendemic regions due to increased international travel and migration [27]. In our case travel history to Mediterranean endemic regions supported suspicion of VL.

The first report of VL in a patient with IBD was on a 27-year-old woman who was not exposed to *Leishmania* sp.

for over 20 years [5]. She was receiving 5 week corticosteroid therapy for UC presented after spontaneous abortion in the seventh month of her first pregnancy. The persistent fever was attributed to documented pyogenic infection. During week 2 progressive marked hepatosplenomegaly occurred. She died 5 weeks after diagnosis. At necropsy, histology showed *Leishmania donovani* organisms in the liver, spleen, bone marrow and lymph nodes [5].

Another reported case was a patient with UC and sepsis with pancytopenia persisting after colectomy due to colonic perforation. Bone marrow biopsy showed an infiltration with *Leishmania* bodies in macrophages, while DNA sequencing confirmed infection [6]. He had history of travel to Mallorca 1.5 years previously. Administration of liposomal amphotericin B cured the patient. Surprisingly, histological examination of the resected colon revealed the presence of an immunoblastic B-cell lymphoma suggesting major immune disturbance [6].

TNF-α inhibitors are potent immunomodulator drugs with growing use in IBD. Juzlova et al. [23] reported a case of 44-year-old man with Crohn's disease treated successfully with infliximab, who developed VL with cutaneous symptoms [23]. He was treated with antimony with a regression of the local findings, but on the 24th day after his admission, the patient suddenly expired due to fatal arrhythmia as a side effect of the treatment with antimony. In a recent report three cases of Catalan coast residents who were treated with TNF inhibitors for Crohn's disease, developed atypical cutaneous lesions of leishmaniasis [13]. In none of the cases *Leishmania* was detected microscopically; diagnosis was confirmed by PCR of skin samples, serology testing and response to treatment. They received systemic treatment with liposomal amphotericin B because of the lack of response to antimony intralesional treatment in 2 patients and because of hepatosplenomegaly in the third [13].

Conclusion

Published data showed that VL is uncommon in patients with UC. Unfavorable prognosis of untreated cases has been reported in the literature. The presented case suggests that VL should be considered in patients with UC if prolonged febrile state with progressing splenomegaly and pancytopenia occur in a patient with travel history to endemic regions.

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Hypokalemic thyrotoxic periodic paralysis in a young Serbian male

Hipokalemijska tireotoksična periodična paraliza kod mladog muškarca u Srbiji

Petar Ristić*†, Tamara Dragović*†, Saša Kiković*, Dragana Ristić†‡, Marija Zdravković§‖, Saša Hinić§, Tijana Durmić¶, Zoran Hajduković*†

Military Medical Academy, *Endocrinology Clinic, ‡Ophtalmology Clinic, Belgrade, Serbia; University Hospital Medical Center "Bežanijska Kosa", §Department of Cardiology, Belgrade, Serbia; University of Defence, †Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; University of Belgrade, ‖Faculty of Medicine, Belgrade, Serbia; University of Belgrade, Faculty of Medicine, ¶Institute of Forensic Medicine "Milovan Milovanović", Belgrade, Serbia

Abstract

Introduction. Hypokalemic thyrotoxic paralysis is a very rare form of periodic paralysis in Caucasian population. In this population, a more frequent form is familiar hypokalemic periodic paralysis with the same clinical presentation. It is flaccid paralysis of proximal muscles in extremities. Having in mind that clinical presentation of hyperthyroidism in these patients is milder than it could be expected with given values of thyroid hormones, differential diagnosis to other forms of hypokalemic paralysis is essential. **Case report.** We presented a case of a young male with hyperthyroidism and severe periodic flaccid paralysis particularly of leg muscles. Laboratory findings showed elevated thyroid hormones levels and hypokalemia during the attacks with normalized potassium levels between attacks. The patient had no relatives with the similar condition. Also, he never had anything like these attacks prior to development of hyperthyroidism. After differential diagnosis, other reasons for hypokalemic periodic paralysis were excluded. We intensified the hyperthyroidism treatment and resolved hypokalemic periodic paralysis attacks with potassium chloride (KCl) infusions. The patient was advised to start a definitive treatment of hyperthyroidism after stabilization of hormonal levels. **Conclusion.** Hypokalemic thyrotoxic paralysis is a rare and potentially dangerous condition which, if recognized, can be prevented by resolving hyperthyroxinemia and the use of nonselective β blockers.

Key words:
hyperthyroidism; hypokalemia; paralysis, drug therapy; treatment outcome.

Apstrakt

Uvod. Hipokalemijska tireotoksična paraliza je veoma retka forma periodične paralize u beloj populaciji. U ovoj populaciji je zastupljenija familijarna hipokalemijska periodična paraliza sa istom kliničkom slikom. To je flacidna paraliza proksimalne muskulature ekstremiteta. Imajući u vidu činjenicu da je kod ovih bolesnika klinička slika hipertireoidizma blaža nego što bi se očekivalo, kada su u pitanju vrednosti hormona štitaste žlezde, veoma je važna diferencijalna dijagnoza u odnosu na ostale forme hipokalemijskih paraliza. **Prikaz bolesnika.** U radu je prikazan mlađi muškarac sa hipertireoidizmom i teškim oblikom flacidne paralize najizraženije u mišićima donjih ekstremiteta. Laboratorijska analiza ukazala je na povišene vrednosti hormona štitaste žlezde i na hipokalemiju tokom napada, uz normalizaciju vrednosti kalijuma u periodima remisije. Porodična anamneza je bila negativna na slična stanja, a bolesnik u prethodnom periodu nije imao ovakve napade. Nakon isključenja drugih potencijalnih uzroka ovog poremećaja, započeto je sa intenzivnom terapijom hipertireoidizma, a napadi hipokalemijske periodične paralize su kupirani primenom infuzije kalijum hlorida (KCl). Bolesniku je savetovano da nakon stabilizacije nivoa hormona štitaste žlezde započne sa definitivnom terapijom hipertireoidizma. **Zaključak.** Hipokalemijska tireotoksična paraliza je retko i potencijalno opasno stanje koje, ukoliko se na vreme prepozna, može biti kupirano terapijom hipertireoze i upotrebom neselektivnih β blokatora.

Ključne reči:
hipertireoidizam; hipokaliemija; paraliza; lečenje lekovima; lečenje, ishod.

Introduction

Hypokalemic thyrotoxic periodic paralysis (HTPP) is a very rare form of periodic paralysis in Caucasian population. In this population, a more common form of hypokalemic paralysis is familiar hypokalemic periodic paralysis (FHPP). Both conditions have similar clinical presentation with flaccid paralysis of proximal muscles on

Correspondence to: Tijana Durmić, University of Belgrade, Faculty of Medicine, Institute of Forensic Medicine "Milovan Milovanović", Dr Subotića 1, 11 000 Belgrade, Serbia; E-mail: tijana.durmic@mfub.bg.ac.rs

extremities, which stresses the importance of differential diagnosis (Table 1) [1].

Table 1
Difference between hypokalemic thyrotoxic periodic paralysis (HTPP) and familiar hypokalemic periodic paralysis (FHPP)

Parameters	HTPP	FHPP
Race	Asian	Caucasian
Age	20–40 years	Adolescence
Heredity	Usually no	Autosomal dominant
Thyrotoxicosis	Always	No effect
Prevention	Normalization of FT3 and FT4 level	Acetazloamide

FT3 – free T3; FT4 – free T4.

Hyperthyroxinemia is necessary for development of HTPP and it can be caused by various reasons: Graves disease, thyroiditis, thyroid stimulating hormone (TSH) secreting pituitary tumor, toxic nodular goiter or toxic adenoma, excessive T4 or excessive iodine ingestion [2–8]. For the occurrence of HTPP, the origin of hyperthyroidism is not important [9].

Pathophysiology of HTPP involves potassium shift in intracellular space and consequent hypokalemia without real potassium loss. In the center of HTPP pathophysiology is Na-K-ATPase pump which can be stimulated by increased insulin and catecholamine response.

HTPP is a treatable condition that can be prevented with the correction of hyperthyroidism and the use of nonselective β blockers.

We reported the case of a young Serbian male with HTPP, provoked by Graves disease.

Case report

A male, age 21, was admitted to our Clinic with severe muscle weakness especially in his legs and worsening of previously treated hyperthyroid state. His hyperthyroidism had been controlled for four years with propylthiouracil, propranolol and an anxiolytic. Parallelly to hyperthyroidism, the patient had periodic attacks of muscle weakness and in his opinion mostly in the evenings. Flaccid paralysis usually ceased spontaneously after a short period of time. Muscles involved in the attacks were predominantly proximal muscles of legs and shoulders. Never before this onset of hyperthyroidism, he had anything like these attacks. None of his relatives had similar condition. He could not relate these episodes to intensive physical activity but insisted that he could feel the incoming attacks. During attacks blood pressure was normal but usually they were followed by tachycardia. Never, even during the most severe attacks, he had deterioration of consciousness. During these four years he was free of HTPP in periods with normalized thyroid hormones levels.

Tree days prior to the admission to our Clinic the patient had developed severe muscle weakness. It was so intense that he could not get up or stand. He denied diarrheas in connection to periodic paralysis. There was no allergy or other significant medical condition prior to onset of hyperthyroidism.

On the day of the admission the patient was anxious, exhausted and hypodynamic. He had faster heart rate and sweating, particularly during the night. There were no signs of dehydration. Palms were warm and moist with discrete tremor of fingers. There were no signs of ophthalmopathy. Palpation revealed diffuse enlargement of thyroid with no signs of infiltration or palpable nodes. Auscultation of lungs and heart showed normal findings. Arterial tension was 120/80 mmHg and heart frequency 118/min. During the attacks the patient had flaccid paralysis and lack of reflexes. Between attacks, neurological exam was normal.

Laboratory examination revealed higher values of thyroid hormones: free T4 (FT4) > 100 pmol/L, free T3 (FT3) > 50 pmol/L with TSH < 0.005. Blood count and basic biochemical values [creatinine, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase, erythrocyte sedimentation rate (ESR)] were in referent range. The lowest plasma potassium level, detected during the attack, was 2.4 mmol/L. This came with the notion that the patient had been prone to self-medication with oral KCl when he believed attack was coming. This behavior usually occurred at night when attacks were most common. After one such night when 3.0 grams of KCL was ingested, potassium level was measured 5.3 mmol/L. Kaliuria was 81.0 mmoL/24 h (normally 25–120 mmoL/24 h) with diuresis of 1,000 mL/24 h.

Electrocardiogram (ECG) findings are presented on Figures 1 and 2.

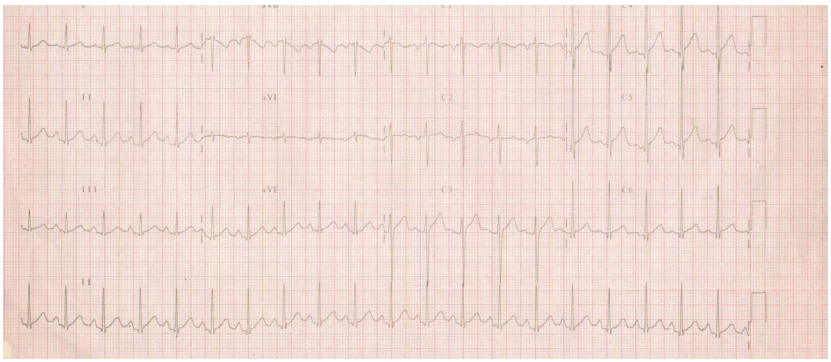

Fig. 1 – Electrocardiogram (ECG) in the period without hypokalemic thyrotoxic periodic paralysis (HTPP).

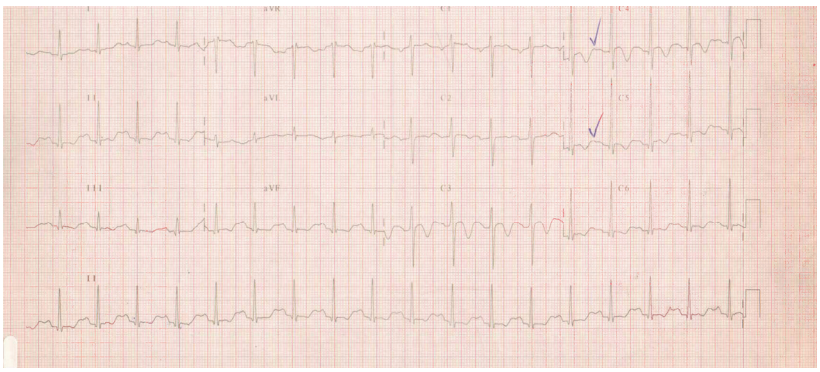

Fig. 2 – Electrocardiogram (ECG) during hypokalemic thyrotoxic periodic paralysis (HTPP) attack.

ECG on admission showed sinus rhythm (heart rate 118/min), PR interval was 145 ms, QRS duration 72 ms, QTc 403 ms, with normal repolarization pattern. During the attack, ECG showed sinus rhythm (heart rate 107/min), PR interval 168 ms, QRS duration 86, QTc 412 ms, negative T waves in D3, aVF and from V2 to V6. U wave was presented in D2, D3, aVF and from V4 to V6.

Ultrasound of thyroid gland showed diffuse enlargement of thyroid. Transversal diameter of the right lobe was 26 x 29 mm and of the left lobe 27 x 26.2 mm. Parenchyma was hypoechogenic and heterogenic with diffusely increased vascularization. No thyroid nodes or regional lymph nodes were found.

During hospitalization HTPP episodes were corrected with 40 mEq of intravenous potassium chloride in the emergency department and was then started on a normal saline infusion with 20 mEq/L of potassium. Also, hyperthyrosis was treated with propylthiouracil (PTU) 300 mcg daily and non-selective β blocker propranolol was given 40 mg twice daily. In support, we temporarily added an anxiolytic. The patient was clinically stabilized and properly informed about necessity of definitive treatment of his hyperthyroidism. Unfortunately there was no compliance from patient. He did not accept to undergo thyroidectomy after reaching euthyroid state nor was interested ine th treatment with I-131 at the time. The patient had such attitude in the first four years prior to the hospitalization in our clinic. Taking medication and KCl supplementation on need was more acceptable to him. Later treatment was conducted in regional medical center. Several years after hospitalization he accepted treatment with I-131, but hyperthyroidism remained. However, he was free of periodic paralysis attacks during the periods with normalized thyroid hormones levels.

Discussion

HTPP is periodic flaccid paralysis caused by transitory hypokalemia and, by rule, it happens during the hyperthyroxinemic state. Common causes for acute systemic paralysis include neurological, metabolic/toxicological or infectious/inflammatory conditions. Neurological ones may be myasthenia gravis, Sy Lambert Eaton, cataplexy, multiple sclerosis, transitory ischemic attack or hyperventilation sy ndrome. In metabolic/toxicological group, there are electrolyte imbalances, porphyries, medicaments, botulismus, alcoholism, opiates, hypoglycemia or some endocrinopathies. Finally, possible causes from infectious/inflammatory group are poliomyelitis, poliomyositis, diphtheria, dermathomyositis, Sy Guillain Barre, and inflammatory myopathy.

Hypokalemia itself might be due to real potassium loss or without real potassium loss. FHPP, HTPP, barium poisoning, insulin excess and alkalosis comprise the latter group. For differential diagnosis it is necessary to explore all reasons for potassium loss including renal ones: mineralocorticoid effect, renal disease, diuretic treatment and hypomagnesemia as well as non renal reasons like reduced food intake, diarrhea, villous adenoma-colon, fistulas, ureterosigmoid stoma or laxative abuse.

HTPP is metabolically induced systemic paralysis without real potassium loss; instead, potassium is shifted to intracellular space. Other causes of hypokalemic periodic paralysis need to be taken in consideration for differential diagnosis.

Rosenfeld [10] was the first who described HTPP in 1902. HTPP is a rare medical condition in our region. Recent epidemiological references have indicated that it is more common in Asian population, up to 1.8–1.9% of thyrotoxic patients in China and Japan compared to 0.1–0.2% in North America [11, 12]. There are reported cases in Caucasians, Aborigines, South American population and rarely in women [13].

Although hyperthyroidism is up to 10 times more common in females, HTPP predominantly affects males [14]. In Chinese population HTPP occurs in 13% of male compared to 0.17% female thyrotoxic patients [15]. Male to female ratio in other studies was reported to be 17–70 : 1 [16]. Usually, HTPP patients are 20–40 years old. Human leukocyte antigen (HLA) evaluation, although with some regularity, is not universally characteristic.

For HTPP to emerge, a patient needs to be in hyperthyroxinemia for some time. Muscle weakness begins usually 3–4 hours after dinner, during rest or sleep. It is believed that this is due to more prominent insulin or adrenalin release. Common provocative factor is a meal rich in carbohydrates, as it was in the case of our patient, and it may also be physical activity or stress. Interestingly, moderate physical activity might stop an incoming attack [17].

Clinical presentation of all hypokalemic paralyses, regardless of their origin (HTPP or FHPP), is the same. It is flaccid paralysis differing in severity of presentation.

Dominantly, it affects proximal muscles of extremities and it is followed by the lack of reflexes. Legs are usually affected first. Walking and standing up from a sitting position are compromised. Respiratory or bulbar muscles are not affected. Patients often claim that they could feel an incoming attack, as it was in the case of our patient. Paralysis usually starts at night and may last form a few minutes up to 48 hours, but usually a few hours. Between attacks, neurological findings are normal [1, 2].

Every HTPP patient has hyperthyroxinaemia. Hyperthyroidism in case of HTPP can be very mild so it can be unnoticed. This requires caution in diagnostic evaluation of hypokalemic paralysis. Our patient had severe hyperthyroxinaemia on admission. Despite the notion that clinical presentation of hyperthyroidism in the case of our patient was clearly present, it could have been more pronounced considering extremely elevated levels of thyroid hormones.

Although paralysis can stop spontaneously, the best way to stop attack is oral or intravenous administration of KCl. Recommended doses are 60–130 mEq orally or 20 mEq dissolved in saline infusion, intravenously [18]. The effect is expected within 15–20 min. The above was confirmed in our experience. Caution is required due to possible hyperkalemia. The other recommended treatment is initial use of non-selective beta blocker propranolol 3 mg/kg, with the notion that such an approach is efficient and free of hyperkalemia. After the normalization of thyroid hormones plasma levels, HTPP patients remain free of attacks. Besides reaching euthyroid state, for the prevention of HTPP, we can use propranolol, too. It is believed that the effects of β2 adrenergic receptor blockade are beneficial in this situation. Sympathetic stimulation of insulin release from β-cells might be the argument for such treatment [1]. Spirinolactone was also reported as useful in prevention of HTPP and, of course, avoiding known provocative factors is highly recommended [19]. On the other hand, taking preventive doses of oral KCl is not effective. Our patient was previously convinced that oral KCl could prevent attack but during the hospitalization in our Clinic we advised him otherwise.

Hypokalemia can be severe during attack but in-between attacks potassium levels are normal. Daily potassium excretion is normal so it is obvious that potassium is shifted from circulation during attacks. It is believed that potassium is shifted to intracellular space. During attacks patients have temporary hypomagnesemia and hypophosphatemia. Patohistological finding by electronic microscopy includes vacuolization of sarcolema and contractile parts of fibrils. Degenerative changes in myofibrils are also seen.

During attacks, ECG changes include tachycardia, negative T waves, U waves, first degree AV block, shortening of QT interval and elongation of QRS complex. Atrial fibrillation may occur; on the other hand, ventricular fibrillation is extremely rare [20]. Our patient during the attacks had U wave, elongation of QRS complex and negative T wave but QT interval was not shortened, instead it was a bit longer. Between attacks, ECG was normal.

Pathogenesis of HTPP is still not clear. Muscle weakness is a consequence of altered depolarization, which disturbs readiness for the next depolarization-contraction cycle. Persistence of mild depolarization state on sarcolema is a combined effect of reduced activity of adenosine triphosphate (ATP), dependent potassium channel and prolonged Na-K-ATPase activity. Earlier, it was found that insulin and catecholamines stimulate Na- K- ATPase, and also that insulin reduces the activity of ATP dependent potassium channel. Thyrotoxicosis in HTPP stimulates the activity of Na-K-ATPase and increases the number and density of adrenergic receptors which all together facilitates development of hypokalemia caused by insulin and catecholamines [21, 22]. Reduced activity of Ca^{2+} pump and increased intracellular Ca^{2+} concentration are reported [23–25].

In 2010 Puwanant and Ruff [26] reported that outward potassium channels (Kir) current is decreased in intercostal myofibrils in both, HTPP and FHPP. In addition to the activation of Na-K ATPase, insulin and catecholamines also decrease the activity of Kir [27]. Genetic mutation of gene encoding Kir2.6 channels was found in 33% Cacausans with HTPP [28].

During rest or sleep, potassium increasingly enters into cells; on the other hand, during physical activity potassium is released in circulation. This data amplify importance of potassium transport in HTPP [23, 29, 30].

For differential diagnosis, the most important condition is FHPP. FHPP is a hereditary condition. In FHPP, mutation is on 1q chromosome and leads to alteration of α subunit on dihydropyridine sensitive L-type calcium (L-Ca) channels. They are slow Ca^{2+} channels. Also, these channels act as voltage sensors for so called excitation-contraction linking. Mutation on genes for Na^+ and K^+ channels are also seen in FHPP. Several patients with HTPP had a mutation on K^+ channel [23]. Presence of hyperthyroxinemia is essential for development of HTPP and has no influence on FHPP.

Our patient had an overwhelming level of evidence for diagnosis of HTPP. The problem with his attitude toward definitive treatment of diffuse toxic goiter kept him away from preventive normalization of thyroid hormones level. The reason for hospitalization in this occasion was a severe and prolonged paralysis caused by worsening of the thyroid hormones level. His previous experience with mild self-ceasing attacks sealed him in his decision to avoid thyreoidectomy. Later attempt in a regional hospital with I-131 did not permanently solve hyperthyroidism and HTPP remained to some extent, except in periods with normalized thyroid hormones levels. At least, during hospitalization in our Clinic, he was educated how to avoid other povocative factors and hopefully reduce his health risk.

Conclusion

HTPP is rare in Caucasians, including Serbs as a part of Slavic population. Hyperthyroidism in HTPP patients is often milder in clinical presentation than it could be expected according to levels of FT4 and FT3. This may be misleading and points to the necessity of careful differential diagnosis in every patient with hypokalemic paralysis. Resolving reasons for hyperthyroxinemia and preventive nonselective β-blockade are recommended.

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CASE REPORT
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Balloon coronary angioplasty and parenteral antiplatelet therapy for intraoperative myocardial infarction during general surgery: an attempt to balance benefits and risks – A case report

Koronarna balon angioplastika i parenteralna antitrombocitna terapija kod intraoperativnog akutnog infarkta miokarda u opštoj hirurgiji: rizici i korist primenjene terapije

Vojislava C. Nešković*†, Slobodan D. Obradović†‡, Ana V. Popadić*, Nenad B. Nikolić*, Dušica Stamenković*†, Zoran R. Kostić†§

Military Medical Academy, *Clinic for Anesthesiology and Critical Care, ‡Clinic for Emergency and Internal Medicine, §Clinic for General Surgery, Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Introduction. Cardiovascular complications remain one of the major risk factors for perioperative morbidity and bad outcome in non-cardiac surgery patients. Here we report a case of the patient suffering intraoperative ST-segment elevation acute myocardial infarction (STEMI) promptly treated with percutaneous balloon angioplasty and intravenous antiplatelet agents. **Case report.** A 62-year-old man, without previous history of cardiovascular morbidity, developed STEMI during abdominal surgery. Due to profound hypotension with mean arterial pressure of less than 40 mmHg, surgery was promptly ended and patient transferred to intensive care unit. Within one hour after the end of the surgery, coronary angiography and successful balloon angioplasty of occluded right coronary artery were performed. Tirofiban infusion was started in recommended dose. Developed hemodynamic instability was related to hypovolemia and excessive drainage, reaching 1,500 mL of blood in the following 15 hours. The following morning, drainage persisted (additional 600 mL of blood) which resulted in profound hypotension (65/40 mmHg). Overall, the patient received 1,970 mL of blood, 6 doses of thrombocytes and 840 mL of fresh frozen plasma. All together, the patient had a favorable outcome, despite the occurrence of bleeding complications and hemodynamic instability. **Conclusion.** The choice of treatment strategy for patients suffering perioperative STEMI during major non-cardiac surgery is challenging. After major non-cardiac surgery, characterized by both high bleeding risk and high risk of stent thrombosis, balloon angioplasty instead of stenting along with parenteral antiplatelet treatment may be a fair therapeutic choice. Clinical choices have to be made individually, according to the weighted risks and benefits.

Key words:
perioperative period; digestive system surgical procedures; myocardial infarction; angioplasty, baloon; tirofiban.

Apstrakt

Uvod. Kardiovaskularne komplikacije predstavljaju jedan od najvažnijih faktora rizika koji utiče na perioperativni morbiditet i ishod lečenja kod bolesnika sa nekardiohirurškim operacijama. Ovde prikazujemo bolesnika sa intraoperativnim akutnim infarktom miokarda i elevacijom ST-segmenta (STEMI) koji je nastao tokom abdominalne hirurške intervencije. U njegovom zbrinjavanju urađena je perkutana balon dilatacija i primenjena intravenska antitrombocitna terapija. **Prikaz slučaja.** Bolesnik, star 62 godine, bez prethodnih kardiovaskularnih tegoba, podvrgnut je hirurškoj intervenciji u abdomenu. Intraoperativno, došlo je do razvoja STEMI. Zbog održavanja naglašene hipotenzije (srednji arterijski pritisak od 40 mmHg), operacija je ubrzano završena i bolesnik preveden u jedinicu intenzivnog lečenja. Unutar prvog sata od završetka operacije, urađena je koronarografija i u istom aktu balon angioplastika okludirane desne koronarne arterije. Uključena je infuzija tirofibana u preporučenoj dozi. Tokom prvih 15 sati, kod bolesnika se održavala hemodinamska nestabilnost usled hipovolemije i ekstenzivne drenaže (1500 mL krvi). Narednog dana,

Correspondence to: Vojislava Nešković, Military Medical Academy, Clinic for Anesthesiology and Critical Care, Crnotravska 17, 11 000 Beograd, Serbia. E-mail: vojkan43@gmail.com

drenaža se održavala (dodatnih 600 mL krvi), što je dovelo do naglašene hipotenzije (65/40 mmHg). Postoperativno, ordinirano je 1970 mL krvi, 6 doza trombocita i 840 mL sveže smrznute plazme. Uprkos naglašenoj hemodinamskoj nestabilnosti i krvarenju, primenjena terapija dovela je do potpunog oporavka. **Zaključak.** Izbor terapije za bolesnike sa perioperativnim STEMI i dalje predstavlja veliki izazov. Kod hirurških bolesnika, kod kojih postoji visok rizik od krvarenja i tromboze, balon dilatacija bez plasiranja stenta, sa primenom peranteralnih antitrombocitnih lekova može biti dobar terapijski pristup. Potreban je individualizovan pristup lečenju, sa procenom rizika i koristi za svakog bolesnika.

Ključne reči:
perioperativni period; hirurgija digestivnog sistema, procedure; infarkt miokarda; angioplastika, balonska; tirofiban.

Introduction

Cardiovascular complications, with its incidence around 4% [1], which accounts to 42% of all perioperative complications [2], remain one of the major risk factors for perioperative morbidity and bad outcome in non-cardiac surgery patients. Since there are more than 200 million patients undergoing major non-cardiac surgery annually [3], the absolute number of patients suffering from perioperative cardiac complications is rather high. Diagnosis and treatment of these complications are challenging [4] and in spite of recently published guidelines on myocardial revascularization [5] and management of ST-segment elevation acute myocardial infarction (STEMI) [6], the optimal treatment strategy for STEMI in the setting of non-cardiac surgery remains unclear.

We report a case of the patient with intraoperative STEMI promptly treated with percutaneous balloon angioplasty and intravenous antiplatelet agents, with favourable outcome despite the occurrence of bleeding complications and hemodynamic instability.

Case report

A 62-year-old man was readmitted to the hospital after the left hemicolectomy, performed one month before due to sigmoid colon carcinoma. The patient had abdominal pain, signs of bowel obstruction, but was otherwise with no major comorbidities. In his previous medical history the patient reported only mild hypertension, not regularly treated. Since abdominal symptoms did not improve on medical treatment, following detailed diagnostic work-up the patient was scheduled for another surgical intervention under suspicion of the presence of intra-abdominal abscess. Preoperatively, he was not taking antiplatelet agents, beta-blockers or statins.

At surgery, smooth intravenous induction (midazolam 2 mg, propofol 1.5 mg/kg, fentanil 2 mcg/kg and rocuronium 0.6 mg/kg) was followed by inhalational anesthesia with sevofluran [expired concentration of 0.6–1.5 minimal alveolar concentration (MAC)]. Abdominal exploration, abscess evacuation and bowel resection with bipolar ileostomy were performed with uneventful surgical course for the next three hours. However, suddenly, the patient became hemodynamically unstable, with profound hypotension (mean arterial pressure dropped to less than 40 mmHg), stable heart rate of 90 bpm, but obvious ST-segment elevation on monitor electrocardiographic (ECG) leads, consistent with evolving myocardial injury. Prolonged hypotension and persistent ECG changes led to the conclusion that STEMI may be developing. Intravenous bolus of 10,000 units of unfractioned heparin was administered and epinephrine boluses were given to correct hypotension. Blood pressure was stabilized, but ECG changes remained unchanged. Surgery was promptly ended and patient was transferred to the surgical intensive care unit (ICU), intubated, mechanically ventilated and sedated.

After arrival in the ICU the 12-lead ECG was performed, showing ST-segment elevation in II, III and AVF leads and ST-segment depression in leads I, AVL, and precordial leads C2-C5 (Figure 1). Blood samples were taken for troponin and creatine kinase measurements. Immediate cardiology consultation was performed and diagnosis of evolving inferior STEMI was confirmed. Within one hour after the end of surgery, the patient was transferred to the cardiac catheterization laboratory and coronary angiography was performed revealing proximal thrombotic occlusion of the dominant right coronary artery (RCA) and no significant coronary artery disease on the left side. No further medication was given. After crossing the RCA occlusion by the coronary guidewire, angioplasty balloon 3.0×18 mm was inflated to 12 atm at the site of the occlusion and distally. Good angiographic result with Thrombolysis in Mycardial Infarction (TIMI) 3 flow was noted, indicating achieved patency of the infarct-related artery (Figure 2).

After percutaneous coronary intervention (PCI), the patient was returned to the ICU, still sedated and mechanically ventilated. Tirofiban infusion was started in recommended doses (bolus 25 mg/kg, followed by infusion of 0.15 mg/kg/min).

After two hours of hemodynamic stability, the patient became hypotensive, with tachycardia (blood pressure 70/50 mmHg, heart rate 115 bpm), which was related to excessive drainage (reaching 1,500 mL of blood in the next 15 hours) resulted in hypovolemia. Tirofiban infusion was stopped after 11 hours because of persistent bleeding and infusion of thrombocytes was given. Also, since the hypotension was difficult to correct with volume replacement only, infusions of dopamine (5 mcg/kg/min) and later, norepinephrine (0.05 mcg/kg/min), were initiated. Effects of tirofiban and thrombocyte infusion were monitored with available point-of-care test (Multiplate) (Figure 3). The following morning, the drainage still persisted (additional 600 mL of blood) which resulted in profound hypotension (65/40 mmHg) and heart rate of 100 beats/min. Volume replacement was continued and norepinephrine infusion was re-started.

Echocardiography revealed left ventricle of normal size with inferior wall akinesis and preserved ejection fraction and left pleural effusion. During the day the patient improved, vasopressor infusion was stopped and the following morning the patient was extubated. Overall, the patient received 1,970 mL of blood, 6 doses of fresh thrombocytes and 840 mL of fresh frozen plasma. Laboratory tests eight hours after the intervention showed peak creatine kinase level of 990 IU/L, creatine kinase MB 138 IU/L and troponin level of 63.96 IU/L.

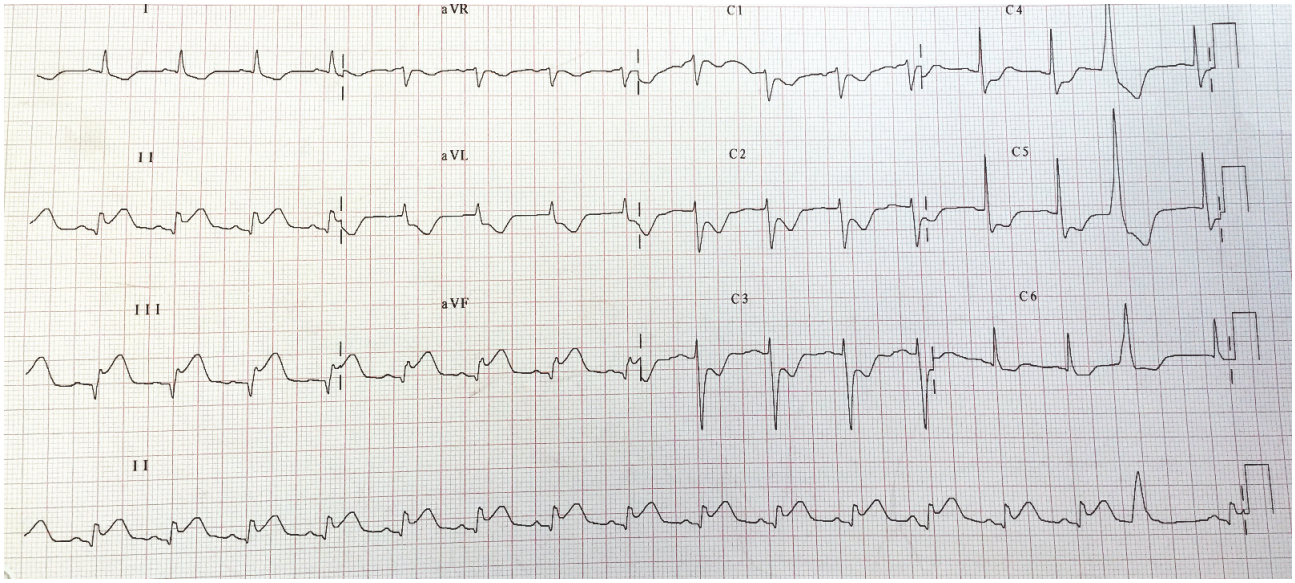

Fig. 1 – Perioperative electrocardiography (ECG) showing ST-segment elevation in leads II, III and AVF and ST-segment depression in precordial leads.

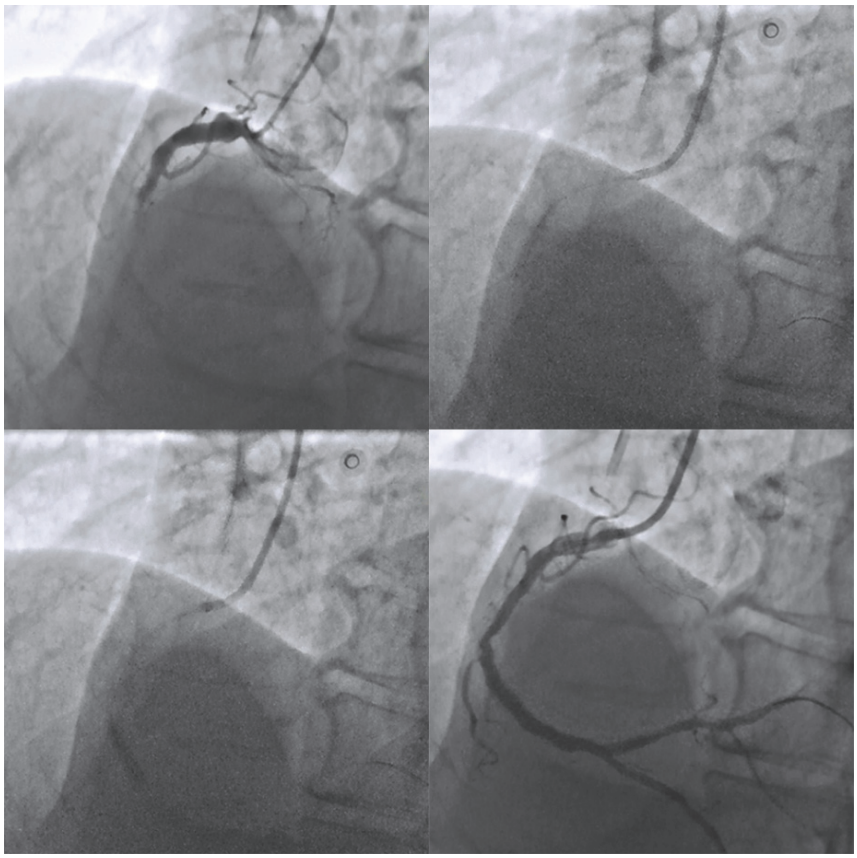

Fig. 2 – Upper left panel: coronary angiography showing thrombotic proximal occlusion of the right coronary artery; Upper right panel: balloon angioplasty at the site of occlusion; Lower left panel: balloon angioplasty distally to the site of occlusion; Lower right panel: final angiographic result with fully patent infarct-related right coronary artery.

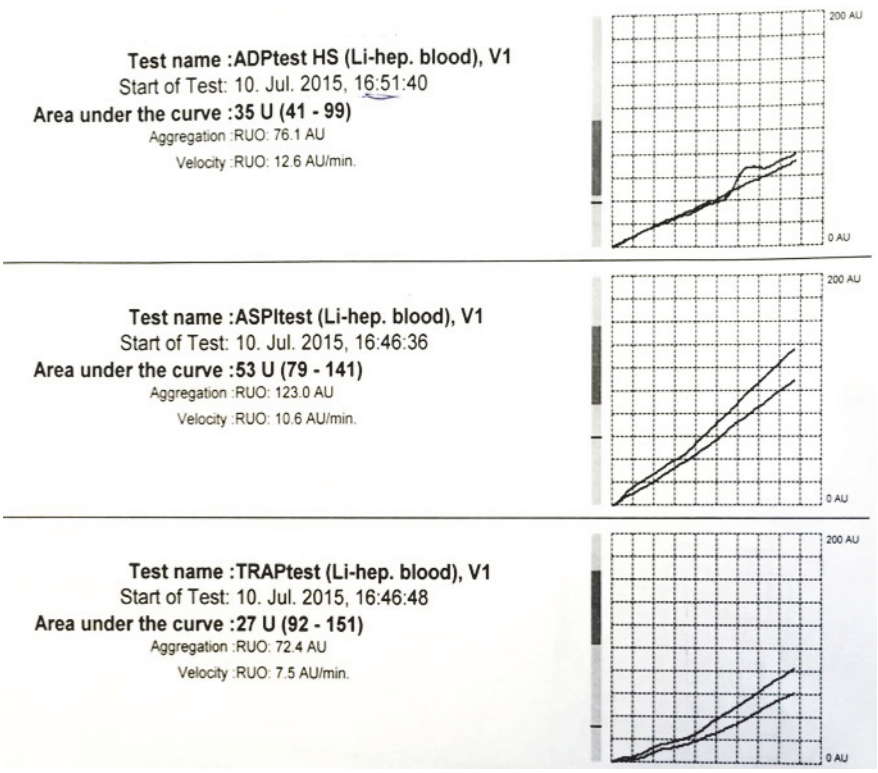

Fig. 3 – Platelet aggregation recordings by Multiplate analyzer showing good response to treatment with tirofiban.

Because of bleeding complications, oral antiplatelet therapy was postponed and acetylsalicylic acid (ASA) was started 100 mg daily on the fourth day after revascularization, followed by clopidogrel 75 mg, twice daily the next day. The patient had uneventful clinical course thereafter and left the ICU after 5 days. Nevertheless, the patient was discharged 46 days after surgery, because of complicated wound healing.

Follow-up coronary angiography performed four months after balloon angioplasty showed patent RCA without significant stenosis.

Discussion

Surgical patients who experience perioperative myocardial ischemia and infarction are at high risk for adverse outcome [7]. The incidence of perioperative myocardial infarction is estimated to be 4% in non-cardiac surgery [7], but may be as high as 8.5% in vascular surgery patients [8] and is strongly associated with mortality [9].

Non-cardiac surgery patients at increased risk for developing new cardiac events may be identified according to their previous medical history, functional reserve and type of surgical intervention [2]. However, patients as presented here, with very few risk factors, rarely develop perioperative myocardial infarction. In addition, in the setting of surgery, typical clinical presentation, such as chest pain, is infrequently seen and may be easily masked in the perioperative period leading to misdiagnosis and delayed treatment. Nevertheless, these patients are at very high risk and require prompt decision regarding the choice of the treatment in order to minimize myocardial loss.

Although data suggest that use of statins, beta blockers and aspirin may reduce the incidence of cardiac complications during non-cardiac surgery [2], they are indicated for patients with known preoperative risk for new cardiac events. However, information regarding optimal treatment for STEMI that occurs during non-cardiac surgery is missing in the current myocardial revascularization and STEMI guidelines [5, 6]. Additionally, data are scarce in the literature regarding safe therapeutic strategy.

It has been shown that successful reperfusion within first two hours from the onset of infarction is the most beneficial [5, 6]. Our patient underwent PCI within one hour from the end of surgery, which fits within recommended door-to-balloon time in PCI-capable centers [5, 10].

One of the key issues in surgical patients would be antithrombotic strategy, because bleeding risk is obvious if effective antiplatelet therapy is administered. It is recommended that patients undergoing primary PCI should receive dual antiplatelet therapy (ASA and P2Y12 receptor blocker) and parenteral anticoagulant as early as possible before angiography [5, 6]. However, in our patient, who underwent abdominal surgery, absorption of the oral medications might be unpredictable. Therefore, since intravenous antiplatelet agents [glycoprotein (GP) IIb/IIIa inhibitors] [5, 6] may be used in selected patients before and during PCI intervention, we decided to administer tirofiban infusion.

The risk of bleeding was well appreciated, which led to the decision to perform plain balloon coronary angioplasty, without coronary artery stenting, in attempt to minimize the need for dual antiplatelet therapy and eliminate possibility of catastrophic stent thrombosis, which may frequently occur in the perioperative period [11]. Of note, in certain circumstances, like the anticipated need for emergency surgery, balloon angioplasty instead of stenting is still guideline recommended approach [5]. Stenting was not done due to concerns related to high risk of postoperative bleeding if optimal dual antiplatelet treatment is administered after major surgery, although there is a single report in the literature of uneventful course of stented patient with STEMI on clopidogrel in the immediate postoperative period [12].

Despite our awareness regarding this problem, bleeding complications were not avoided, manifested by excessive drainage and hemodynamic instability and solved by prompt blood product replacement and prolonged mechanical ventilation. Eventually, the result of treatment was a good one, with the patent infarct-related coronary artery four months after the intervention. We believe that in complex circumstances after major non-cardiac surgery, characterized by both high bleeding risk and high risk of stent thrombosis, balloon angioplasty instead of stenting along with parenteral antiplatelet treatment may be a fair therapeutic choice for patients presenting with STEMI.

Conclusion

The choice of treatment strategy for patients suffering perioperative STEMI during major non-cardiac surgery is challenging. Since this clinical presentation as in our case is rarely seen, there can hardly be expected that clinical studies will be performed regarding the optimal treatment. Nowadays, clinical choices have to be made individually, according to the weighted risks and benefits.

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Zdravstveno-higijenske prilike u srpskoj vojsci krajem 19. veka (prema pisanju „Ratnika“)

Health and hygiene opportunities in the Serbian Army at the end of the 19th century (according to the “Warrior” writing)

Nenad Ž. Petrović

Univerzitet odbrane, Institut za strategijska istraživanja, Beograd, Srbija

Key words:
history of medicine; history 19th century; military health services; morbidity; mortality.

Ključne reči:
istorija medicine; istorija, IX vek; sanitet, vojni; morbiditet; mortalitet.

Uvod

Na prostorima današnje Srbije, još od srednjeg veka, higijenske i zdravstvene prilike nisu bile dobre. Često su harale epidemije koje su kosile živote. Stanje se nije bitnije promenilo ni uspostavljanjem nacionalne države tokom 19. veka [1]. Jedan od pionira u istraživanju razvoja medicine, a posebno vojnog lekarstva u Srbiji, svakako je doktor Vladan Đorđević. Prema njegovim svedočenjima, između 1840. i 1875. godine je u vojnim bolnicama lečeno ukupno 104 154 pripadnika vojske, od čega se za 82 436 zna dijagnoza (u ovaj broj, dakle, nisu bili uključeni vojnici koji su bili ambulantno lečeni). Prema bolestima, najviše je bilo hospitalizovanih od nastupne groznice i „dalka“,12 006, a umrlo je 33; od zapaljenja pluća i plućne maramice bolovalo je 7 605, a preminulo 707; od šuge 5 594, ali bez smrtnog ishoda; katara na želucu 5 643, a umrlo njih devet; od raznih venеričnih bolesti 5 594, ali bez umiranja. Zatim slede srdobolja (dizenterija) sa ukupno 826 bolnički lečenih osoba, a umrlih 41; od „poljačine“ (skorbuta) lečeno je njih 724, a umrlo 24; od velikih boginja (*Variola vera*) hospitalizovano je bilo 470, a umrlo 46 (što čini skoro 10%); od raznih nervnih bolesti (u šta su uračunate neuroze, neuralgije, išijas, lumbago, potres mozga lečilo se u vojnim bolnicama 412, a umrlo sedam; od zapaljenja mozga i kičmene moždine 168, a umrlo 107 (što daje najveći postotak od preko 50%); od kile (*Hernia*) 109, sa jednim smrtnim slučajem; od padavice (*Epilepsia*) 96 obolelih, da tri mrtva. Zanimljiv je podatak da su od posledica šibanja, kao vojničkog kažnjavanja, dok je tako nešto postojalo kao kazna, bilo hospitalizovano 70 vojnika, a jedan je preminuo (1854. godine); od iznurenosti i velike slabosti bila su hospitalizovana 43 obolela, sa devet smrtnih ishoda; od šuljeva (*Haemorroidales*) se lečilo 35 obolelih, bez smrtnih ishoda; od duševnih bolesti (koje autor odvaja od nervnih, pa ih definiše kao melanholiju, idiotizam, psihopatiju) bilo je lečeno 36 vojnika. Konačno, kao razlog oboljevanja navodi se i „tuga za kućom“, stručno nazvana *Nostalgia*, od koje je u razmatranom periodu bolovao 21 vojnik, a umrla dvojica (1843. i 1847. godine) [2].

Stanje zdravlja regruta i pitomaca Vojne akademije

Do izvesnog napretka dolazi u četvrt veka mirnodopskog razvoja posle 1885. godine. Međutim, ni tada pokazatelji zdravstveno-higijenskih prilika u srpskoj vojsci nisu bili sjajni. „Rak rana“ ostala je nehigijena i nesmetano širenje „prilepčivih“ bolesti – pojave rasprostranjenije nego u bilo kojoj drugoj evropskoj vojsci. U to vreme tražio se odgovor na pitanje o fizičkim sposobnostima regruta, njihovoj spremi i uzrocima velikog poboljevanja i smrtnosti [3]. Zaključeno je kako brojne okolnosti doprinose tome da fizičke osobine dvadesetogodišnjih mladića budu neodgovarajuće. Lazarević na statistikama regrutacije pokazuje prosečne i apsolutne cifre. Naglašava da je kod fizičkih osobina presudno nasleđe, odnosno „karakter rase“, a, osim toga, ishrana, piće, teški radovi, klima i uslovi stanovanja. Posebno se naglašava da je

Correspondence to: Nenad Ž. Petrović, Univerzitet odbrane, Institut za strategijska istraživanja, Neznanog junaka 38, 11 000 Beograd, Srbija. E-mail: npetrovic21@yahoo.com

telesna visina uslovljena osobinom određene „rase“ (narodnosti). U tabeli tadašnje ukupne prosečne visine naroda (muške i ženske populacije) na prvom mestu nalaze se severni Nemci sa 162,1 cm do 173 cm, potom Šveđani sa 160,8 cm do 170,2 cm, pa Englezi sa 160–172,7 cm i Severnoamerikanaci (SAD) sa srednjom visinom između 160–173 cm. U donjem delu tabele nalazili su se Španci i Italijani sa zajedničkim prosekom između 156–162 cm, potom Austro-Ugari sa 155,3 cm do 172 cm i, kao poslednji, Francuzi sa 154 cm do 168 cm. Srbi (muškarci i žene) su tada u proseku imali visinu između 160 cm i 170 cm. Ako se uzme prosečna visina samo vojnika, onda je na prvom mestu bila Norveška sa 171,3 cm, ispred Švedske sa 169,9 cm, Danske sa 169,5 cm, Engleske 169,3 cm, Holandije 169,2 cm, iza kojih, sa neznatnom razlikom, slede Mađarska, Nemačka, Rusija, Švajcarska, i Francuska sa 168–169 cm, dok su na začelju ostale Italija sa 167,6 cm i Španija sa 166,7 cm. Srbija je tada imala vojnika sa prosečnom visinom od 169 cm i prema ovoj statistici nalazila se u sredini tabele izjednačena sa Nemačkom, a ispred Rusije, Švajcarske i Francuske. Mora se imati u vidu da su u ono vreme svuda u Evropi prosečne visine bile niže nego danas, zbog manjeg konzumiranja mesa, odnosno manjeg unosa belančevina. Lazarević je sredio podatke o merenja visine, težine i obima grudi prilikom regrutacije 23 281 vojnika starosti 20–22 godine. Dajući tabelarni pregled odnosa između visine i težine kod srpskih regruta Lazarević zapaža da težina i visina nisu u srazmeri. Tako je sa statističkim prosekom visine od 1,69 m bilo 1 255 regruta, ali od tog broja samo 728 je imalo odgovarajuću težinu od 69 kg. Kod statističkog proseka visine opaža se pothranjenost, dok, naprotiv, za niže vrednosti visine, kao npr. 1,55 m, statistika pokazuje gojaznost. Doktor Lazarević je uporedivši regrutne nalaze po okruzima izveo zaključak da su regruti iz pretežno planinskih krajeva snažniji, jače muskulature i širih grudi, dok su u ravničarskim predelima muškarci niži rastom, slabijih kostiju i užih grudi, ali zato uhranjeniji. Među prve srezove po prosečnoj visini regruta stavlja Pčinjski, Poljanički, Vlasotinački, Crnogorski, Račanski i Zlatiborski, a među one sa najboljim obimom grudi Dragačevski, Ražanjski, Žički, Golubački i Užički. Visina i kapacitet pluća imali su najneposredniji odraz na osnovni zahtev pešadije, a to je marševanje. Zbog toga je zanimljiva i statistika dužine koraka i brzine kretanja kod raznih armija. Jednako rastojanje za minut ruski pešadinci pređu u 112–116 koraka, nemački u 114, austrijski u 115, dok francuskim i italijanskim treba po 120 koraka što je posledica njihovog nižeg rasta a samim tim i manjeg raspona nogu [4]. Da je stanje regruta bilo jako loše dalo bi se zaključiti iz tabele u kojoj se daju podaci o četvorici vojnika sa sela iz zapadne Srbije (srezovi Takovski, Kačerski, Ljubićki i Dragačevski). Njihove visine kretale su se od 1,53 m do 1,60 m, a težina između 65,32 i 73,25 kg. Marković navodi da je prema saznanjima iz njegove prakse najbolja srazmera prisutna kod regruta iz radničkog i seljačkog staleža gde na svakih 10 centimetara visine bude po 3,8 kg telesne mase. Međutim, razlika u obimu grudnog koša između najdubljeg udisaja i izdisaja za vojnike do visine od 1,60 m trebala bi da iznosi 6,5 centimetara što nije bio slučaj ni kod jednog vojnika uzetog za ovu statističku analizu. Ovaj parametar se kretao između 5,2 cm i 5,9 cm [5]. Gerasimović konstatuje da je ogroman broj srpskih regruta iz zemljoradničkog staleža i da su na odsluženje roka stigli sa dosta razvijenom muskulaturom. „Oni nisu izranjeni, većina ih se loše i oskudno hranila, ali na rad i naprezanja su oni, ko više ko manje, još od kuće naviknuti. Među njima ima ih i sa jednostrano razvijenom muskulaturom prema pozivu i radovima, kojima su se bavili do dolaska u kasarnu. Ali je kod sviju njih najmanje vežbano srce i pluća, a među tim ove dve funkcije stoje u vrlo tesnoj vezi sa svakim mišićnim radom“ [6].

Doktor Pecić, pišući o parametrima razvijenosti regruta u Beogradskom pukovskom okrugu, kritikuje najnovija pravila o regrutovanju, odnosno o oslobađanju od služenja vojnog roka. Naime, 1901. godine prvi put su primenjena nova pravila i autor je, kao član komisije, uporednim tabelama i rezultatima pregleda regruta rođenih 1880. i 1881. godine iz Beogradskog okruga došao do određenih zaključaka. Najopštije zapažanje bilo je da najveći broj privremeno nesposobnih dolazi iz redova onih koji su započeli služenje roka, pa su usled oboljevanja otpušteni. Drugo važno zapažanje bilo je da zakonski minimum od 154 cm visine i 78 cm obima grudi za regrutovanje nije odgovarajući tako što je suviše nizak. Autor je došao do zaključka da se ne bi izgubilo mnogo na broju vojnika ukoliko bi bili oslobođeni službe svi oni sa visinom do zaključno 160 cm. Najveći broj regruta je, ionako, spadao u tadašnju kategoriju „srednjeg rasta“, visine između 161 cm i 170 cm – takvih je bilo 53,88%, dok je onih „visokog rasta“, odnosno od 171 cm i više, na istom uzorku bilo 32,85 % [7].

Statistikom bolovanja i smrtnosti regruta u 1893. i 1894. godini dokazivalo se kako nije tačno rasprostranjeno mišljenje da se srpski narod degenerisao u odnosu na stanje zdravlja i lepote kakve je, tobože, posedovao u srednjem veku. Pitanje je, naglašava suvislo Popović, da li se može poverovati narodnim pesmama kada opisuju gorostasne junake i koliko su takvi tipovi bili pravilo, a koliko izuzeci? I koncem 19. veka bilo je u narodu, ali kao retkost, dvadesetogodišnjaka sa preko 1,90 m visine. Autor konstatuje poražavajuću činjenicu da je Šumadija, kao jezgro Srbije, dala u oba posmatrana godišta najveći broj telesno zaostalih u razvoju, bez izgleda na popravak. Popović objašnjava zašto je to tako: „rana ženidba našeg seljaka, kojoj je izvor često veoma trivijalnog i spekulativnog karaktera.“ [8] Ipak, i to je bilo bolje od statistika za Austriju, Francusku, Italiju i Švajcarsku. Posmatrano po divizijskim oblastima (Moravska, Drinska, Dunavska, Šumadijska i Timočka) lekar je izveo statistiku bolesti i mana zbog kojih je najčešće dolazilo do oslobađanja od služenja vojske: deformiteti na gornjim i donjim ekstremitetima tela; kilavost (*Hernia*); zaostalost u telesnom razvoju; slepilo na oba oka; deformiteti kostura; tuberkuloza i škrofuloza; nemost i gluvonemost; hronične bolesti krvnih sudova; nervne i duševne bolesti; gušavost (Hruma). Osim slepila i druge očne mane i bolesti mogle su biti smetnja za regrutovanje. U raznim vojskama se različito gledalo na podobnost kratkovidih i dalekovidih za služenje roka. Do 1879. godine, u Nemačkoj i Francuskoj vojnici nisu smeli nositi naočare što znači da do tada pojedinci i sa najmanjom dioptrijom nisu regrutovani. Uvidelo se da je ovo bilo neosnovano, a poseb-

no u civilizovanim zemljama gde je zbog masovnog školovanja rastao broj onih koji su morali nositi ova pomagala. Međutim, posmatrano od zemlje do zemlje, granice za oslobađanje zbog poremećaja vida bile su različite. U Srbiji onoga doba nije bilo nekih posebnih propisa i normi, ali su u praksi, oni koji su bili i najmanje kratkovidi i nosili naočare oglašavani za nesposobne. Svakako da je to bila posledica toga što je bilo malo kratkovidih [11]. Kao granica za oslobađanje od služenja vojske predlagana je dioptrija od – 4 odn. + 6 na oba oka, a za one koji su daltonisti, nesposobnost da razlikuju crvenu, zelenu i ljubičastu boju, kao i za još neke poremećaje vida [9].

Po nekim pitanjima pojavljivala su se oprečna mišljenja. Dok su jedni autori krivili lošu higijenu u kasarnama za visok procenat poboljevanja i smrtnosti vojnika, drugi su to osporavali. Članak sanitetskog majora Gerasimovića opisuje širok aspekt čuvanja zdravlja vojnika opisujući podrobno sklop ljudskog tela, ulogu pojedinih organa, ishranu, piće, oblačenje, spavanje, kupanje, pranje kose, rad i odmor, način stanovanja u kasarnama, higijenu tokom marševanja, bivakovanja, logorovanja i kantonovanja. Autor ističe potrebu da vojnik bude zdrav i da sačuva zdravlje, odbacuje mišljenja da su uslovi života u kasarnama i naprezanja odgovorni za obolevanje i smrtnost regruta. Po njegovom mišljenju, oni koji su oboleli tokom služenja vojnog roka ili su bolest doneli iz civilstva ili su je pak sopstvenom nemarnošću prouzrokovali. Inače, smatrao je da je vojnički život u suštini zdrav, iako jeste zamoran, ali nije nehigijenski [10]. Merenja razvoja mišića kod novih vojnika pokazuju da se butni mišići nogu razvijaju kod svih, međutim da kod ruku to nije uvek slučaj. Uzrok ovome leži u napornom egzerciru kojem su izloženi pešadinci tokom obuke, kada su najopterećenije noge. Ipak, zbog ishrane koja je za mnoge regrute bila bolja od one koju su imali kod kuća, najčešće ne dolazi do mršavljenja, već naprotiv, do debljanja. Ovo važi za čak 70% regruta koji su posle tri meseca u kasarni dobili u proseku 3,27 kg, dok je kod ostalih primetan gubitak težine u proseku za 2,78 kg. Autor ovde stavlja još jednu ogradu, da kod onih koji su u proseku dobili na težini uzrok može biti u pojačanoj ishrani putem paketa od kuće ili kupovinom hrane. Takođe, kod mnogih koji su izgubili na težini, zapravo je bila reč o pozitivnom trendu poboljšanja zdravlja jer su na odsluženje vojnog roka došli sa viškom kilograma [11].

Stanje zdravlja pitomaca najviše vojne škole, kao i oficira, takođe nije bilo zadovoljavajuće. Iako je za službu u armiji potrebno savršeno zdravlje, to se nije uvažavalo pa su tako u Akademiju dolazili i pitomci narušenog zdravlja. To ne treba da čudi, ako se imaju u vidu teške higijenske prilike u narodu što se moralo odraziti i na stanje pitomaca. Početkom devedesetih godina 19. veka primećeno je učestalije poboljevanje pitomaca, pa i smrtni slučajevi. Ovo se dovodilo u vezu sa regrutovanjem već bolesnih mladića kao i sa iscrpljujućim životom tokom školovanja, kada su pitomci po čitav dan bili izloženi teškim naporima. Zbog ovoga se predlagalo da lekarske komisije budu stručnije te da u njima, pored specijalista za očne, ušne i unutrašnje bolesti, bude i lekar bakteriolog, kao i da svaki kandidat podnese podatke o zdravlju porodice, a naročito u pogledu prisutnosti tuberkuloze, škrofula i sifilisa. Treba primati samo najzdravije sa najrazvijenijom muskulaturom, sa dobrim obimom grudi, punokrvne. Kako je u samoj Akademiji bilo obolevanja od tuberkuloze, preporučivalo se da treba izvršiti temeljne dezinfekcije učionica, spavaonica i odeće pitomaca. Lekaru je bila dužnost da na prvom mestu spreči, a tek posle da leči. Lekar, takođe, treba da ume da barata laboratorijom za ispitivanje uzoraka vazduha i prašine, a za to mu je neophodna i sva oprema koje, nažalost, u Srbiji manjka. Predlog je bio i da se smanji broj školskih časova zbog preopterećenosti mladića [12].

Bolesti i smrtnost vojnika

Lekarski pregledi budućih vojnika vršeni krajem 19. veka demantovali su teorije o zdravlju koje vlada na selu nasuprot nezdravom gradu. Smrtnost vojnika bila je velika. Najviša je bila 1891. godine, kada je od obolelih vojnika umrlo 261 ili 1,9%. Od smrtonosnih bolesti na prvom mestu je bila upala pluća, potom tuberkuloza, tifus i meningitis. Tako je u 1898. godini od 213 umrlih, od pomenutih bolesti umrlo njih 126, a od ostalih 87 vojnika [13]. Opšte nepovoljne zdravstvene prilike očituju se i na statistici nataliteta i mortaliteta. Iako je vojni razlog za brigu o natalitetu sa moralne tačke problematičan, jer vojsci treba „topovsko meso“, ipak cifre mnogo toga kazuju: prema statistikama za godine 1907. i 1908. na svakih hiljadu stanovnika u Nemačkoj se rađalo 32,3, a umiralo 18 što je davalo plus od 14,3 ljudi. Slične su cifre bile i kod ostalih anglosaksonskih i nordijskih naroda. Kod Francuza se na hiljadu stanovnika rađalo 20,2, a umiralo čak 19,7 što je davalo plus od samo 0,5 čoveka. U Rusiji se rađalo godišnje 48, a umiralo 29,5, što je davalo višak od 18,5 ljudi. U Kraljevini Srbiji se 1908. godine na hiljadu stanovnika rodilo 36,8, a umrlo 23,7 što je činilo prirast od 13,1 čoveka.. Autor je stoga zaključio da u pogledu „rashoda umiranja“ Srbija stoji najgore: „Da nije preterano velikog broja rađanja, mi bismo po ogromnom rashodu u umiranju pretekli u opadanju i samu Francusku [1].“

U časopisu „Ratnik“ nalazimo tekstove i o velikom broju obolelih i umrlih tokom služenja vojnog roka. Takav je tekst tadašnjeg upravnika Beogradske vojne bolnice, sanitetskog pukovnika doktora Mihaila Markovića [18]. Iako se u članku to izričito ne tvrdi, logično je pretpostaviti da je uvođenje stajaće vojske, sa obaveznim dvogodišnjim rokom za mladiće od 21 godine života, a u uslovima nehigijenskih kasarni, dovelo do ovolikog broja obolelih i umrlih. Imajući u vidu da je tadašnju populaciju činilo uglavnom seosko stanovništvo sa nerazvijenom svešću i navikama o čistoći, jedan broj regruta mogao je da već bolestan dođe na odsluženje vojnog roka jer komisije nisu rado oslobađale od vojne obaveze. Za ovako nešto potvrdu nalazimo u rečima dr Markovića, kada opisuje stanje vojnika: „Ovi žalosni kandidati za smrt, ili su klicu bolesti sa sobom od svojih kuća doneli, koju regrutna komisija pri ovlašćenom pregledu nije uočila, ili su je docnije u kasarni dobili. Jadnim ovim ljudima možda bi se u začetku njihove bolesti, – domaćom negom, dobrom i podesnom hranom, i čistim vazduhom, – još i pomoći moglo, ili, što je vrlo verovatno, kod njih se u povoljnim higijenskim okolnostima bolest još za dugo vreme ne bi ni razvila /.../

Uzevši u obzir, da u vojsku dolaze mladići u 21-voj godini starosti – dakle u najboljoj snazi i razviću njihovom, procent pobolevanja među njima i suviše je veliki, a, na žalost, procent umiranja još veći.“ Osim ovoga, Marković i nebrigu trupnih lekara vidi kao uzrok velikog poboljevanja jer vojnike šalju na stacionarno lečenje, tek kada je bolest uznapredovala i kada joj leka nema. Dajući mišljenje o načinima prevazilaženja on se na prvom mestu zalaže za prepravke postojećih kasarni koje su nehigijenske [5]. Kao uzrok lošeg stanja zdravlja čitavog naroda, a onda i regruta, isticana je loša voda iz nehigijenskih bunara. Za vojsku je bilo od posebne važnosti da, dok je u pokretu, ima zdravu pijaću vodu. Kada to nije moguće obezbediti, tada je neophodno njeno pročišćavanje. Zbog toga su lekari, pozivajući se na evropske uzore, zahtevali uvođenje lako prenosivih filtera, jednostavnih za održavanje [14]. Kratak osvrt na jednu polemiku u najmnogoljudnijoj armiji sveta vredan je pažnje zbog oslikavanja dve škole mišljenja: modernizatorske i tradicionalističke. Do koje mere su se one sukobljavale vidi se čak i na pitanju pročišćavanja vode za vojnike. Dok su se jedni zalagali za filtrovanje vode kao neizbežno i korisno sredstvo, drugi su odbacivali ovu novotariju smatrajući filter izlišnim (npr. Rusi kod kojih je ispijanje čaja navika koja će zameniti pročišćavanje vode). Suprotno, pristalice praćenja novina, isticale su da se u uslovima ratovanja ne može uvek obezbediti prokuvana voda za spravljanje čaja [15]. Treba smanjiti broj garnizona, odnosno grupisati vojsku u manji broj mesta, čime bi se zdravstvena služba olakšala i unapredila. Kao drugu meru predlagano je da „regrutne komisije najbrižljivije i najsavesnije, a pod ličnom odgovornošću predsednika i članova komisije, - naročito člana lekara – regrute pregledaju, i samo one za sposobne oglašuju, koji su faktički za vojnu službu sposobni, a sve iole slabije i nerazvijenije, ostavljaju kao privremeno nesposobne, za iduću godinu [5].“ Jedno radikalnije viđenje izneto na stranicama časopisa optužuje surov postupak oficira prema regrutima tokom obuke: „Prenagljeno i nerazumno muštranje i dresiranje vojnika, pored pomenutih zlih posledica, doprinosi, te su nam redovno za vreme rekrutske škole pune bolnice [16].“ Među bolestima koje su među vojskom osobito opake i smrtonosne, dr Marković pominje one koje su krajem 19. veka bile rasprostanjene širom Evrope: katar pluća, zapaljenje pluća i plućne maramice; vrućica (trbušni tifus); jektika (tuberkuloza); nastupna groznica; male boginje; srdobolja (dizenterija). U petogodišnjem periodu 1888–1892. najveći broj umrlih ubedljivo je bio od katara pluća i to 479 na 15 594 obolelih i hospitalizovanih (ovaj redosled je izveden prema apsolutnim vrednostima, dok je prema broju obolelih, odnosno procentualno, na prvom mestu bila tuberkuloza). Sledeća bolest, koja je kosila vojsku u mirnodopskom razdoblju, bio je trbušni tifus od koga je za pet godina umrlo 117 vojnika od njih 1 036 hospitalizovanih. Od tuberkuloze, tada široko rasprostranjene socijalne bolesti koja je morila stanovništo, umrlo je čak 83 vojnika od 309 lečenih [5].

Da je stanje higijene u vojsci bilo turobno pokazuje tekst u kojem se apeluje da se vojska podvrgne temeljnim pregledima čistoće tela, da se svakom vojniku obezbedi sapun, peškir i voda za pranje, kao i da se vojnici vaspitaju da održavaju ličnu higijenu [17]. U srpskoj vojsci zabeležena je najveća stopa smrtnosti u Evropi od čak 29 promila (29 na hiljadu lečenih) što je mnogo više od najlošije rangirane zapadne vojske – španske kod je smrtnost iznosila 13 promila. Prema podacima o smrtnosi, sledeća je bila a Austro-Ugarska sa 12,5 promila, Italija sa 11,1 promil, Francuska sa 9,2 promila, Rusija sa 9 promila, dok je najbolje stajala Nemačka sa 5,3 promila. Jedan od uzroka lošeg zdravstvenog stanja vojnika predstavljale su nehigijenske kasarne građene od lošeg i nezdravog materijala u blizini močvara i sa ustajalim vazduhom [18]. Još je gore bilo stanje zdravlja zuba kod vojnika, o čemu postoji samo jedan parcijalan izveštaj. Nađeno je da su u valjevskom garnizonu (a tako je po svoj prilici bilo i drugde) zubi bili potpuno ispravni kod samo 9,72% vojnika, dok su kod 90,28% bili pokvareni i izvađeni u većoj ili manjoj meri. Ovakvo loše stanje bilo je posledica nedovoljne profilakse i nedostatka stručnih zubnih lekara. Praksa je bila da se i zbog najmanjeg kvara zub vadi. Ovo autor sa punim pravom naziva „sakaćenjem“. Da bi se ovoj žalosnoj pojavi stalo na put predlagao je za početak uvođenje po jednog stomatologa u svakoj diviziji, a kasnije i više. Da je u Srbiji onoga vremena bilo izuzetno malo zubnih lekara govori podatak da su oficiri zbog popravke zuba morali putovati u Beograd [19].

Analiza stanja u različitim vojskama u devetnaestom veku pokazala je kako je pruska vojska najdalje odmakla u zdravstvenoj zaštiti svojih vojnika. Još 1843. godine pruska armija beležila je najnižu stopu smrtnosti u Evropi, svega 13,95 promila, dok je u isto vreme ona u engleskoj vojsci iznosila 15,3 promila, a u francuskoj čak 19,5 promila! Do kraja tog veka svuda je, zahvaljujući napretku medicine i poboljšanju higijene, došlo do opadanja smrtnosti, kako u građanstvu, tako i u vojsci. Opet je Nemačka imala najbolji rezultat jer je u godini 1889/90. stopa mortaliteta u njenoj vojsci iznosila 3,3 promila, u francuskoj vojsci 5,4 promila, engleskoj 5,7 promila, austro-ugarskoj 6,3 promila, italijanskoj 7,5 promila, ruskoj 7,7 promila, u španskoj vojsci čak 10,4 promila. Kako se vidi, čak i najveći stepen mortaliteta je bio znatno niži od najmanjeg (pruskog) mortaliteta pola veka ranije. Samo za Prusku, pokazano je da je na uporednom uzorku civila starosti između 20 i 25 godina života i vojnika istog životnog doba, stopa smrtnosti bila skoro dvostruko veća u civilstvu: 3,9 prema 7,5 promila! Međutim, nije jasno da li su se pod civilnom populacijom od 20 do 25 godina računali samo muškarce ili i žene? Ako je ovo drugo, onda bi to donekle relativizovalo nalaze autora. Prema navedenim podacima, među umrlim u građanstvu, čak 6,4 promila umrlo je od bolesti, dok je među njihovim vršnjacima u kasarnama ta vrednost iznosila samo 3 promila. Međutim, u civilstvu kod populacije starosti 20–25 godina bilo je samo 0,22 promila samoubica, naspram 0,6 promila u kasarnama. Autor navodi da se ovakav odnos ne može smatrati sam po sebi razumljiv i objašnjiv time da su regrutovani samo najzdraviji i najsnažniji muškarci, već razloge vidi u poboljšanju uslova života u kasarnama. Tome je uzrok i decentralizovani sistem podizanja kasarni, odnosno njihovog zidanja na većem rastojanju i u što više mesta umesto nekadašnjeg sabijanja. Opao je i broj lečenih vojnika, kao i broj provedenih bolničkih dana po pacijentu u Nemačkoj [20]. Međutim, Vladimir Popović je tvrdio kako se kao štetna pokazala praksa u Srbiji da se na zahtev

pojedinih opština otvaraju kasarne, kako bi ta mesta „oživela“. On je smatrao da bi sa higijenskog stanovišta bilo pametnije skoncentrisati vojsku u manje gradova, gde bi se teren za izgradnju brižljivo odabrao, a medicinsko osoblje bi se moglo usredsrediti isključivo na posao. Takođe, i sa strateške tačke posmatrano, koncentrisanje trupa je poželjnije od disperzije Iznošenje uporedne satatistike poboljevanja u evropskim vojskama ukazalo je na loše stanje u Srbiji, a za njega je okrivljavana najpre loša higijena u stanovništvu, a posebno u vojsci, aljkavo regrutovanje (gde se i bolesni primaju u vojsku), loše građenje kasarni... Podaci o stopi smrtnosti u srpskoj vojsci za šestogodišnji 1890–1895. ovore pokazali su da se prosečan mortalitet kretao između 8,1 promila (1890) i 17,7 promila (1893). Iskazano apsolutnim brojevima, godišnje je umiralo 138-305 vojnika, u proseku 238. Prosečno, za ovaj period stopa smrtnosti bi iznosila 14 promila što je, u to vreme, bilo najviše u Evropi, više i od Španije! Još se lošija slika dobija kada se broju umrlih pridoda broj otpuštenih pre odsluženja roka i onih koji su onesposobljeni (stekli invaliditet) tokom vojnog roka. Tada se celokupan gubitak penjao na čak 42 promila! Ako se uzme u obzir da je prosečna brojnost stajaće vojske iznosila 17 000 vojnika, onda bi to značilo da se od ovog broja svake godine gubilo preko 700 ljudi! [21].

Pored „očekivanih“ bolesti kao što su tuberkuloza, zapaljenje pluća, malarija, dizenterija, u to vreme dosta su bile rasprostranjene razne venerične bolesti, a među njima posebno sifilis. U tri godine (1892–1894) od ovih bolesti, u srpskoj vojsci je bolovalo ukupno 3 303 lica mesto evropskih armija po broju ovih „sramnih“ bolesti! Sifilis se u Srbiji onoga vremena dobijao najviše prenošenjem neseksualnim putem, odnosno nehigijenom koja se ogledala u korišćenju zajedničkih čaša i pribora za jelo i prljavštinom [22]. Takođe, prosek oboljevanja od kožnih bolesti bio je veći no u bilo kojoj drugoj vojsci, izričito tvrdi Popović, navodeći cifru od čak 70 promila: „Ovako veliki procenat kožnih i potkožnih bolesti prirodna je posledica zanemarene telesne čistoće naših vojnika. Naš vojnik je `do zla Boga` prljav. Drugače (SIC) i ne može biti – kad se zna da se naš vojnik kupa samo za vreme leta, a međutim ceo vojnički rad skopčan je sa mogućnošću prljanja i odela i tela.“ Kao mere za popravku stanja predlagao je ustanovljenje kolektivnih montažnih kupatila – tuševa [21].

Zaključak

Kroz veći broj stručnih priloga objavljenih u časopisu „Ratnik“ moguće je pratiti brigu za poboljšanje zdravstvenog stanja u vojsci. Prilike u celokupnoj populaciji nisu bile zadovoljavajuće, kako zbog niske svesti o higijeni, tako i zbog slabe opremljenosti kadrovima i potrebnim materijalima. Bilo je premalo lekara i lekarskih pomoćnika, nije se dovoljno izdvajalo za zdravstvo. Kako je najveći deo stanovništva živeo na selu, bez razvijene svesti o potrebi održavanja higijene, ali i bez dovoljno sredstava za očuvanje čistoće, to se stanje iz civilstva preslikavalo i na stanje u kasarnama. Vojni objekti za smeštaj regruta nisu građeni u skladu sa najvišim zahtevima za očuvanje zdravlja, kasarne su podizane na močvarnom i nezdravom terenu, nisu bile dovoljno provetravane i osvetljene suncem, vojnicima nije bilo obezbeđeno redovno kupanje. Prilikom regrutacija, dešavalo se da oboleli mladići dođu na odsluženje vojnog roka gde im se stanje samo pogoršavalo. Broj obolelih i umrlih u vojsci Srbije na prelazu 19. u 20. vek bio je veoma visok. Čitav niz stručnih članaka napisan od strane vojnih lekara ukazivao je na ove poražavajuće činjenice. Pitanje ishrane vojnika, takođe, nije bilo rešeno na zadovoljavajući način. Do početka ratova u 20. veku u kojima je Srbija učestvovala, stanje na polju saniteta nije se bitnije popravilo.

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