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

MEDICAL REVIEW

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

MEDICAL REVIEW is published bimonthly (six issues per year) with a circulation of 1.000 copies. The annual payment fee in 2021, for individuals from the territory of Serbia, is 3,000.00 dinars (the value-added tax included), 4,000.00 dinars for individuals from Serbia who are not members of the Society of Physicians of Vojvodina of the Medical Society of Serbia, 60 Euros for members outside the territory of Serbia, and 8,000.00 dinars (+ VAT) for institutions. The payment account is: 340-1861-70 or 115-13858-06, ”Annual membership fee for Medical Review”.

The manuscripts are submitted at: aseestant.ceon.rs/index.php/medpreg/. Editorial Office Address: Društvo lekara Vojvodine Srpskog lekarskog društva, 21000 Novi Sad, Vase Stajića 9, Tel. 021/521-096; 063/81 33 875, E-mail: dlvsldnovisad@gmail.com; Website: www.dlv.org.rs

Printed by: “Feljton” Novi Sad

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

MEDICINSKI PREGLED

ČASOPIS DRUŠTVA LEKARA VOJVODINE SRPSKOG LEKARSKOG DRUŠTVA
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E-mail: dlvsldnovisad@gmail.com; Web: www.dlv.org.rs

Štamparija: »Feljton« Novi Sad

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

Med Pregl 2021; LXXIV (7-8): 217-280 Novi Sad: July-August.

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Novi Sad *Vase Stajića 9* *Srbija*

Med Pregl 2021; LXXIV (7-8): 217-280. Novi Sad: juli-avgust.

SADRŽAJ

ORIGINAL STUDIES
ORIGINALNI NAUČNI RADOVI

University of Novi Sad, Faculty of Medicine Novi Sad[1]
Clinical Center of Vojvodina, Novi Sad
Emergency Center[2]
Radiology Center[3]
Vascular and Endovascular Surgery Clinic[4]

Original study
Originalni naučni rad
UDK 616.1-005.4-02:[616.98:578.834]-07/-08
https://doi.org/10.2298/MPNS2108221N

ACUTE LIMB ISHEMIA ASSOCIATED WITH SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 INFECTION

AKUTNA ISHEMIJA UDOVA POVEZANA SA COVID-19 INFEKCIJOM

Dragan NIKOLIĆ[1, 2], Marijana BASTA NIKOLIĆ[1, 3], Đorđe MILOŠEVIĆ[1, 4], Nikola BATINIĆ[1, 4] and Slavko BUDINSKI[1, 4]

Summary

Introduction. Severe acute respiratory syndrome coronavirus 2 infection-induced coagulopathy may be the underlying cause of acute limb ischemia, a sudden decrease in limb perfusion. The aim of this study was to present a case series of acute limb ischemia associated with severe acute respiratory syndrome coronavirus 2 infection. **Material and Methods.** This unicentric, observational cohort study evaluated the incidence, risk factors, clinical, laboratory and radiological findings, problems in diagnosis, treatment and outcome of patients with severe acute respiratory syndrome coronavirus 2 infection-induced acute limb ischemia. **Results.** The study included 14 patients with a diagnosis of severe acute respiratory syndrome coronavirus 2 infection-induced acute limb ischemia in a 20-month period. The majority of patients were male (78.6%), with a mean age of 67 years, body mass index > 30, and typical cardiovascular risk factors. Only 64.3% of acute limb ischemia patients with coronavirus disease 2019 underwent attempts of limb salvage; 14.3% required primary amputation; 21.4% were treated with systemic anticoagulant therapy alone. The mortality rate was high (42.9%). Revascularization was successful in 7 (50%) patients with acute limb ischemia. The overall amputation rate was 28.6%. **Conclusion.** The high incidence of acute limb ischemia associated with severe acute respiratory syndrome coronavirus 2 infection, even with thromboprophylaxis, high mortality rates and poor limb salvage outcomes, encourage clinicians to apply individually tailored diagnostic and therapeutic approaches. Prolonged systemic use of heparin may improve the effectiveness of surgical treatment, limb salvage, and overall survival.

Key words: COVID-19; Severe Acute Respiratory Syndrome; Extremities; Ischemia; Embolism and Thrombosis; Arterial Occlusive Diseases; Risk Factors; Early Diagnosis; Signs and Symptoms; Treatment Outcome

Sažetak

Uvod. COVID-19 infekcijom indukovana koagulopatija može može biti osnovni uzrok akutne ishemije ekstremiteta, odnosno iznenadnog smanjenja perfuzije ekstremiteta. Cilj rada je prezentacija serije slučajeva akutne ishemije ekstremiteta povezanih sa COVID-19 infekcijom. **Materijal i metode.** Ovom unicentričnom, opservacionom, kohortnom studijom evaluirani su incidencija, faktori rizika, klinička, laboratorijska i radiološka prezentacija, problemi u dijagnostici, tretmanu i ishodu obolelih od akutne ishemije ekstremiteta udružene sa COVID-19 infekcijom. **Rezultati.** Studija je obuhvatila 14 pacijenata kod kojih je tokom perioda od 20 meseci dijagnostikovana akutna ishemija ekstremiteta udružena sa COVID-19 infekcijom. Većina pacijenata bili su muškarci (78,6%), prosečne starosti 67 godina, sa indeksom telesne mase > 30 i tipičnim kardiovaskularnim faktorima rizika. Samo 64,3% pacijenata sa akutnom ishemijom ekstremiteta podvrgnuto je pokušajima spasavanja udova; 14,3% je zahtevalo primarnu amputaciju; 21,4% je lečeno samo sistemskom antikoagulantnom terapijom. Stopa mortaliteta bila je visoka (42,9%). Revaskularizacija je bila uspešna kod 7 (50%) pacijenata sa akutnom ishemijom ekstremiteta. Ukupna stopa amputacija bila je 28,6%. **Zaključak.** Visoka incidencija akutne ishemije ekstremiteta povezane sa COVID-19 infekcijom, čak i pri primeni tromboprofilakse, visoka stopa mortaliteta i loši ishodi spasavanja ekstremiteta, podstiču klinicare da primenjuju individualno prilagođen dijagnostički pristup i terapijski tretman za svakog pacijenta pojedinačno. Produžena sistemska upotreba heparina može poboljšati efikasnost hirurškog lečenja, spasavanje udova i ukupno preživljavanje.

Ključne reči: COVID-19; teški akutni respiratorni sindrom; ekstremiteti; ishemija; embolija i tromboza; arterijske okluzivne bolesti; faktori rizika; rana dijagnoza; znaci i simptomi; ishod lečenja

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Abbreviations

SARS-CoV-2	– severe acute respiratory syndrome coronavirus 2
ALI	– acute limb ischemia
COVID-19	– coronavirus disease 2019
PT	– prothrombin time
aPTT	– activated partial thromboplastin time
CRP	– C-reactive protein
LDH	– lactate dehydrogenase
BMI	– body mass index
CT	– computed tomography

Introduction

Acute limb ischemia (ALI) is a life and limb threatening condition caused by a sudden decrease in limb perfusion [1].

A range of thrombotic complications in patients with coronavirus disease 2019 (COVID-19) infectionis is wide, from the most common presentation, such as venous thromboembolism, to arterial thrombosis manifested by cerebral, visceral, coronary and limb ischemia. Coagulation system disorders have been registered in the following viral infections: severe acute respiratory syndrome coronavirus 1 (SARS CoV-1), Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory virus syndrome coronavirus 2 (SARS-CoV-2). The following factors contribute to the development of induced thrombosis: endothelial dysfunction, inflammation, cytokine release, hypercoagulability, and hypoxia. The COVID-19 infection induces hypercoagulability, elevation of D-dimer, fibrinogen level, fibrin/fibrinogen degradation products, antiphospholipid antibodies and thrombocytopenia, which increase the risk of thrombus formation [2, 3].

Clinical presentation of ALI varies, depending on the location and number (multilevel lesions) of affected arterial blood vessels, effects of compensatory vascular mechanisms, presence of associated thrombotic processes and severity of COVID-19 infection [4].

Early recognition of ALI and intervention when possible, can reduce mortality in these critically ill patients and improve chances of limb salvage.

The aim of our study was to determine the incidence, risk factors, clinical presentation, laboratory and radiological findings, problems in diagnosis, treatment, and outcomes of ALI associated with COVID-19 infection.

Material and Methods

This unicentric observational cohort study was conducted at the Clinical Centre of Vojvodina, Novi Sad, Serbia. Data of all patients who tested positive for COVID-19 and presented with ALI were collected from a prospectively maintained database. We extracted data of 14 ALI patients with COVID-19 infection from the hospital information system from March 6, 2020 to December 6, 2021. The individual case data included patient demographics, comorbidities, risk factors, clinical presentations, the Society for Vascular Surgery (SVS) and the International Society for Clinical Vascular Surgery (ISCVS) i.e. Rutherford classification of clinical presentations, location of arterial occlusion, radiological and laboratory findings (white cell count, absolute lymphocyte count, platelets, prothrombin time (PT), activated partial thromboplastin time (aPTT), c-reactive protein (CRP), D-dimer, fibrinogen, lactate dehydrogenase (LDH), and ferritin), treatment (anticoagulation therapy, endovascular treatment, or open surgery revascularization) and inpatient mortality. The COVID-19 infection was confirmed by real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay. The radiologic diagnosis of ALI was confirmed by Duplex ultrasound and/or computed tomography (CT) angiography.

Results

Patient characteristics and risk factors

Fourteen patients with confirmed COVID-19 infection developed ALI. Most of the patients were male (N = 11 or 78.6%), with a mean age of 67 years (range: 28 – 82), with body mass index (BMI) > 30 (median BMI: 32.3 kg/m^2), and typical cardiovascular risk factors including hypertension (71.4%), ischemic heart disease (28.6%), peripheral arterial disease 4 (28.6%), and diabetes mellitus (57.1%). Six COVID-19 patients presenting with ALI (42.8%) had multiple comorbidities **(Table 1)**. A COVID-19 associated ALI occurred in 3 (21.4%) young patients without typical risk factors for arterial disease and thrombosis.

Age ≥ 70 years (hazard ratio 2.65, 95% confidence interval (CI) 1.62 – 4.35), male sex, obesity, history of cardivascular disease, diabetes mellitus, and elevated D-dimer were significantly associated with an increased risk of developing an arterial thrombotic event associated with ALI. A COVID-19 associated ALI occurred in 50% of patients receiving thromboprophylaxis.

Anatomic distribution

Predominant localizations of arterial thrombotic processes in patients with COVID-19 infection were large (aorta, iliac, subclavian, axillary) and medium-sized (superficial femoral, brachial) arteries of the upper and lower extremities. The distribution of ischemic limb events included: lower extremity (78.6%) and upper extremity (21.4%). Multiple locations occurred in 14.3% of patients **(Table 1)**. Concomitant deep vein thrombosis occurred in 14.3% of patients. Thrombosis of prior vascular reconstruction (stents/bypass grafts) occurred in 14.3% of patients.

Clinical presentations

The classic clinical features of ALI included the "6 Ps": pain, pallor, poikilothermia, pulselessness, paresthesia, and paralysis. The severity of ALI, by Rutherford classification, ranging from a viable, not immediately threatened limb (stage I) to a profoundly ischemic limb with irreversible damage (stage III), is shown in **Table 1**.

Table 1. Baseline characteristics of patients with acute limb ishemia associated with COVID-19 infection
Tabela 1*. Osnovne karakteristike pacijenata sa akutnom ishemijom ekstremiteta udruženom sa COVID-19 infekcijom*

Patients characteristics/Unit/*Karakteristike pacijenata/Jedinica*	Value/*Vrednost*
Demographics N (%)/*Demografija Br (%)*	
Sex/*Pol*	
Male/*Muškarci*	11 (78.6%)
Female/*Žene*	3 (21.4%)
Mean age (years) *Prosečna starost (godine*)	67 Range: 28 – 82/*Raspon*: 28–82
Comorbidities N (%)/*Komorbiditeti Br* (%)	
Median BMI (kg/m^2) *Prosečna vrednost BMI (kg/m^2*)	32.3 (Interquartile range: 28 – 33)/(*Interkvartilni raspo*n: 28–33)
Hypertension/*Hipertenzija*	10 (71.4%)
Ischemic heart disease/*Ishemijska bolest srca*	4 (28.6%)
Diabetes mellitus/*Šećerna bolest*	8 (57.1%)
Peripheral vascular disease/*Periferna vaskularna okluzivna bolest*	4 (28.6%)
Chronic kidney disease/*Hronična bubrežna insuficijencija*	2 (14.3%)
Multiple comorbidities/*Višestruki komorbiditeti*	6 (42.8%)
No past medical history/*Bez prethodnih oboljenja*	3 (21.4%)
Severity of COVID-19 infection N (%)/*Težina kliničke slike COVID-19 infekcije Br (%)*	
Severe/*Teška*	7 (50%)
Moderate/*Srednja*	4 (28.6%)
Asymptomatic/Mild/*Asimptomatska/Blaga*	3 (21.4%)
Classification of acute limb ischemia N (%)/*Klasifikacija akutne ishemije udova Br (%)*	
Viable (I)/*Udovi nisu ugroženi (I)*	2 (14.3%)
Marginally threatened (IIa) *Granica do koje udovi nisu ugroženi (IIa)*	4 (28.6%)
Immediately threatened (IIb)/*Udovi su ugroženi (IIb)*	6 (42.8%)
Nonviable (III)/*Nepovratna ishemija udova (III)*	2 (14.3%)
Diagnosis N (%)/*Dijagnoza Br (%)*	
Clinical diagnosis only (instability) *Samo klinička dijagnoza (nestabilnost)*	5 (35.7%)
Duplex ultrasound/*Dupleks ultrazvuk*	4 (28.6%)
Computed tomography angiography *Kompjutersko tomografska angiografija*	5 (35.7%)
Median D-dimer (ng/mL) *Prosečna vrednost D-dimera (ng/mL)*	3520 (Interquartile range: 1723 - 9737) (*Interkvartilni raspon: 1723–9737)*
Localization N (%)/*Lokalizacija Br (%)*	
Lower extremity/*Donji ekstremitet*	11 (78.6%)
Upper extremity/*Gornji ekstremitet*	3 (21.4%)
Multiple localizations/*Višestruke lokalizacije*	2 (14.3%)
Treatment N (%)/*Lečenje Br (%)*	
Anticoagulation only/*Samo antikoagulantna terapija*	3 (21.4%)
Primary amputation/*Primarna amputacija*	2 (14.3%)
Endovascular/*Endovaskularno*	1 (7.1%)
Open surgery revascularization *Revaskularizacija – otvorena hirurgija*	8 (57.1%)
Outcome N (%)/*Ishod Br (%)*	
Successful revascularization (surgery/endovascular) *Uspešna revaslularizacija (hirurgija/endovaskularna)*	7 (50%)
Limb loss/*Gubitak ekstremiteta*	4 (28.6%)
In-hospital mortality/*Bolnički mortalitet*	6 (42.8%)

Legenda: MBI – Indeks telesne mase

In hospitalized patients, ALI was a complication in patients with severe COVID-19 in 50% of cases, in 28.6% patients with moderate clinical presentation of COVID-19, and it was a primary clinical manifestation of COVID-19 in the absence of respiratory symptoms in 14.3% of patients. In 28.6% patients, ALI occurred concurrently with ischemic symptoms in other vascular beds (arterial, venous). The symptoms of ALI occurred at a median of 13 (6 – 19) days after the first symptoms of COVID-19 infection.

Diagnostic evaluation

Clinical diagnosis of ALI (physical examination findings, measurement of ankle-brachial index), without additional imaging confirmation, was made in 5 (35.7%) patients. Imaging vascular examinations depended on the stability of patients for transfer to the imaging department. In that respect, only 9 (64.2%) patients underwent confirmatory imaging. Duplex ultrasound (28.6%) and CT angiography (35.7%) were the most common imaging modalities **(Table 1)**.

Inflammatory biomarkers (CRP, erythrocyte sedimentation rate, ferritin, LDH) were elevated in all patients compared to the reference ranges. D-dimer was significantly elevated above reference ranges **(Table 1)** and it correlated with worse outcomes (mortality). Values of the following coagulation studies: aPTT, PT, international normalized ratio and platelets were within normal limits.

The initial treatment for ALI patients was anticoagulant therapy with intravenous unfractionated heparin, unless there were contraindications (active bleeding in the previous 24 to 48 hours, recent surgery, history of heparin-induced thrombocytopenia). Despite the use of anticoagulant therapy, new thromboembolic events occurred in 3 (21.4%) patients with COVID-19 infection.

The approach to limb management (conservative, endovascular or open approach) was based on the patients' overall stability (severity of systemic illness - COVID-19), severity of ischemia, and limb viability. Limb viability was classified and managed using the Rutherford classification **(Table 1)**. Critically ill patients were not candidates for revascularization.

In one series only 64.3% of ALI patients with COVID-19 underwent attempts for limb salvage; up to 14.3% required primary amputation; 21.4% were treated with systemic anticoagulant therapy only. Amputation after revascularization attempt was performed in 2 (14.3%) patients. Revascularization options included the following:

- *Surgical revascularization* (57.1%) for ALI - open thrombectomy with/without endarterectomy/patch angioplasty and/or surgical bypass;
- *Endovascular revascularization* (7.1%) - percutaneous mechanical thrombectomy.

After the intervention, the ALI patients received anticoagulant therapy according to the protocol. The use of continuous postoperative heparin was associated with increased limb salvage and survival.

Limb outcomes and mortality

The mortality rate among patients with COVID-19 infection who developed ALI was high (42.9%). Revascularization (surgery/endovascular) was successful in 7 (50%) ALI patients, and 2 patients with failed revascularization required amputation. Two patients (14.3%) required reintervention due to recurrent arterial thrombosis within the first 48 hours. The overall rate of major amputations (below-knee amputation, above-knee amputation) was 28.6% **(Table 1)**.

Discussion

The ALI is a thrombotic complication typically associated with severe COVID-19 infection. However, critical limb ischemia often did not correlate with the severity of COVID-19 infection. The severity of respiratory infection and other symptoms of COVID-19 infection are often not consistent with the severity and level of vascular involvement. Moreover, ALI can be the primary presenting symptom of COVID-19 even in the absence of respiratory symptoms. In a study of 49 patients with ALI and COVID-19 infection, ALI was the initial presentation in 22 (45 %) patients. Thrombotic events in the setting of COVID-19 infection occurred at a median of 11 (5 to 20) days after the first symptoms of infection [5, 6].

The incidence of patients with COVID-19 associated with ALI who require hospitalization ranges from 3 – 15%. The prevalence of ALI is 4 – 21 per 100,000 hospitalized patients with COVID-19. Risk factors for ALI associated with COVID-19 infection include older age (≥ 75 years), obesity, diabetes mellitus and cardiovascular comorbidities. About 20% of patients, predominantly younger ones, without standard risk factors for arterial thrombosis, may develop ALI associated with mild COVID-19 infection [7]. Because ALI can occur without the common risk factors associated with arterial thrombosis, a high index of suspicion is necessary to detect nonspecific clinical forms of ALI and to provide timely targeted therapeutic treatment.

Thrombotic etiology of ALI, versus embolic, is predominant in patients with COVID-19 infection. Arterial thromboembolic events may occur despite the use of preventive or therapeutic doses of anticoagulant therapy [8].

Thrombosis of large (aorta, iliac, subclavian, axillary) and medium-sized (superficial femoral, brachial) arteries of the upper and lower extremities is the most common localization of thromboembolic processes that have been presented in previous studies. The lower extremities are affected more commonly (> 70%) compared to the upper extremities. Thrombosis of previous vascular reconstructions (stents, bypass grafts) has also been reported as a high-risk site for the thrombotic process. Thrombosis of small vessels with consequent distal trophic changes is often associated with the use of vasopressor agents. Thrombotic complications occur either as isolated arterial thrombosis or combined with thrombosis in other arterial or venous vascular beds [9].

The diagnosis of ALI is predominantly clinical. Vascular imaging (duplex ultrasound, CT angiography) confirms the location and extent of arterial obstruction. The severity of COVID-19 infection (stability of the patient) and severity of ALI determine the urgency and selection of vascular imaging modality and therapeutic approach. Laboratory findings are characterized by two states: hypercoagulable state and a state of systemic inflammation. Patients with COVID-19 may have a variety of abnormal coagulation patterns. D-dimer levels are significantly associated with increased mortality [10–12].

Anticoagulant therapy should be initiated immediately after the diagnosis of ALI is established. Decision on revascularization treatment (endovascular or open approach) of ALI associated with COVID-19 infection is based on the patient's overall stability (severity of systemic illness), degree of ischemia, and limb viability. Based on literature reviews, less than 50% of ALI patients with COVID-19 underwent limb salvage procedures and were treated with systemic anticoagulants only. Basic therapeutic approach in the treatment of arterial thrombosis is therapeutic anticoagulation followed by oral anticoagulants or low molecular weight heparin, with or without addition of an antiplatelet agent to reduce the incidence of recurrent thromotic incidents [13].

Despite attempts at revascularization (endovascular, open surgical, combination endovascular-open surgery), ALI associated with COVID-19 has high rates of limb loss, up to 35% of patients. Rethromboembolic events may occur despite the use of anticoagulant therapy. The mortality rate of ALI patients associated with severe COVID-19 is about 50% [6, 7].

Conclusion

The high incidence, even with thromboprophylaxis, high mortality rate and poor limb salvage outcomes in acute limb ischemia associated with severe acute respiratory syndrome coronavirus 2, encourage clinicians to apply individually tailored diagnostic and therapeutic approaches. Prolonged systemic use of heparin may improve the effectiveness of surgical treatment, limb salvage, and overall survival.

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Rad je primljen 5. I 2022.
Recenziran 10. I 2022.
Prihvaćen za štampu 13. I 2022.
BIBLID.0025-8105:(2021):LXIX:7-8:221-225.

Special Gynecological Hospital "Genesis", Novi Sad[1]

Original study
Originalni naučni rad
UDK 618.146-006-089.85
https://doi.org/10.2298/MPNS2108226D

LOOP ELECTROSURGICAL EXCISION IN THE TREATMENT OF PRECANCEROUS LESIONS OF THE CERVIX

ELEKTROEKSCIZIJA OMČOM U HIRURŠKOM TRETMANU PREKANCEROZNIH PROMENA NA GRLIĆU MATERICE

Srđan ĐURĐEVIĆ[1] and Jelena NIŠEVIĆ[1]

Summary

Introduction. In the period from 2010 to 2021, 150 patients were treated with loop electrosurgical excision of the cervix at the Special Gynecological Hospital "Genesis", Novi Sad. The aim of this study was to present the results of treatment, incidence of complications and recurrences after loop excision in the treatment of cervical dysplasia. **Material and Methods.** The study group A included 87 (58%) patients with cervical intraepithelial neoplasia, and the control group B included 63 (42%) patients with benign lesions of the cervix. **Results**. The total number of complications (bleeding, pain, infection) in the group A was recorded in 16 (20.7%) and in group B in 4 (6.4%) patients, and this difference was statistically significant. The absolute risk of complications in the group A was 18.39% and 6.34% in the group B. The relative risk of complications was 2.9, meaning that women in group A had a 3 times higher relative risk of complications compared to women in the group B. The absolute risk of recurrence in the group A was 4.56% and 4.76% in the group B. The relative risk of recurrence was 0.96. Women in the group A had a 4% lower relative risk of recurrence than women in the group B. The recurrence rate was lower in the group B (4.8%) compared to the group A (5.9%), but this difference was not statistically significant. **Conclusion**. Loop electrosurgical excision is the treatment of choice in the treatment of cervical dysplasia with good therapeutic outcomes and a low complication and recurrence rate.

Key words: Uterine Cervical Dysplasia; Cervical Intraepithelial Neoplasia; Colposcopy; Electrosurgery; Cervix Uteri; Risk Factors; Treatment Outcome; Postoperative Complications; Recurrence

Sažetak

Uvod. U periodu od 2010. do 2021. godine, u Specijalnoj ginekološkoj bolnici *Genesis* u Novom Sadu, 150 pacijentkinja je lečeno lup (*loop*) elektro-ekscizijom grlića materice. Cilj ove studije bio je predstavljanje rezultata lečenja, pojave komplikacija i recidiva nakon primene lup ekscizije u lečenju cervikalne displazije. **Materijal i metode.** Ispitivanu grupu A činilo je 87 (58%) pacijentkinja sa cervikalnom displazijom, a kontrolnu grupu B 63 (42%) pacijentkinje sa benignim promenama na grliću materice. **Rezultati.** Broj ukupnih komplikacija (krvarenje, bol, infekcija) u grupi A zabeležen je kod 16 (20,7%), a u grupi B kod četiri pacijentkinje (6,4%) i ta razlika je statistički značajna. Apsolutni rizik za pojavu komplikacija u grupi A bio je 18,39%, a u grupi B 6,34%. Relativni rizik od komplikacija bio je 2,9, što znači da žene u grupi A imaju tri puta veći relativni rizik od pojave komplikacija u odnosu na žene u grupi B. Apsolutni rizik od recidiva u grupi A je 4,56%, a u grupi B 4,76%. Relativni rizik od recidiva je 0,96. Žene u grupi A imale su 4% manji relativni rizik od recidiva nego žene u grupi B. Stopa recidiva bila je niža u grupi B (4,8%) u poređenju sa grupom A (5,9%), ali ova razlika nije statistički značajna. **Zaključak.** Lup elektro ekscizija je tretman izbora u lečenju displazije grlića materice, sa dobrim terapijskim rezultatima i niskom stopom komplikacija i recidiva.

Ključne reči: displazija grlića materice; cervikalna intraepitelijalna neoplazija; kolposkopija; elektrohirurgija; grlić materice; faktori rizika; ishod lečenja; postoperativne komplikacije; recidiv

Introduction

Human papillomaviruses (HPVs) cause a variety of lesions, from transient infections with minimal disorders to a complete change in the cervical mucosa with immature malignant cells, which are called cervical dysplasia, or cervical intraepithelial neoplasia (CIN) [1, 2]. Depending on whether only the lower or middle third of the epithelium is affected, or more than two-thirds, these changes are classified into 3 grades (CIN 1 – 3). They precede the onset of cancer because the lower border of the mucosa and cervical tissue (the so-called basement membrane) has not yet been damaged [3]. The diagnosis of CIN is made by taking a Papanicolaou cytological smear, colposcopic examination of the cervical mucosa, control of HPV smear, and taking samples for histopathological analysis, which makes a definite diagnosis [4]. The treatment of cervical dysplasia caused by HPV depends on the location and severity. Changes that do not go deep into the cervical canal and that are colposcopically visible are removed by electrosurgical excision [5]. There are several definitions in the medical literature re-

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Abbreviations

HPV – human papillomavirus
CIN – cervical intraepithelial neoplasia
ATZ – atypical transformation zone
ASC-US – atypical squamous cells of undetermined significance

ferring to electrosurgical excision with wire electrodes of different shapes in the treatment of various changes in the cervix [1–8]. This creates some confusion, because different terms are used for the same or similar surgical procedure on the uterine cervix, such as loop diathermy, large loop excision of transformation zone, loop electrosurgical excision procedure, loop conization, etc.

All these electrosurgical excision techniques cut and coagulate the tissue with a wire electrode at the same time. This provides removal of changes in the cervix detected by cytology and colposcopy and collection of a tissue sample for a definitive histopathological diagnosis [6, 7]. Loop-shaped wire electrodes are used to perform loop excision, while thin triangle-shaped loops are used for loop conization to remove abnormal tissue inside the cervical canal with the help of low-voltage electricity [5]. Due to its simple application, short duration, patient comfort, and treatment outcome, loop electrosurgical excision has a great application in the treatment of precancerous CIN and advantage over other methods such as classical knife conization, cryotherapy, and laser application [3–5]. The intervention is planned immediately after the end of menstruation and there must be no acute infection of the mucous membrane of the cervix and vagina. Indications for loop excision include precancerous lesions, cervical ectopy, polyps, chronically altered mucosa (cervicitis, erosive changes, postpartum obstruction, large retention cysts), etc. [3, 4]. Loop electrosurgical excision is performed under short-term intravenous anesthesia, but it can also be performed under local intracervical anesthesia. During the intervention, the altered part of the cervix is removed with a special electric loop and it is sent for histopathological examination **(Figure 1)**. Two hours after intervention, the patient goes home, rests during the day, and the next day she can perform usual activities. Complications of loop excision are rare and include bleeding, usually 7 – 20 days after the intervention [5, 8]. The aim of this study was to present the results of treatment, incidence of complications and recurrences after loop excision in the treatment of cervical dysplasia.

Material and Methods

This retrospective-prospective study was performed at the Special Gynecological Hospital "Genesis" in Novi Sad in the period from 2010 to 2021. One hundred and fifty patients with various changes on the cervix were treated with loop electrosurgical excision. The group A included 87 (58%) patients with CIN 1 – 3, while the control group B included a total of 63 (42%) patients with various benign lesions in the cervix (ectopia of the cylindrical mucosa, polyps, chronically inflamed mucosa, cervicitis, large retention cysts). Inclusion criteria for the study group A were one or more suspicious colposcopic findings on the surface of the vaginal part of the cervix (leukoplakia, mosaic, punctures, acetowhite epithelium, atypical transformation zone (ATZ), etc.) and/or a suspicious cytological smear (Papanicolaou smear grade 3A or higher, or Bethesda classification: atypical squamous cells of undetermined significance (ASC-US)). Exclusion criteria were acute inflammation of the cervical mucosa, positive cervical swabs, as well as biopsy-proven microinvasive or invasive cervical cancer on definite histopathological findings.

Patient preparation prior to the procedure included laboratory blood and urine tests, microbiological and cytological cervical smears, colposcopic examination of the mucous membrane of the lower genital organs (vulva, vagina, and cervix), electrocardiography of the cardiac activity, and examination by an anesthesiologist. In all patients, the procedure was performed under total intravenous anesthesia lasting from 5 to 10 minutes (propofol, midazolam, and fentanyl/remifentanil) and all patients were advised not to consume food or drink fluids 6 hours before the intervention. Prior to the procedure, a single dose intravenous antibiotic was prescribed (cephalosporin 1.0 g) and in obese patients and patients with associated comorbidities (hypertension, diabetes, other chronic diseases), prevention of venous thromboembolism was done by elastic bandages.

In the operating room, the patients are positioned in a gynecological position, with legs spread. After the introduction of total intravenous anesthesia, the vagina is wiped with a disinfectant (sol. octanisept, manufactured by Schulke) and, if necessary, immediately before the intervention, the cervix is stained with the so-called Lugol's solution containing iodine, in order to precisely mark the border of the altered tissue. Then, the cervix is grasped with a single-tooth tenaculum above the visible change and gently pulled forward. Using an electric loop and monopolar energy (Erbe model VIO® 3 V 1.0.x with current

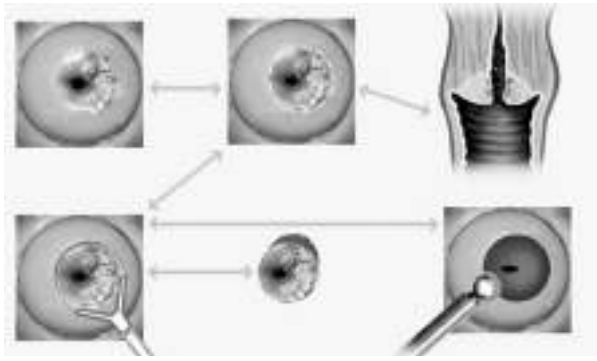

Figure 1. Schematic representation of loop electrosurgical excision of changes on the cervix
Slika 1. *Šematski prikaz elektroekscizije omčom promena na grliću materice*

Table 1. Suspicious colposcopic images, cytological findings, and HPV in the examined groups
Tabela 1. *Sumnjive kolposkopske slike, citološki nalaz i prisustvo humanih papilloma virusa prema ispitivanim grupama*

Tested Groups *Ispitivane grupe*	Colposcopy *Kolposkopija*		Cytology *Citologija*		HPV type *HPV tipizacija*	
	+	–	PA II or NILM *PA II ili NILM*	PA III or ASC-US and more/*PA III ili ASCUS i više*	+	–
A	69 (79.3%)	16 (20.7%)	38 (43.6%)	47 (56.4%)	82 (94.2%)	5 (5.8%)
B	2 (3.2%)	61 (96.8%)	60 (95.2%)	3 (4.8%)	5 (7.9%)	58 (92.1%)

Legend: Colposcopy + – presence of at least one suspicious colposcopic image (leukoplakia, mosaic, puncture, acetowhite epithelium, atypical transformation zone (ATZ)), Colposcopy – absence of suspicious colposcopic images, Cytology Papanicolaou smear - PA II/negative for intraepithelial lesion or malignancy (NILM) – negative finding, Cytology PA III, atypical squamous cells of undetermined significance (ASC-US) – positive finding, HPV + – presence of 1 or more human papillomaviruses, HPV – absence of human papillomaviruses
Legenda: *Kolposkopija + – prisustvo bar jedne sumnjive kolposkopske slike/leukoplakija, mozaik, punktacija, aceto white epitel, atipična zona transformacije), Kolposkopija - odsustvo sumnjivih kolposkopskih slika; Citološki Papanikolau razmaz II/NILM – uredan negativan nalaz, Citološki Papanikolau razmaz III, AS-CUS – atipične skvamozne ćelije neodređenog značaja – pozitivan nalaz, HPV + – prisustvo 1 ili vise humanih papilloma virusa, HPV – odsustvo prisutnih humanih papilloma virusa*

strength adjusted to each patient individually), a thin layer of abnormal cervical tissue is removed. Then, coagulation spray with the ball electrode stops capillary bleeding and forms a scar. **Figure 2** shows loop electrosurgical excision performed in the Special Gynecological Hospital "Genesis".

The IBM SPSS Statistics 22 software was used for statistical analysis. Differences between groups for continuous variables (age) were compared using Student's t-test for independent samples. For the analysis of categorical variables (incidence of bleeding, pain, infection, and any of the complications) the χ^2 test was applied between the groups. A p-value less than 0.05 was considered statistically significant.

Results

The average age in the study group A was 34.1 years, and in the control group B 31.7 years. In terms of the age, there was no statistically significant difference between the groups (A = 34.08 ± 11.06, B = 31.73 ± 8.08 years, p = 0.155). **Table 1** shows colposcopic and cytological findings, as well as the presence of HPV. Sixty-nine (79.3%) patients from the group A, and only two (3.2%) from the group B had one or more suspicious colposcopic findings, while 47 (56.4%) patients from group A and only 3 (4.8%) from the group B had a suspicious cytological finding. **Table 2** shows the histopathological finding after loop electrosurgical excision in the examined groups. **Table 3** shows the incidence of various complications and recurrences after loop excision in the examined groups. Complications occurred in 16 patients in the group A and in 4 patients in the group B. The absolute risk of complications in the group A was 18.39% and 6.34% in the group B. The relative risk of complications was 2.9, which indicates that women in the group A had a 3 times higher relative risk of complications compared to women in the group B. Pain was not observed in any of the groups. The incidence of bleeding was statistically significantly lower in the group B (4.8%) compared to the group A (18.5%), $\chi^2 = 6.132$, p = 0.013, while the incidence of infections showed no statistically significant difference between the groups: $\chi^2 = 0.032$, p = 0.858. The incidence of total complications was statistically significantly higher in the group A (18.8%) compared to the group B (6.3%), $\chi^2 = 4.817$, p = 0.028. There was no statistically significant difference between the age and the occurrence of bleeding (without bleeding: 32.76 ± 9.78, with bleeding: 35.06 ± 11.89 years, p = 0.21), infection (without infection: 33.11 ± 10.10, with infection: 29.00 ± 0.00 years, p = 0.092) or any of the complications (no complications: 32.48 ± 9.72, with complications: 34.45 ± 11.41 years, p = 0.28). The absolute risk of recurrence in the group A was 4.56% and 4.76% in the group B. The relative risk of recurrence was 0.96, which indicates that women in the group A had a 4% lower relative risk of recurrence compared to women in the group B. The recurrence rate was

Table 2. Histopathological finding
Tabela 2*. Patohistološki nalaz*

Tested Groups *Ispitivane grupe*	L SIL CIN 1	H SIL CIN 2, CIN 3	Ectopy *Ektopija*	Polyp *Polip*	Other findings: Nabothian cysts, chronic inflammation *Ostali nalazi: Ovule Nabothii, hronična inflamacija*
A	55 (63.3%)	32 (36.7%)	0	0	0
B	0	0	50 (79.4%)	5 (7.9%)	8 (12.7%)

Legend: L SIL - low-grade squamous lesion, H SIL - high-grade squamous lesion, CIN - cervical intraepithelial neoplasia, Nabothian cysts - retention cysts
Legenda: *L SIL – skvamozna lezija niskog stepena, H SIL– skvamozna lezija viskog stepena, CIN – cervikalna intraepitelijalna neoplazija, Ovule Nabothii – retencione ciste*

Table 3. Complications and recurrences after loop excision
Tabela 3. *Komplikacije i pojava recidiva nakon lup ekscizije*

Tested Groups/*Ispitivane grupe*	Bleeding/*Krvarenje*	Pain/*Bol*	Infection/*Infekcija*	Recurrence/*Recidiv*
A	15 (17.2%)	0	1 (1.1%)	4 (4.6%)
B	3 (4.8%)	0	1 (1.6%)	3 (4.8%)

Legend: Pain - appearance of pain associated with the intervention that requires the use of analgesics in the first 72 hours after the intervention, Recurrence of removed changes at check-ups 6 months and over after the intervention
Legenda: Bol – pojava bolnosti u vezi sa intervencijom koja zahteva primenu analgetika prvih 72 sata nakon intervencije, Recidiv – ponovna pojava uklonjenih promena na kontrolnim pregledima šest i više meseci nakon intervencije

lower in the group B (4.8%) compared to the group A (5.9%), but this difference was not statistically significant $\chi^2 = 0.008$, $p = 0.766$. There was no statistically significant difference in the age concerning the incidence of recurrences (without recurrence: 33.09 ± 9.97, with recurrence: 33.00 ± 10.14 years, $p = 0.981$).

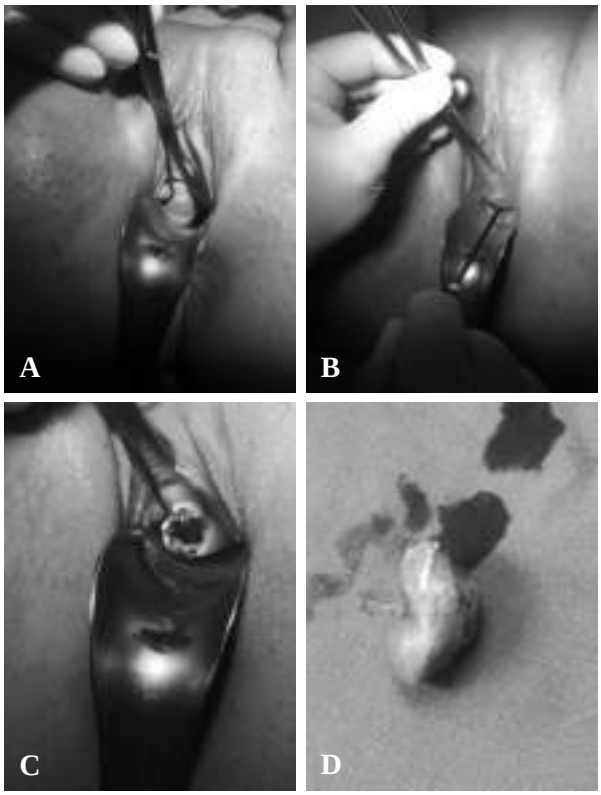

Figure 2. Loop electrosurgical excision (step by step)
Slika 2. *Elektrohirurška ekscizija omčom (korak po korak)*
A - Gentle grasp of the anterior lip of the cervix with a single-toothed tenaculum
A – Lagano prihvatanje i povlačenje grlića materice jednozubom zupčastom hvataljkom
B - Loop excision with a wire loop heated by electric current
B – Lup ekscizija elektroomčicom grlića materice
C - Spray coagulation of the cervix
C – Sprej-koagulacija lože promene
D - Removed part of the cervix for histopathological analysis
D – Odstranjeni deo (preparat) grlića materice za patohistološku analizu

Discussion

In 1989, W. Prendiville was among the first to publish a professional article on the application of loop electrosurgical excision in the treatment of cervical dysplasia [10]. He described a large loop of wire in the form of electrode that enables a deep incision of the transformation zone of the cervix with minimal thermal effects on the surrounding tissue, thus enabling a histopathological examination of the removed tissue. Over the past 30 years, after a great number of published professional articles, loop excision has become a standard, widely accepted minimally invasive surgical method in the treatment of cervical dysplasia [1–12]. In a large retrospective study, including 557 professional papers, Dunn et al. found that various complications have been reported in 9.7% of patients [13]. Less serious complications (9.6%) included abdominal pain and vaginal bleeding, while serious complications included profuse vaginal discharge and bladder spasm. In our study, including a total of 150 patients, various complications (bleeding, infection) were recorded in 20 patients (13.3%), which is slightly more than in the study of Dunn et al., but pain was not reported by any of our patients. According to the official recommendations of the Royal College of Gynecologists and Obstetricians in London, United Kingdom, light bleeding occurs in about 85% of all patients in the period of 2 – 30 days after loop excision [8]. In our study, after loop excision, bleeding was recorded in 20 (12%) patients, which is significantly less than in the reports and recommendations of the Royal College of Gynecologists and Obstetricians in London. The bleeding is related to the mechanism of healing and epithelialization of the cervical tissue after the electro excision procedure. The risk of narrowing or stenosis of the cervical canal after loop excision is 2 – 14% and is increased after repeated interventions, removal of a large volume of cervical tissue, and in the postmenopausal period, but we have not found this complication in our study. During the period of tissue recovery and epithelialization, great physical exertion and exercises, sexual intercourse, swimming and baths are prohibited. About 33% of patients have transient pain during sexual intercourse

in the first 3 months after the intervention, although this was not recorded in our patients [3, 5, 10]. Yap et al. found a statistically significant decrease in fear and anxiety in a total of 105 patients who were informed on the procedure before loop excision was performed under local anesthesia [14]. In their study, Nicols et al. investigated the application of ultrasound in the assessment of cervical tissue regeneration after loop excision in the treatment of CIN [15]. They concluded that loop excision results in a significant shortening of the cervix length. Stansiou et al. published a study that included 3,861 patients who underwent loop excision in the treatment of CIN in a period of 22 years [16]. The average age of the patients was 36.2 years, similar to 34.1 years in our study. After the intervention, the so-called »clean edges« without atypical cells were confirmed in 3,166 – 82%, in 437 – 11.3% the edges were positive, and in 258 – 6.7% the finding was unclear. A total of 239 – 6.2% of patients underwent another loop excision or conization of the cervix. The study shows that 90% of patients with positive or suspicious edges who underwent the second procedure were detected within 24 months after the first intervention. Kudoh et al., on the other hand claim that persistent HPV infection after primary treatment is a statistically significant and positive predictor of recurrence of abnormal cytology and CIN recurrence, where HPV 16 is directly responsible for the recurrence of CIN 2 and 3 changes [17, 18]. Bjorner et al. emphasize the importance of separating the so-called residual disease from CIN recurrence after the primary treatment with loop excision [19]. Women with residual CIN changes can benefit significantly from early colposcopy, biopsy, or diagnostic conization, because in addition to residual changes, small-volume occult cervical cancer can be detected. In our study group A (patients with treated CIN changes), negative edges were present in all cases after loop excision, and recurrence was recorded in 4 (4.6%) patients in the period of 12 – 24 months after the intervention. The recurrences were detected using colposcopy, cytology, and HPV typing in all cases, they were local in nature (near the edges of the resection) and were resolved by repeat loop excision.

Conclusion

There was no statistically significant difference between the groups with respect to age. In the group A, one or more suspicious colposcopic findings or a suspicious cytological Papanicolaou smear, indicating cervical intraepithelial neoplasia, were found in 69 (79.3%) and 47 (56.4%) patients, respectively, while a positive human papillomavirus smear was found in 82 (94.2%) patients. Patients in the group A had a three times higher relative risk of complications (bleeding, infection) compared to women in the group B, while pain was not recorded in any of the observed groups. The incidence of bleeding, as one of the most significant complications, was statistically significantly lower in the control group B compared to the group A, while the incidence of infection was not statistically significantly different between groups. The absolute risk of recurrence in the group A was 4.56% and 4.76% in the group B. The relative risk of recurrence was 0.96 and women in the group A had a 4% lower relative risk of recurrence compared to women in the group B. The incidence of recurrences was not statistically significant in either group. In the study group A, in patients with treated cervical intraepithelial neoplasia, negative edges were found after loop excision in all cases, and recurrence was recorded in 4 (4.6%) patients in the period of 12 – 24 months after the intervention. The recurrences were local, found near the edges of the resection, and they resolved after a repeat loop excision. Loop electrosurgical excision is a treatment of choice in the treatment of cervical dysplasia, with good therapeutic outcomes and a low complication and recurrence rate.

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Rad je primljen 26. X 2021.
Recenziran 25. XI 2021.
Prihvaćen za štampu 25. XI 2021.
BIBLID.0025-8105:(2021):LXIX:7-8:226-231.

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Original study
Originalni naučni rad
UDK 616-083.98-053.4:572.512.087
https://doi.org/10.2298/MPNS2108232B

THE ACCURACY OF THE BROSELOW TAPE IN WEIGHT ESTIMATION AMONG PEDIATRIC POPULATION

TAČNOST BROSELOW TRAKE U PROCENI TELESNE TEŽINE U PEDIJATRIJSKOJ POPULACIJI

Branislava BRESTOVAČKI SVITLICA[1, 2] and Zorana STRAJNIĆ[3]

Summary

Introduction. Accurate body weight estimation in pediatric emergencies is of crucial importance for successful interventions. One of the most commonly used one-dimensional methods for quick estimation of body weight is the Broselow pediatric emergency tape that is based on correlation between body weight and body height. The objective of this study was to determine the accuracy and precision of the estimated body weight with the Broselow tape in children. **Material and Methods.** The study was conducted as an observational cross-sectional study at the Novi Sad Health Center. Data on body weight and height were collected according to a pre-prepared protocol from randomly selected medical records of 750 children who visited the Counseling Center in the period of six months and classified into three age categories. **Results.** The accuracy of the Broselow tape was 46.3% (95%, confidence interval 42.7 - 49.9), i.e. in 46.3% of children the deviation of the estimated values based on the Broselow tape in relation to the reference (measured) body weight did not exceed 10%. The average percentage error was -3.10 ± 17.4% and further analysis revealed a significant difference in the three analyzed groups ($F = 49.182$, $p < 0.001$). The average absolute percentage error was 13.7 ± 11.0% and further analysis revealed a significant difference in the three analyzed groups ($F = 13.116$, $p < 0.001$). **Conclusion.** The highest reliability of the Broselow tape was found in the group of children aged 2 to 6 years. Although this method offers several advantages over other methods for assessing body weight in pediatric emergencies, healthcare professionals must be aware of its limitations.

Key words: Anthropometry; Body Weight; Body Height; Child; Pediatric Emergency Medicine; Medication Errors; Risk Factors

Sažetak

Uvod. Za uspešno sprovođenje hitne intervencije u pedijatrijskim urgentnim situacijama od presudnog značaja je podatak o telesnoj težini pacijenta. Jedna od najčešće korišćenih jedno-dimenzionalnih metoda za brzu procenu telesne težine je *Broselow* traka koja radi po principu korelacije telesne težine i telesne visine. Cilj rada je utvrđivanje tačnosti i preciznosti procenjene telesne težine *Broselow* trakom kod dece u ispitivanoj populaciji. **Materijal i metode.** Istraživanje je sprovedeno kao opservaciona studija poprečnog preseka, u Domu zdravlja Novi Sad. Podaci o telesnoj težini i visini prikupljani su iz nasumice izabranih kartona 750 dece prema unapred pripremljenom protokolu, stratifikovani u tri starosne kategorije i koja su posećivala savetovalište u periodu od šest meseci. **Rezultati.** Tačnost *Broselow* trake iznosi 46,3% (95% interval poverenja: 42,7–49,9), odnosno kod 46,3% dece važi da odstupanje ocenjenih vrednosti na osnovu *Broselow* trake u odnosu na referentnu (izmerenu) telesnu težinu ne prelazi 10%. Prosečna procentualna greška iznosi -3,10 ± 17,4% i daljom analizom utvrđena je značajna razlika u odnosu na tri analizirane grupe ($F = 49,182$, $p < 0,001$). Prosečna apsolutna procentualna greška iznosi 13,7 ± 11% i daljom analizom utvrđena je značajna razlika u odnosu na tri analizirane grupe ($F = 13,116$, $p < 0,001$). **Zaključak.** Najveća pouzdanost *Broselow* trake utvrđena je u grupi dece starosti od 2 do 6 godina. Iako ova metoda nudi veliki broj prednosti u odnosu na ostale metode za procenu telesne težine u urgentnim stanjima kod dece, zdravstveni radnici moraju biti svesni i njenih ograničenja.

Ključne reči: antropometrija; telesna težina; telesna visina; dete; pedijatrijska urgentna medicina; greške u primeni lekova; faktori rizika

Introduction

A pediatric emergency deparbody weightent is a professional challenge for all health professionals for several reasons. Firstly, there are many different clinical conditions that are not common and healthcare professionals have no experience in conducting interventions [1, 2]. Secondly, it is necessary to quickly determine various parameters such as drug dosage, equipment size, defibrillator energy, all based on age and body weight [3, 4]. Ideally, the child should be weighed; however, this is not always possible in emergency management, such as during resuscitation or trauma. Over the past decades, various methods have been used to assess body weight, each with its advantages and limitations [5]. The most commonly used methods include assumptions or estimates [6] of parents or health professionals that are not completely reliable [7, 8]. Body weight estimation formulas (advanced pediatric life support, Luscombe and Owens, Nelson formula, and others) are considered the oldest methods and are generally recommended in pediatric literature, although many studies indicate insufficient reliability [9, 10].

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Abbreviations

MPE – mean percentage error
MAPE – mean absolute percentage error
ANOVA – one-way analysis of variance
CI – confidence interval

The Broselow tape is a color-coded zone system which was first introduced in 1988 as a rapid method for determining drug dosages and emergency equipment sizing in pediatric emergencies based on the patient's length. The Broselow tape was developed based on the 50th percentile of children's weight at the National Center for Health Statistics in the United States [11], and it is recommended by the Advanced Trauma Life Support and the Pediatric Advanced Life Support in the United States and Europe [2]. In emergencies, when every second counts, it is not reasonable to spend valuable time calculating the body weight, the dose of medication, estimating the required size of equipment, the required energy for the defibrillator, and so on [11]. The Broselow tape allows estimation of the body weight quickly and easily and then determining standardized emergency drug dosages and equipment size using a color-coded zone system based on height–weight correlation [2, 5]. Furthermore, in addition to saving time, the possibility of error in calculating the dose and preparation of the drug is significantly reduced, and so is the stress of health workers which is mainly present during child resuscitation.

A number of studies performed in developed countries showed that the tape is valuable for estimating children's weight [2, 12–15]. However, recent studies suggest that the tape may have partial reliability in children of different ethnicity and nutritional status. For that reason, debate still continues about its validity in different age groups, weight groups and populations in different countries [16, 17]. If the weight estimation is inaccurate, or the methodology is prone to error when used during emergency situations, then the child is at risk of medical error. Therefore, it is important to use an accurate and simple method for assessing body weight in emergency situations [16]. The aim of this study was to determine the accuracy and precision of the estimated body weight using the Broselow tape in children aged 1 month to 12 years.

Material and Methods

This was an observational, "virtual" weight estimation study (one in which weight estimations are calculated from a database of anthropometric measurements, rather than a study in which a weight estimation system is directly applied to an individual child). The research included a random selection of health records of 750 children who visited the Counseling Center of the Health Center Novi Sad, in the period from January to June 2019.

The sample was stratified by age: 250 children from 1 month to 24 months of age (up to 2 years and ≤ 12 kg); 250 children from 25 months to 72 months (from 2 to 6 years and 12.1 kg to 18 kg); 250 children from 73 months to 144 months of age (6 to 12 years ≥ 19 kg). Inclusion criteria: pediatric population, aged one month to 12 years of both genders. Exclusion criteria: children younger than 1 month and children older than 12 years, children with amputated limbs, with congenital or acquired deformity, cerebral palsy, and children with cancer.

Data were collected according to a pre-prepared protocol including gender, age, body length/height and body weight. Objective measurement of children's body weight and length/height was performed according to the accredited procedure of the institution (number 2120/154). Based on the collected data, each child was assigned a color zone according to the Broselow tape (Broselow Pediatric Emergency Tape, 2007, Edition B, Armstrong, Medical Industries, IL, USA). The data used from the literature are valid for conducting a virtual study. A study by Wells et al. confirmed the validity of such studies [18].

The study was approved by the Ethics Committee of the Health Center Novi Sad (No 21/29-2) and Ethics Committee of the Faculty of Medicine in Novi Sad. In order to provide anonymity of the respondents, data were collected without any personal identification, and all data were treated with high confidentiality.

Statistical data processing was performed using the statistical package IBM SPSS Statistics 21. The data analysis followed the recommended methods for weight estimation which focus on evaluating the bias, precision and overall accuracy of the weight estimation systems. Pearson's correlation was applied to determine the relationship between measured and estimated body weight values based on the Broselow tape. T-test for dependent samples was used to compare the measured and estimated body weight values. The following parameters were used to measure bias: the difference between the measured and estimated value (residual), mean percentage error (MPE), the difference between the measured and estimated value expressed as percentage. A positive MPE indicates an overestimation of weights on average, and a negative MPE indicates underestimation. The mean absolute percentage error (MAPE) was used as a measure of precision, that is, the absolute value of the difference between the measured and estimated value divided by the measured value expressed as a percentage. To measure the accuracy of the method, the percentage of the estimated body weight with ≤ 10% and ≤ 20% of the measured value was used (PW10, PW20). A PW10 of > 70% and a PW20 > 95% was considered to be an acceptable accuracy for the weight estimation method [16]. The Bland-Albody weightan method is a graphical method that compares two methods (or measurements) showing the average difference. This method is used to calculate the average difference between the two bias methods with limits of agreement defined by a 95% prediction. One-way analysis of variance (ANOVA) was used to compare the MPE and MAPE between the three

analyzed groups. Further analysis included the application of the Tukey test. The chi-square test was used to compare accuracy measures (PW10, PW20) between the three analyzed groups. The p value < 0.05 was considered statistically significant.

Results

The sample included data from the health records of 750 children. There were slightly more females in the total sample (N - 388; 51.7%). The average age of children was 3.6 years ± 3.3. The average measured body weight in the sample was 18.2 ± 11.5 kg. **Table 1** shows the distribution of respondents stratified into three age categories in relation to the estimated values of body weight according to the Broselow tape.

Scatter plot of measured and estimated values, template modeling body weight score, based on Broselow tape, confirmed a high correlation between these values in the youngest age group ($r = 0.811$, $p < 0.001$), group II ($r = 0.593$, $p < 0.001$) and group III ($r = 0.716$, $p < 0.001$). The average difference between measured and estimated values of body weight in the first group was 0.11 (95% confidence interval (CI): - 0.27 and 0.05), 0.09 in group II (95% CI: -0.39, 0.22) and -4.97 kg in the third group (95% CI: -6.02, 3.93). Using the t-test for related samples, no significant difference in the measured and estimated values of body weight was found in the first ($p = 0.189$) and the second ($p = 0.585$) age group, while in the third group there was a statistically significant difference ($t = 9.398$, $p < 0.001$).

Comparison between estimated and measured weight based on the Broselow tape

A measure of bias

The MPE was -3.10 ± 17.4% (Med = -3.7%) **(Table 2)**. Using ANOVA, a significant difference in the MPE was found in relation to the three analyzed groups ($F = 49.182$, $p < 0.001$). Further analysis revealed a significant difference in MPE between group I (1.1 ± 15.7%) and group III ($p < 0.001$), as well as between group II (1.1 ± 15.6%) and group III ($p < 0.001$), with a significantly higher value in group III (-11.4 ± 17.6%; Med = -10.8%).

A measure of precision

The MAPE was 13.7 ± 11.0% (Med = 10.8%), with the highest value determined in group III (16.6 ± 12.8%; Med = 14.3%) **(Table 2)**. Using ANOVA, a significant difference in the MAPE was found in relation to the three analyzed groups ($F = 13.116$, $p < 0.001$). Further analysis revealed a significant difference in MAPE between groups I and III ($p < 0.001$), as well as between groups II and III ($p < 0.001$).

A measure of accuracy

The accuracy of the Broselow tape body weight score in the sample ($n = 750$) was 46.3% (95% CI: 42.7-49.9), namely, in 46.3% of children the deviation of the estimated values based on the Broselow tape in relation to the reference (measured) body weight did not exceed 10% **(Table 2)**. Furthermore, a significant difference was found in the accuracy of the body weight estimate using the Broselow tape (PW10) between the three analyzed groups ($\chi^2 = 11.145$, $p = 0.004$), with the highest accuracy achieved in group II (53.2%) and the lowest in group III (38.4%). The **Table 2** shows the measured bias, precision, and accuracy of the Broselow tape.

Bland-Albody weightan method

Graph 1 shows Bland-Albody weightan plots of percentage error for actual weights in group I. The solid line represents the MPE and the dashed lines represent the 95% limits of agreement, the lower limit of agreement of values (D = d-1.96 * SD), and the upper limit of agreement (G = d + 1.96 * SD). In 95% the differences between the estimated and measured body weight values in group I were within the limits of -2.7 kg and 2.5 kg.

The Bland-Albody weightan diagram in group II is shown in **Graph 2.** In 95% the differences

Table 1. Distribution of respondents in relation to the estimated and measured body weight/color zone using the Broselow tape

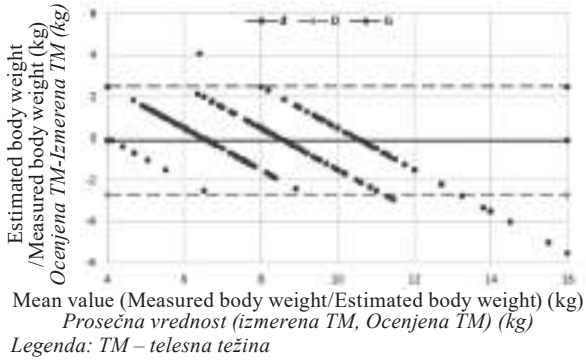
Tabela 1. *Distribucija ispitanika u odnosu na procenjene vrednosti telesne mase pomoću Broselow trake i izmerene telesne težine po kolor zonama*

Broselow-color zone (values kg) *Broselow-boja (opseg vrednosti telesne težine)*	Measured values *Procenjena vrednost (kg)*	Total *Ukupno* n (%)	Group I *Grupa I* n (%)	Group II *Grupa II* n (%)	Group III *Grupa III* n (%)
Gray (3 - 5 kg)/*Siva*	4	7 (0.9)	7 (2.8)		
Pink (6 - 7 kg)/*Roze*	6.5	88 (11.7)	88 (35.2)		
Red (8 - 9 kg)/*Crvena*	8.5	86 (11.5)	86 (34.4)		
Purple (10 - 11 kg)/*Ljubičasta*	10.5	70 (9.3)	69 (27.6)	1 (0.4)	
Yellow (12 - 14 kg)/*Žuta*	13	85 (11.3)		85 (34.0)	
White (15 - 18 kg)/*Bela*	16.5	141 (18.8)		141 (56.4)	
Blue (19 - 23 kg)/*Plava*	21	152 (20.4)		23 (9.2)	129 (51.6)
Orange (24 - 29 kg)/*Narandžasta*	26.5	45 (6.0)			45 (18.0)
Green (30 - 36 kg)/*Zelena*	33	76 (10.1)			76 (30.4)

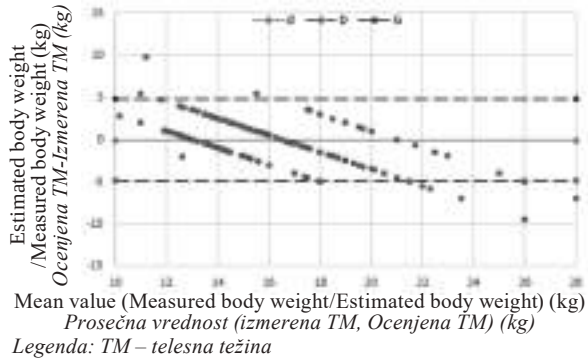
Table 2. Measures of bias, precision, and accuracy of the Broselow tape
Tabela 2. *Mere pristrasnosti, preciznosti i tačnosti Broselow trake*

Parameters *Parametri*	Age group I *I starosna grupa*	Age group II *II starosna grupa*	Age group III *III starosna grupa*	Total *Ukupno*	p
	$\overline{X} \pm SD$	$\overline{X} \pm SD$	$\overline{X} \pm SD$	$\overline{X} \pm SD$	
MPE[1]	1.1 ± 15.7	1.1 ± 15.6	-11.4 ± -17.6	-3.10 ± 17.4%	F = 49.182, p < 0.001
MAPE[2]	12.5 ± 9.5	12.1 ± 9.9	16.6 ± 12.8	13.7 ± 11.0%	F = 13.116, p < 0.001
PW10[3] (95% CI)[4]	47.2 (40.9, 53.6)	53.2 (46.8, 59.5)	38.4 (32.3, 44.7)	46.3 (42.7, 49.9)	$\chi 2$ = 11.145, p = 0.004
PW20 (95% CI)	77.6 (73.2, 83.7)	77.6 (73.2, 83.7)	77.6 (73.2, 83.7)	75.5 (72.2, 78.5)	$\chi 2$ = 27.928, p < 0.001
PW30 (95% CI)	95.6 (92.3, 97.8)	96.8 (93.8, 98.6)	96.8 (93.8, 98.6)	92.7 (90.6, 94.4)	$\chi 2$ = 27.822, p < 0.001

Legend**:** MPE[1] - average percentage error; MAPE[2] - average absolute percentage error; Accuracy[3] - percentage of the estimated value ≤ 10% (PW10), ≤ 20% (PW20) and ≤ 30% (PW30); CI - confidence interval
*Legenda**:** [1]MPE - Prosečna procentualna greška; [2] MAPE – Prosečna apsolutna procentualna greška; [3] Tačnost – kategorija procentualne greške – unutar 10% (PW10), unutar 20% (PW20) i unutar 30% (PW30), CI - interval poverenja*



Graph 1. Bland-Altman plot of agreement in group I
Grafikon 1. *Bland-Altman dijagram za I grupu*



Graph 2. Bland-Altman plot of agreement in group II
Grafikon 2. *Bland-Altman dijagram za II grupu*

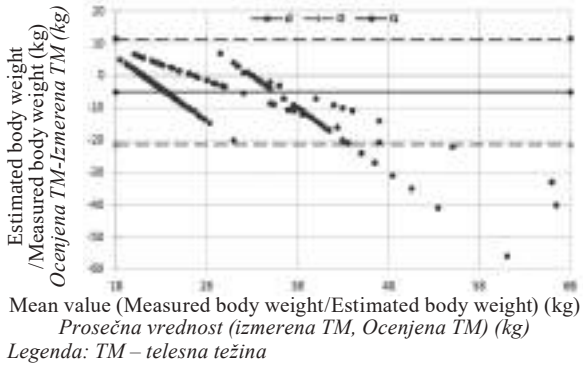
between the estimated and measured body weight values in group II were within the limits of -4.9 kg and 4.8 kg.

The Bland-Albody weightan diagram in group III is shown in **Graph 3**. In 95% the differences between the estimated and measured body weight values in group III were within the limits of -21.4 kg and 11.4 kg.

Discussion

Overestimation or underestimation of body weight can have severe negative consequences in the pediatric population [17]. Some authors believe that all factors that potentially affect patient safety must be addressed and minimized, especially medication dosage calculation errors. Although the Broselow tape is widely used around the world, it is necessary to determine its accuracy and reliability before using it in a certain population.

In this study, by means of statistical analysis, significant correlation between the measured and estimated body weight values, using the Broselow tape, was confirmed. However, there are some differences in the reliability of the Broselow tape in relation to the age and body weight of children. The Broselow tape has the highest reliability in children up to 6 years of age and body weight up to 18 kg, while in older children there are certain deviations. The accuracy and precision of the estimated body weight decreases with increasing body length/height and weight, and thus it is lowest in children with body weight ≥ 19 kg. The Broselow tape estimated weight of 46% of the study population within 10% of a child's measured weight. A significant difference was found in the accuracy of the estimated body weight between the three analyzed groups, with the highest accuracy being achieved in the group of children from 2 to 6 years, 12.1 kg to 18 kg (53.2%), respectively. The study of Khouli et al. showed that Broselow tape included 46% of children within 10% of a child's measured weight [13], and the study of Rosenberg et al. showed that Broselow tape



Graph 3. Bland-Altman plot of agreement in group III
Grafikon 3. *Bland-Altman dijagram za III grupu*

included more than half of the children within 10% of a child's measured weight [7]. In a review paper based on 46 analyzed studies, Young et al. [4] found an average accuracy of estimated body weight using the Broselow tape in 54% of children and so the tape showed to be significantly more precise in relation to the formula based on the age.

Comparing the obtained results with the results of other studies there are certain similarities regarding reliability of the Broselow tape. The study conducted by Lubiz et al. in 1988 showed that the Broselow tape is a simple, safe and reliable method for assessing body weight, with reliability being highest in the group of children from 3.5 to 10 kg, while in children over 25 kg there were some deviations [19]. In fact, most studies indicate that the use of the Broselow tape is more reliable in younger children and children with lower body weight [2, 3, 8, 15–18]. A number of studies suggest that in high-income countries, the tape underestimates weight, resulting in medication underdosing and unsuccessful resuscitation. Taking into account the results of a large number of studies and the trend of increasing obesity in children in developed countries, the authors of the tape made several corrections [11]. On the other hand, there is evidence that the tape significantly overestimates body weight in middle- and low-income countries and the latest version can lead to medication overdosing [12]. Surveys conducted in Sudan, Nigeria, Nepal, South Africa, and India show that increased weight and age of children significantly decreases the reliability of the tape [14, 15, 20–22]. Several researchers have therefore suggested that the estimated body weight of Indian children should be corrected by 10% [20]. From the above, it can be seen that the results of research conducted in different economic and ethnic environments are not consistent. The variations may be due to varied number of reasons. As a result, in additional research, similar methods have been developed based on the Broselow tape (Kinder-Sicher KS; T.O. Zugck, Heide, Germany) including the ruler "Pediatape", Pädiatrisches Notfalllineal (PNL; Alpha 1 Werbedesigne K., Falkenberg, Germany) and Paulino System (PS; Paulino-System UG & Altonaer Werbewerkstatt, Hamburg, Germany) [23]. However, they did not show significant precision compared to the Broselow tape. In addition, two-dimensional approaches are being developed, such as the Mercy method, Pediatric advanced weight prediction in the emergency room tape, Wozniak method, and others, which include two factors in the evaluation of body weight: body length (humerus and ulna length) and the upper arm and waist [3, 23]. These methods have shown to be more accurate on the one hand, but the inclusion of more determining factors increases the risk of inaccuracy on the other hand. In situations where the clinical status of patients requires making decisions based on many unknown factors, methods that help in more precise determination of the child's body weight, contribute significantly to reducing potential errors [16]. The choice of method for assessing the weight to be used during child care in emergencies is very important because it must be reliable, accurate, accessible, easy to read, and easy to use [3].

Conclusion

The Broselow tape is most reliable in children weighing from 12.1 kg to 18 kg. With increasing the weight, the reliability of the prediction decreases, especially in children weighing > 18 kg. The Broselow tape, used in the estimation of body weight in children, is a simple, useful, and generally accurate tool in emergency care, helping health care providers who care for critically ill children, especially in settings where the age is unknown and relatives are not available. Emergency care providers should be aware of the potential benefits and limitations of this commonly used tool in pediatric resuscitation.

This study has several limitations. First, the study was conducted in a medical institution and the results cannot be generalized for all the pediatric population. Second, our study did not test the dosages and equipment size, whether or not appropriate for the study population. Despite the shortcomings, this study may represent a significant contribution to improving the quality of care in pediatric emergency deparbody weightents. In order to establish the applicability and reliability of the Broselow tape to avoid the negative consequences of incorrectly estimated body weight, there is a need for further research by expanding the sample.

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Rad je primljen 21. X 2021.
Recenziran 4. XI 2021.
Prihvaćen za štampu 15. XI 2021.
BIBLID.0025-8105:(2021):LXIX:7-8:232-237.

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Original study
Originalni naučni rad
UDK 612.349.8.08
https://doi.org/10.2298/MPNS2108238I

APPROPRIATE USE OF THE HOMEOSTASIS MODEL ASSESSMENT OF INSULIN RESISTANCE INDICES

ODGOVARAJUĆA UPOTREBA INDEKSA HOMEOSTAZNIH MODELA PROCENE INSULINSKE REZISTENCIJE

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Summary

Introduction. Timely detection of insulin resistance is of great importance and a number of indices have been developed for its evaluation, among which the homeostasis model assessment of insulin resistance index is the most commonly used in clinical practice. However, it can be calculated via two different models – homeostasis model assessment 1 and homeostasis model assessment 2. Most studies determine the cut-off values of the study population using the homeostasis model assessment 1, while recently most physicians use homeostasis model assessment 2 in everyday clinical practice. The aim of our study was to examine whether there was a difference in the values of homeostasis model assessment of insulin resistance and homeostasis model assessment of panceratic beta cells function calculated using these two models. **Material and Methods.** Laboratory findings of 42 patients who were diagnosed with glycemia and insulinemia were used in this study. Fasting and postprandial glycemia and insulinemia were used to calculate homeostasis model assessment indices using homeostasis model assessment 1 and homeostasis model assessment 2. **Results**. When comparing the values of the homeostasis model assessment of insulin resistance and homeostasis model assessment B indices, calculated via homeostasis model assessment 1 and homeostasis model assessment 2, we found a statistically significant difference ($p < 0.001$) which was also obtained when comparing the values of the homeostasis model assessment B index. Linear correlation analysis showed a significant positive correlation between the measured values of the homeostasis model assessment of insulin resistance (calculated via both models) and postprandial insulinemia at 120 minutes ($p < 0.005$). **Conclusion.** The results indicate that homeostasis model assessment 2 yields significantly lower homeostasis model assessment of insulin resistance and homeostasis model assessment B index values than when calculated by the homeostasis model assessment, which may be a stumbling block in the use of homeostasis model assessment index. It is necessary to pay attention which homeostasis model assessment model was used to define the cut-off values of these indices, and to use the same model in the diagnosis of insulin resistance in each patient in everyday clinical practice.

Key words: Insulin Resistance; Blood Glucose; Postprandial Period; Glucose Tolerance Test; Insulin; Correlation of Data; Clinical Laboratory Techniques

Sažetak

Uvod. Pravovremeno otkrivanje insulinske rezistencije je od višestrukog značaja, stoga su razvijeni brojni indeksi za njenu procenu među kojima je u kliničkoj praksi najčešće korišćen indeks homeostaznog modela za procenu insulinske rezistencije. On se može računati na dva načina – putem homeostaznog modela za procenu insulinske rezistencije 1 i homeostaznog modela za procenu insulinske rezistencije 2 modela. Većina studija definiše *cut-off* vrednosti svoje populacije koristeći model 1, dok je u poslednje vreme upotreba modela 2 sve prisutnija. Cilj našeg rada je ispitivanje da li postoji razlika u vrednostima indeksa dobijenih homeostaznim modelom za procenu insulinske rezistencije i homeostaznim modelom za procenu funkcije beta ćelija pankreasa računatih pomoću ova dva modela. **Materijal i metode.** Za studiju su upotrebljeni laboratorijski nalazi 42 pacijenta kojima su određivane vrednosti glikemije i insulinemije našte i u toku postprandijalnog testa, a potom računate vrednosti modelima 1 i 2**. Rezultati.** Prilikom poređenja vrednosti indeksa homeostaznog modela za procenu insulinske rezistencije, računatog putem modela 1 i 2, uočili smo njihovu statistički značajnu razliku ($p < 0,001$) što je dobijeno i pri poređenju vrednosti indeksa homeostaznog modela za procenu funkcije beta ćelija pankreasa. Linearnom korelacionom analizom utvrđen je statistički značajan stepen pozitivne korelacije između izmerenih vrednosti indeksa homeostaznog modela za procenu insulinske rezistencije računatog putem oba modela sa insulinemijom u 120. minutu postprandijalnog testa ($p < 0,005$). **Zaključak.** Dobijeni rezultati pokazuju da indeksi homeostaznih modela za procenu insulinske rezistencije koji se računaju modelom 2 daju statistički značajno niže vrednosti indeksa nego kada se računaju pomoću modela 1, a to može da stvori problem prilikom upotrebe ovog indeksa. Neophodno je obratiti pažnju na to koji je od dva homeostazna modela za ocenu insulinske rezistencije korišćen za definisanje granične, *cut off,* vrednosti ovih indeksa, te isti model primeniti i pri ispitivanju insulinske rezistencije kod svakog pacijenta u svakodnevnoj kliničkoj praksi.

Ključne reči: insulinska rezistencija; glikemija; postprandijalni period; test tolerancije na glukozu; insulin; korelacija; kliničke laboratorijske tehnike

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Abbreviations

HOMA	– homeostasis model assessment
HOMA-IR	– homeostasis model assessment of insulin resistance
OGTT	– oral glucose tolerance test
TyG	– triglyceride and glucose
SD	– standard deviation

Introduction

Metabolic syndrome, closely linked to the lack of physical activity and hypercaloric diet rich in simple carbohydrates, is a major challenge for modern medicine [1]. The root of this condition is insulin resistance accompanied by dyslipidemia, hypertension, hypercoagulability, and visceral obesity. Furthermore, altered hormonal activity of the adipose tissue in obesity may lead to chronic inflammation [1, 2]. Due to the many complications stemming from this condition, early diagnosis is of utmost importance, particularly the presence of insulin resistance. In clinical practice, latent impairments of glucose metabolism are commonly discovered by using different tolerance tests, e.g. the oral glucose tolerance test (OGTT), especially when measuring insulinemia as well. However, reference values for insulinemia at 120 minutes of the OGTT are not defined, forcing physicians to rely solely on their clinical experience.

In order to define insulin resistance better and ease the path to the diagnosis, several indices of insulin resistance have been developed. These indices use the values of fasting glycemia and insulinemia alone or combined with the values measured after oral glucose uptake in order to detect insulin resistance in its early phase. Two of the most well-known indices of insulin resistance are the homeostasis model assessment of insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) which use only fasting glucose and fasting insulinemia. Other indices, such as Stumvoll and Matsuda are more precise, since they combine fasting values of glycemia and insulinemia with the values obtained after and during OGTT as well as some other parameters [3–5]. McAuley and triglyceride and glucose (TyG) indices include triglyceride concentration to assess insulin resistance [6, 7]. Of the indices mentioned above, HOMA-IR is the most frequently used in clinical practice. It can be used via two available modalities: HOMA-IR formula (HOMA1 model) and HOMA calculator (HOMA2 model). With the HOMA-IR formula, we can calculate HOMA-IR index (estimate of insulin resistance) and HOMA-B index (estimate of insulin secretion). On the other hand, HOMA calculator is an improved model compared with the formula, but the equations that the calculator uses are unknown [8, 9]. Besides HOMA-IR and HOMA-B, the calculator also yields a HOMA-S index which is an estimate of insulin sensitivity. It must be pointed out that the cut-off values of HOMA-IR need to be defined separately for each study population, since the values depend on ethnicity, genetic predisposition, food culture etc. [10]. Most studies use the HOMA-IR formula when trying to define the cut-off values for their respective values [10, 11], even though lately the use of HOMA calculator is more prevalent in everyday practice. Therefore, the aim of this paper is to examine differences in HOMA-IR and HOMA-B values obtained by using the HOMA1 model (formula) compared with HOMA2 model (calculator).

Material and Methods

This study included laboratory results of 42 patients (aged from 21 to 66 years), 28 female and 14 male. The laboratory measurements, fasting and postprandial glucose and insulin levels, were performed in the Institute of Laboratory Diagnostics "Biotest" in Novi Sad in order to identify abnormalities in glucose tolerance. Samples were taken between November 2020 and March 2021, with the consent of the Institute director.

Fasting (at least 8 hours after the last meal) and postprandial (120 minutes after the beginning of the postprandial test) glucose and insulin levels were measured. During the postprandial test patients ate a meal containing at least 75 grams of carbohydrates. After that, they did not take any food, sugary drinks, and were not physically active for 120 minutes when a new blood sample was taken [12]. Serum glucose levels were measured using spectrophotometry (reference range: 3.9 – 6.1 mmol/L (fasting); < 7.8 mmol/L (postprandial); Cobas Integra 400, SPFT), while serum insulin was measured using electrochemiluminescence (reference range: 2.0 – 25.0 μIU/mL (fasting); Cobas e411, ECLIA). Exclusion criteria were patients with fasting and postprandial hyperglycemia and fasting hyperinsulinemia.

Fasting glycemia and fasting insulinemia were used to obtain HOMA indices, using two available models. HOMA1 model calculates the HOMA-IR and HOMA-B indices by using a well-known formula: HOMA-IR: fasting glycemia [mmol/L] × fasting insulin [μU/mL]/22.5; HOMA-B (%): (20 × fasting insulin [μU/mL])/(fasting glucose [mmol/L] – 3.5) [8]. HOMA2 model calculates HOMA-IR, HOMA-B and HOMA-S indices by using the HOMA calculator downloaded from the official website of the Oxford Centre for Diabetes, Endocrinology and Metabolism [9]. The calculator uses formulas that are unknown to the users but are considered an improvement to the HOMA1 model [8].

All data were analyzed using the Jeffreys' Amazing Statistics Program, version 0.14.1. After confirming the normal distribution of variables, all data were presented as mean ± standard deviation (SD). T-test for dependent samples was used to determine if there were statistically significant differences. Linear correlation analysis was used to measure the correlation between HOMA indices and laboratory parameters not included in the HOMA index formula (postprandial glycemia and postprandial insulinemia).

Results

For each of the participants, HOMA indices were calculated using both HOMA1 and HOMA2 models with subsequent comparison of obtained values.

Table 1. Mean. minimum and maximum values of laboratory parameters and calculated indices
Tabela 1. *Srednje, najniže i najviše vrednosti laboratorijskih parametara i računatih indeksa*

Variable *Varijabla*	$\overline{X}$ ± SD N/*B* = 42	Minimum value *Najniža vrednost*	Maximum value *Najviša vrednost*
Fasting glucose/*Glukoza našte*	4.962 ± 0.622	3.200	6.100
Postprandial glucose/*Glukoza nakon obroka*	5.114 ± 1.307	2.870	7.100
Fasting insulin/*Insulin našte*	12.445 ± 5.031	4.500	22.600
Postprandial insulin/*Insulin nakon obroka*	54.086 ± 40.516	4.700	183.400
HOMA-IR1	2.807 ± 1.293	0.811	5.725
HOMA-B1	182.953 ± 81.729	75.000	473.333
HOMA-IR2	1.599 ± 0.654	0.580	3.020
HOMA-B2	133.957 ± 41.709	75.100	308.100
HOMA-S2	75.669 ± 37.414	33.100	172.800

Legend/*Legenda*: HOMA-IR1. HOMA-B1 – indices obtained via HOMA1 model/*Indeksi dobijeni putem HOMA1 modela*; HOMA-IR2. HOMA-B2. HOMA-S2 – indices obtained via HOMA2 model/*Indeksi dobijeni putem HOMA2 modela*

Later, linear correlation analysis was performed to examine the relationship between HOMA indices and postprandial glycemia and insulinemia.

Using the T-test for dependent samples, a statistically significant difference was found between both HOMA-IR and HOMA-B indices calculated using the formula (HOMA1 model) and using the calculator (HOMA2 model) ($p < 0.01$ for both) **(Tables 1 and 2)**. These results showed that the calculator (HOMA2 model) yields significantly lower values compared with formula (HOMA1 model).

Linear correlation analysis found a statistically significant moderate positive correlation between HOMA-IR values obtained by HOMA1 model and postprandial insulinemia ($r = 0.450$; $p < 0.005$). Also, HOMA-IR values obtained by HOMA2 showed a significant moderate positive correlation with postprandial insulinemia ($r = 0.473$; $p < 0.005$) **(Tables 3 and 4)**. We did not find a significant correlation between HOMA-B (obtained via either HOMA1 or HOMA2) and postprandial insulinemia **(Tables 5 and 6)**. However, we found a significant but weak negative correlation between HOMA-B

Table 2. T-test for dependent samples
Tabela 2. *T-test za zavisne uzorke*

Variable *Varijabla*	HOMA1 model $\overline{X}$ ± SD N/*B* = 42	HOMA2 model $\overline{X}$ ± SD N/*B* = 42	p-value *p-vrednost*
HOMA-IR index/*indeks homeostaznog modela insulinske rezistencije*	2.807 ± 1.293	1.599 ± 0.654	$p < 0.001$
HOMA-B index/*indeks homeostaznog modela za funkciju beta ćelija pankreasa*	182.953 ± 81.729	133.957 ± 41.709	$p < 0.001$

Table 3. Linear correlation analysis (HOMA-IR1)
Tabela 3. *Linearna korelaciona analiza homeostaznog modela za procenu insulinske rezistencije*

	HOMA-IR1/*HOMA-IR1* N/*B* = 42	
	r	p
Postprandial glycemia/*Postprandijalna glikemija* (mmol/L)	0.252	p = 0.107
Postprandial insulinemia/*Postprandijalna insulinemija (mIU/L)*	0.450	p = 0.003

Tabela 4. Linear correlation analysis (HOMA-IR2)
Tabela 4. *Linearna korelaciona analiza homeostaznog modela za procenu insulinske rezistencije*

	HOMA-IR2/*HOMA-IR2* N/*B* = 42	
	r	p
Postprandial glycemia/*Postprandijalna glikemija* (mmol/L)	0.188	p = 0.233
Postprandial insulinemia/*Postprandijalna insulinemija (mIU/L)*	0.473	p = 0.004

Tabela 5. Linear correlation analysis (HOMA-B1)
Tabela 5. *Linearna korelaciona analiza homeostaznog modela za procenu funkcije beta ćelija pankreasa*

	HOMA-B1/*HOMA-B1* N/*B* = 42	
	R	p
Postprandial glycemia/*Postprandijalna glikemija* (mmol/L)	-0.328	p = 0.037
Postprandial insulinemia/*Postprandijalna insulinemija (mIU/L)*	0.072	p = 0.654

Tabela 6. Linear correlation analysis (HOMA-B2)
Tabela 6. *Linearna korelaciona analiza homeostaznog modela za procenu funkcije beta ćelija pankreasa*

	HOMA-B2 N/*B* = 42	
	r	p
Postprandial glycemia/*Postprandijalna glikemija* (mmol/L)	-0.220	p = 0.162
Postprandial insulinemia/*Postprandijalna insulinemija (mIU/L)*	0.253	p = 0.105

obtained via HOMA1 model and postprandial glycemia (r = -0.328; p = 0.037) **(Table 5)**.

Discussion

Our study shows that HOMA1 and HOMA2 models give significantly different results for HOMA-IR and HOMA-B indices. We already pointed out that cut-off values must be established before routinely using these indices in the study population. Studies that were conducted to establish cut-off values of HOMA-IR predominantly used the HOMA1 method [13]. The apperance of HOMA2 model resulted in its growing use in clinical practice [8]. In the population of the Autonomous Province of Vojvodina (Serbia), the cut-off value of 2 was established using the HOMA2 model [14]. However, HOMA2 model yields significantly lower cut-off values compared to HOMA1 model. A study which was dealing with this problem used both HOMA1 and HOMA2 models to establish the cut-off value of HOMA-IR. In that study, HOMA1 gave a cut-off value of > 2.5 while HOMA2 model gave a cut-off value of > 1.4 showing a statistically significant difference [15]. This clearly shows that a physician must be cautious when interpreting the HOMA-IR index values and always use the same HOMA model that was used to establish cut-off values in the population that his or her patients are a part of in order to avoid a false diagnosis of insulin resistance. Hence, if the cut-off value was established using the HOMA2 model, the physicians must use the same model when calculating HOMA indices for their patients, or else they face the risk of false diagnosis of insulin resistance in their patients.

Another important issue, besides how to interpret the results of HOMA indices, is the type of sample used to measure the concentration of glucose and insulin. Namely, both HOMA1 and HOMA2 model use plasma samples, not serum samples. However, the majority of laboratories today use serum samples. This brings up the question if it is appropriate to use (in the formula or the calculator) glucose and insulin concentrations obtained via serum samples. Regarding the differences in glucose concentration based on the sample type, some studies showed that glucose concentration is higher in plasma compared with serum, but other studies did not find a difference [16]. Regardless of these findings, it is not recommended to use serum glucose concentration in diagnosing diabetes [17, 18]. For example, in a study by Kim HS., when using plasma glucose concentration, 20.9% of participants were diagnosed with diabetes, while the serum glucose levels did not detect it in the same participants [16]. On the other hand, many studies that dealt with HOMA indices and insulin resistance were based on serum concentrations of glucose and insulin [15, 19–22]. Regarding insulin concentration, the problem is not only the difference in values when using plasma vs. serum, but also the fact that different serum methods can yield values that differ as much as 200% compared to other methods [23]. This is further complicated by the fact that there are many different serum methods and that serum is the predominant sample when measuring insulin concentration in clinical practice. Furthermore, some of these serum methods exhibit a cross-reactivity with proinsulin, which further impacts the precision of these methods and renders them inadequate for usage in HOMA index calculation [24]. Manley et al. concluded that physicians must always be aware of differences between these methods, especially in research, as the variability of insulin concentrations measured might affect the conclusions of the researchers. Also, on the official website of the Oxford Centre for Diabetes, Endocrinology and Metabolism, it is stressed that cut-off values of a population must be established based on the laboratory assay used for measuring insulin concentration [25]. Therefore, it is clear that any difference in methods used for the measurement of glucose and insulin concentrations is going to affect the HOMA index value as well.

Using linear correlation analysis, a significant positive correlation between HOMA-IR (both in HOMA1 and HOMA2 models) and postprandial insulin concentration was found. However, we did not find a significant correlation between HOMA-B and postprandial glucose and insulin. These results would certainly be more valid if insulinemia and glycemia at 120 minutes of OGTT were used instead of postprandial glycemia and insulinemia. A study by Lima LMTR [26] found a significant correlation between HOMA-IR and insulinemia at 120 minutes of OGTT, while a study by Williams RA et al. [27] did not find a significant correlation between HOMA-IR and postprandial glycemia and insulinemia in the postprandial test. They reported an interesting finding that HOMA-IR, waist circumference, and physical activity were good predictors of postprandial insulinemia, while none of the variables in question could accurately predict postprandial glycemia, as in our study. They also pointed out that no other study tried to elucidate which variables could be the predictors of postprandial glycemia and insulinemia in adolescents, even though they could be regarded as risk factors for developing cardiovascular disease. Also, they stated that postprandial hyperinsulinemia appears before postprandial hyperglycemia and as such should be assessed in adolescents and young adults.

Conclusion

Although the use of homeostasis model assessment indices may seem rather simple, there are many questions that need to be answered before we can use them to diagnose insulin resistance.

The first question is whether the cut-off value was defined using plasma or serum samples for measuring glycemia and insulinemia. The same sample type should then be used for measuring glycemia and insulinemia. If the sample is serum, special attention must be paid to the method used.

The second question is whether the cut-off value of the study population was established using the most prevalent laboratory method for measuring serum insulin, as differences between methods can be as great as 200%.

Finally, the third question is whether the study that established the cut-off value used homeostasis model assessment 1 or homeostasis model assessment 2, as it is imperative to use the same model used in clinical practice when working with patients from the population in question, because the two models produce significantly different values of homeostasis model assessment of insulin resistance and homeostasis model assessment B.

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Rad je primljen 13. X 2021.
Recenziran 15. XI 2021.
Prihvaćen za štampu 17. XI 2021.
BIBLID.0025-8105:(2021):LXIX:7-8:238-243.

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Original study
Originalni naučni rad
UDK 616.314-007.26
UDK 616.317:616.742]-073.7
https://doi.org/10.2298/MPNS2108244R

THE IMPORTANCE OF LIP MUSCLE ACTIVITY IN DETERMINING THE POSITION OF THE ANTERIOR TEETH IN DEEP BITE

ZNAČAJ AKTIVNOSTI MIŠIĆA USANA U ODREĐIVANJU POLOŽAJA FRONTALNIH ZUBA KOD STRMOG ZAGRIŽAJA

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Summary

Introduction. Low-strength forces of the soft tissues are closely related to the stomatognathic system morphology. The muscles of the lips have a significant influence on the position of the front teeth. The goals of our research were to: 1. Examine the electromyographic activity of the circular muscle of the lips in Class I and Class II division 2 patients with incisor retrusion in the position of physiological rest and centric occlusion at maximum voluntary muscle contraction; 2. Examine the existence of a correlation between the electromyographic activity of the lip muscles and the position of the incisors in the examined groups. **Material and Methods.** The action potentials of the circular muscle of the lips were registered at different positions of the lower jaw. Intramuscular coaxial electrodes were placed according to the Greenfield scheme. Measurements were performed bilaterally, summed up with ten muscle levels, in 100 patients aged 8 - 12 years; 30 patients were in the control group with neutroclusion, i.e. Class I and 70 patients in the study group with Class II division 2, according to Angle. **Results.** The results are given in microvolts as average cumulative voltage of action potential amplitudes, which were used to determine changes in the orbicular muscle activity. After statistical analysis of the obtained data, it was concluded that the bioelectrical activity of the examined muscles at all measured positions was lower in patients with distoclusion, except for the lower lip in patients with Class II division 2, where this activity was significantly higher. **Conclusion.** The results of the study show that the determined changes in the action potentials of the circular muscles of the lips in subjects with distoclusion indicate a cause-and-effect relationship between muscle function and occlusion. A significantly increased activity of the lower lip in deep bite can be considered responsible for the steep position of the upper incisors.

Key words: Lip; Masticatory Muscles; Malocclusion, Angle Class II; Dental Occlusion; Electromyography; Incisor; Muscle Contraction; Bite Force

Sažetak

Sile malih jačina, poreklom od mekih tkiva, u uskoj su vezi sa morfologijom stomatognatog skeleta. Mišići usana imaju bitan uticaj na položaj frontalnih zuba. Stoga je cilj naših istraživanja bio da se ispita elektromiografska aktivnost kružnih mišića usana kod pacijenata sa neutrookluzijom (klasa 1) i distookluzijom, praćenom retruzijom sekutića (klasa II/2 – strmi zagrižaj), i to u položaju fiziološkog mirovanja i centralne okluzije pri maksimalnoj voljnoj kontrakciji mišića i da se ispita postojanje korelacije između elektromiografske aktivnosti mišića usana i položaja sekutića kod ispitivanih grupa. **Materijal i metode.** Akcioni potencijali kružnog mišića usana registrovani su pri različitim položajima donje vilice. Intramuskularne koaksijalne elektrode postavljene po Grinfildovoj šemi. Merenje je vršeno obostrano, sumirano sa deset nivoa mišića, kod 100 pacijenata uzrasta 8–12 godina i to 30 pacijenata kontrolne grupe sa neutrookluzijom i 70 pacijenata eksperimentalne grupe sa distokluzijom dentoalveolarnih odnosa po Anglu. **Rezultati** istraživanja predstavljeni su u mikrovoltima kao srednje kumulativne voltaže amplituda akcionih potencijala, na osnovu kojih su utvrđene promene aktivnosti kružnog mišića. Nakon statističke analize dobijenih podataka, konstatovano je da je bioelektrična aktivnost ispitivanih mišića u svim mernim pozicijama klase II/2 bila manja od aktivnosti kod pacijenata sa distookluzijom, osim donje usne kod klase II/2, gde je ta aktivnost značajno veća. **Zaključci** ukazuju na to da utvrđene promene akcionih potencijala kružnih mišića usana kod ispitanika sa distookluzijom, ukazuju na uzročno-posledičnu vezu između mišićne funkcije i okluzije. Značajno povećana aktivnost donje usne kod strmog zagrižaja može se smatrati odgovornom za strmi položaj gornjih sekutića.

Ključne reči: usna; mastikatorni mišići; malokluzija II klase po Anglu; dentalna okluzija; elektromiografija; sekutići; mišićna kontrakcija; sila zagrižaja

Introduction

The muscles of the orofacial region, especially circular muscles of the lips and muscles of mastication, together with other factors (shape and position of dental arches, shape of the craniofacial skeleton, etc.), play an important role in the position of teeth, modifications in the shape of dental arches and other dentoalveolar structures, relationship between the jaws, etc. [1, 2].

The etiology of orthodontic anomalies has not been fully clarified because it has been noticed that normal occlusion is often accompanied by markedly impaired

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Abbreviations

Class I – neutroclusion
Class II – distoclusion
EMG – electromyography
CO – centric occlusion
MVMC – maximum voluntary muscle contraction
mV – microvolt

functions of the orofacial musculature. Therefore, electromyography (EMG) recording of the activity of orofacial muscles is of particular importance in scientific research and increasingly important in everyday clinical practice [3–6].

Dr. Edward Angle pointed out that muscles have a great influence on the development of dental arches and the formation of occlusion. Today, it is known that improper muscle function is one of the important etiological factors in the development of malocclusions [7–10].

The importance of the low-strength forces of the soft tissues, in the physiological rest position of the mandible, is especially emphasized, since the muscles are only occasionally functional during 24 hours. The form-function relationship is still unclear and opinions are divided. This relationship is largely controlled by genetics [4, 9]. The circular muscle of the lips plays a significant role in determining the position of the incisors. The circular muscle of the mouth is a wide and flat muscular ring. Class II malocclusions, division 2, according to Angle, are also called "deckbiss" (German), deep or overlap bite. These are dentoalveolar gnathofacial anomalies, due to characteristic changes that include the teeth, alveolar extensions, jaws and face. Distoclusion is accompanied by retrusion and deep overlap of the incisors. Epidemiological studies indicate a high prevalence of Class II malocclusions, which varies from 10 to 27% in different populations [11]. Today, it is considered that the muscles of the lips, especially the lower lip, play an important role in the formation of the inclination and angulation of the incisors, that is, in determining the position of the incisors in deep bite [12–15]. The EMG activity of the lip muscles has a significant effect on incisor position.

Research objectives

The goals of our research were:

1. To examine the electromyographic activity of the circular muscles of the lips in patients with neutroclusion (Class 1) and distoclusion associated with incisor retrusion (Class II 2 - deep bite) in the position of physiological rest, centric occlusion (CO), and maximum voluntary muscle contraction (MVMC);

2. To examine the existence of a correlation between the EMG activity of the lip muscles and the position of the incisors in the examined groups.

Material and Methods

First, by means of random selection, 100 patients from current case studies of the Dental Clinic in Novi Sad and students of the Elementary School "Vasa Stajić" in Novi Sad, who meet the required conditions and provided a written parental consent, were selected. The control group included patients with neutroclusion and complete dentition. The EMG analysis was performed in a total of 30 patients in the control group, 15 male and 15 female, aged 8 – 12 years, in the position of physiological rest, CO of the mandible, with MVMC when swallowing saliva.

The study group included patients with distoclusion and retrusion of the upper incisors (deep bite), with complete dentition, selected by random selection from the current case studies of the Dental Clinic, with their consent. The EMG analysis was performed in a total of 70 patients in the study group, 35 male and 35 female, aged 8 – 12 years, in the position of physiological rest, CO of the mandible, with MVMC of the muscles when swallowing saliva.

The action potential of the orbicularis oris muscle (pars inferior) in the position of physiological rest, CO of the mandible and MVMC when swallowing saliva was analyzed by EMG analysis, in normal occlusion and distoclusion (Class II 2, according to Angle). The measurement was performed at a constant temperature of 25 degrees Celsius, using a Medelec Synergy type apparatus **(Table 1)**.

To register the action potential of the examined orofacial muscles, facial, intramuscular coaxial electrodes were used, placed according to the Greenfield scheme (1956). The EMG measurement was performed in the area of the upper and lower lip, for orbicular oris muscle, in physiological rest position, CO of the mandible and with MVMC followed by swallowing saliva, for 20 seconds, because after that muscle fatigue occurs.

When registering EMG activity, the filters of the EMG device were set at 100 Hz – 2 KHz. To assess EMG activity in relaxation (physiological rest), the activity was registered at 10 microvolts (mVs) amplification of the oscilloscope, CO, and MVMC at 250 – 500 mV, depending on the length of EMG activity in the examinee. The EMG measurement was recorded simultaneously via a tape printer, and the tape speed was 2.5 mm/sec. To register the total bioelectric potential of these muscles, an integrator was used, and the mean value was obtained from 3 consecutive measurements.

Table 1. Number and gender distribution of examinees
Tabela 1. *Broj i polna struktura ispitanika*

Gender/*Pol*	Number of examinees/*Broj ispitanika*	Class I/*Klasa I*	Class II 2/*Klasa II 2*
Male/*Muški*	50	15	35
Female/*Ženski*	50	15	35
Total/*Ukupno*	100	30	70

Table 2. Bioelectrical activity of orbicularis oris muscle (lower lip) found in eugnathic and Class II examinees of both genders
Tabela 2. *Bioelektrična aktivnost m. orbicularis oris (donja usna) kod ispitanika oba pola sa eugnatim odnosom vilica i klasom II*

	Orbicularis oris muscle (lower lip)/*m. orbicularis oris (donja usna*)					
	Class I - both genders/*Klasa I – oba pola*			Class II - both genders/*Klasa II – oba pola*		
	Female and male *Muški i ženski*	Centric occlusion *Centralna okluzija*	Maximum voluntary muscle contraction *Maksimalna voljna kontrakcija mišića*	Female and male *Muški i ženski*	Centric occlusion *Centralna okluzija*	Maximum voluntary muscle contraction *Maksimalna voljna kontrakcija mišića*
	3.36	19.20	197.40	4.08	28.14	317.41
Standard deviation *Standardna devijacija*	1.52	2.77	41.54	1.53	7.29	92.69
Coefficient of variation *Koeficijent varijacije*	46.04%	14.44%	21.05%	37.51%	25.89%	29.20%
Standard error *Standardna greška*	0.21	0.39	5.88	0.22	1.03	13.11
Confidence interval *Interval poverenja*	3.04	5.55	83.09	3.06	14.57	185.38
Significance of differences *Značajnost razlika*	-1.65	-4.59	-1.79			
Significance of differences for *Značajnost razlika za* $p < 0.05$	2.02	2.02	2.02			
Correlation/*Korelacija*	0.25	0.41	0.33			
Statistical significance *Statistički značaj*	$p < 0.05$	$p > 0.05$	$p > 0.05$			

Results

The results of our research were analyzed using oscilloscope diagrams and graph presentation, presented in mV, as mean cumulative action potential voltage amplitude.

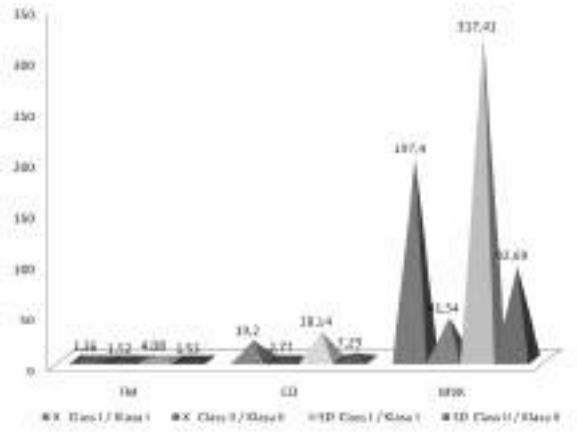
Using the registered values of the action potential, the changes in the circular muscle of the lips were compared, in physiological rest position, mandibular CO, and with MVMC of the muscles when swallowing saliva in Class I and Class II 2 occlusion according to Angle. Based on the data on bioelectrical activity of muscles, a quantitative analysis was performed, with description of the results that were classified according to the parameters provided in the research protocol, presented in tables and graphs.

Qualitative analysis was conducted using modern, standard statistical methods for individual and comparative analysis of the obtained data. The statistical significance of the obtained results was confirmed by the Student's t-test. Statistical data processing included:

1. Arithmetic mean ($\overline{X}$)
2. Standard deviation (SD)
3. Standard error (SE)
4. Coefficient of variation (CV)
5. Confidence interval (CI) with 95% probability
6. T-test - significance of differences (t)
7. Confidence interval (p), $p < 0.05$
8. Correlation coefficient between two variables (r).

When analyzing the degree of correlation between the examined phenomena, the interpretation of the results was performed using the following scale:

- If "r" is from ± 0,01 to ± 0,2 there is no correlation
- If "r" is from ± 0,2 to ± 0,4 there is a weak correlation
- If "r" is from ± 0,4 to ± 0,7 there is a significant correlation
- If "r" is from ± 0,7 to ± 1,0 there is a very significant correlation **(Table 2, Graph 1)**.



Graph 1. Bioelectrical activity of orbicularis oris muscle (lower lip) found in eugnathic and Class II examinees of both genders
Grafikon 1. *Bioelektrična aktivnost m. orbicularis oris (donja usna) kod ispitanika oba pola sa eugnatim odnosom vilica i klasom II*
Legenda: FM – oba pola, CO – centralna okuzija, MVK – maksimalna voljna kontrakcija mišića

Discussion

The EMG study of muscle activity in deep bite confirmed our main hypothesis that irregular function of the orofacial muscles has an impact on occlusion. Action potentials measured in the orbicularis oris muscle in predicted time periods are approximately the same in both genders, in both examined classes, in all measured positions, i.e. positions of the mandible, which is in accordance with previous researches [1, 16, 17].

The bioelectrical activity in the orbicularis oris muscle in deep bite showed significantly higher values compared to normal bite, with a significantly higher activity of the lower part of this muscle, i.e. the lower lip, than of the upper lip. This finding can be explained by the position of the lower lip and its morphology, i.e. the position of the lower lip line, which is also indicated by Lapatki et al. [18] and Mills [19] and Posen et al. [12, 13].

The amplitudes of action potentials of the examined orofacial muscles in both classes and all groups of subjects are the lowest in the position of physiological rest of the mandible, and the highest in the position of CO and with MVMC, which is explained by their function.

Comparative analysis of EMG activity of the circular muscle of the lips in the position of physiological rest of the mandible, CO and with MVMC when swallowing saliva, in persons with deep bite, compared to normal occlusion, showed significantly higher values of action potentials, especially in function, in examinees with Class II 2 occlusal relations according to Angle.

Lowe and Takada examined the EMG activity of the orbicularis oris muscle in children with different craniofacial morphology and found a significantly higher activity of this muscle in the position of physiological rest of the mandible and at maximum intercuspation in persons with Class II 2 compared to examinees with Class I, II 1 and Class III occlusal relations according to Angle [20].

Dutra et al. research showed that mouth breathing causes an increase in the EMG activity of the lower bundle of the orbicularis oris muscle, and a decrease in the activity of the upper bundle, thus showing a direct dependence of EMG activity on the magnitude of the function of the examined muscle [21].

Even today, there are research dilemmas lasting for decades regarding the position of the upper incisors in deep bite and the question whether this position of the incisors is only inherited as a part of a comprehensive clinical picture of Class II 2 malocclusion, or it is the result of abnormal perioral musculature, and especially the circular muscle of the lips. Numerous authors have studied this issue, including Marković et al. [22], Graber et al. [23], and others, and they believe that the etiology of deep bite is primarily genetic in origin [22, 23].

In his research, Marx found a lower level of lip strength in position of physiological rest of the mandible in examinees with Class II 2 occlusal relations according to Angle [24]. Simpson found that the muscular activity of the upper lip was not related to the degree of retraction of the upper incisors. He also found no correlation between the shape of the dental arches and the function of the perioral musculature. However, this author noted higher values of lip strength in examinees with shorter maxillary arch, maxillary prognathism and low mandibular inclination, which are the characteristics of a Class II 2 malocclusion according to Angle [25].

Ingervall and Janson believe that it is unlikely that retroclination of the incisors in deep bite is caused by a strong upper lip, because in his research they registered negative upper lip pressure in these examinees. This authors also claim that one cannot eliminate the possibility that the position of the upper incisors in Class II 2 malocclusion is determined by a strong lower lip pressure. Theydid not find a correlation between the EMG muscle activity of the lips in the position of physiological rest of the mandible and during chewing, which led them to the conclusion that there was an adaptation of lip pressure and that lip pressure at rest was the result of incisor position and passive soft tissue dropping over the teeth [26].

Posen found that teeth were in balance with the forces acting on them externally and internally, thus determining their position. He registered high lip strength values in persons with deep bite and low in persons with bimaxillary protrusion. According to him, the strength of the lips represented the tone of the lips, and similarly the force that acted on the teeth. This author has established a correlation between the lip strength, its EMG activity, position and morphology and dentoalveolar and cephalometric characteristics [12, 13].

Mills claimed that the lower lip, i.e. the relatively high oral line in relation to the front teeth is solely and exclusively responsible for the retrusion of the upper incisors in persons with Class II 2 malocclusion according to Angle [19].

These findings are fully consistent with the findings of Lapatki et al., who registered a significantly higher pressure of the lips, especially the lower lip, on the upper incisors, in people with deep bite, which is associated with a high position of the lip line. These authors did not find a difference in the activity of perioral musculature at rest in different study groups [18]. In his research, Thueer found that lip pressure at rest depends solely on their tone and that it is different from lip pressure during chewing and swallowing, which depends on muscle activity and correlates with EMG activity of lip muscles and morphology of bites that is considered to be an adaptive process [14, 27, 28].

Conclusion

Our findings showed that the changes in the action potentials of the muscles of the lips in examinees with distoclusion indicate a causal relationship between muscle function and occlusion. A significantly increased activity of the lower lip in deep bite may be considered responsible for the steep position of the upper incisors.

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Rad je primljen 15. XI 2021.
Recenziran 18. XI 2021.
Prihvaćen za štampu 26. XI 2021.
BIBLID.0025-8105:(2021):LXIX:7-8:244-248.

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Original study
Originalni naučni rad
UDK 616.896:316.36
https://doi.org/10.2298/MPNS2108249L

BEHAVIORAL ADJUSTMENT OF SIBLINGS OF CHILDREN WITH AUTISM SPECTRUM DISORDER

PRILAGOĐAVNJE PONAŠANJA BRAĆE I SESTARA DECE SA POREMEĆAJEM IZ SPEKTRA AUTIZMA

Jelena LUKIĆ[1], Nina BRKIĆ JOVANOVIĆ[2], Miljana TASIĆ[3], Daniela TAMAŠ[4] and Mila VESELINOVIĆ[4]

Summary

Introduction. Challenges in the development of a child with autism spectrum disorder require adjustment of the entire family, parents and siblings. So far, the researchers' efforts have mostly been focused on children with autism spectrum disorder or their parents, and less frequently on the siblings living in a family with a child with autism spectrum disorder. The goal of this research was to identify problems in the functioning of siblings of persons with autism spectrum disorder and the relationships of these problems with various sociodemographic and family characteristics. **Material and Methods.** The research was conducted as a cross-sectional study which included thirty children aged 4 to 18 years with a sibling with autism spectrum disorder. The Strengths and Difficulties Questionnaire was used to assess the functioning of siblings of children with autism, while family characteristics were collected through a sociodemographic questionnaire created for research purposes. Statistical package for the social sciences 20.0 software was used for data entry and processing. **Results.** There are no significant differences in the functioning of siblings of children with autism spectrum disorder in relation to their gender, age, family status and the level of functionality of a child with autism spectrum disorder. In most cases, there is a low risk of clinically significant problems (63.3%), in 10% of cases the risk is medium, and in 26.7% of cases it is high. **Conclusion.** Problems in the functioning of siblings of children with autism spectrum disorder do not depend on sociodemographic or family characteristics. Models of support, based on the research findings along with community education, strengthen the siblings through their experience of growing up with a sibling with autism spectrum disorder.

Key words: Autism Spectrum Disorder; Siblings; Adaptation, Psychological; Sibling Relations; Parent-Child Relations; Child; Risk Factors

Sažetak

Uvod. Izazovi u razvoju deteta sa poremećajem iz spektra autizma (engl. *autism spectrum disorder*) zahtevaju prilagođavanje cele porodice, roditelja i sibilinga. Dosadašnji napori istraživača, najčešće su bili usmereni na decu sa poremećajem iz spektra autizma ili na roditelje, ređe na perspektivu sibilinga koji žive u zajednici sa detetom sa poremećajem iz spektra autizma. Cilj našeg istraživanja podrazumeva utvrđivanje problema u funkcionisanju sibilinga osoba sa poremećajem iz spektra autizma i odnos ovih problema sa različitim sociodemografskim i porodičnim karakteristikama. **Materijal i metode**. Istraživanje je sprovedeno kao studija preseka, u okviru kojeg je obuhvaćeno tridesetoro dece čiji brat ili sestra imaju poremećaj iz spektra autizma, starosti od 4 do 18 godina. U svrhu procene funkcionisanja sibilinga dece sa autizmom korišćen je Upitnik o snagama i teškoćama (*The Strength and Difficulties Questionnaire*), dok su sociodemografskim upitnikom, kreiranim za potrebe istraživanja, prikupljeni podaci o karakteristikama porodice. Za unos i obradu podataka korišćen je programski paket SPSS 20.0. **Rezultati.** Ne postoje značajne razlike u funkcionisanju sibilinga dece sa poremećajem iz spektra autizma, u odnosu na pol, uzrast, porodični status i stepen funcionalnosti deteta sa poremećajem iz spektra autizma. U najvećem procentu slučajeva postoji nizak rizik za postojanje klinički signifikatnih problema (63,3%), u 10% slučajeva rizik je srednji, a u 26,7% slučajeva je visok. **Zaključak.** Problemi u funkcionisanju sibilinga dece sa poremećajem iz spektra autizma ne zavise od sociodemografskih i porodičnih karakteristika. Modeli podrške, zasnovani na nalazima istraživanja, zajedno sa edukacijom zajednice, osnaživali bi sibilinge u iskustvu odrastanja sa bratom/sestrom sa poremećajem iz spektra autizma.

Ključne reči: poremećaji iz autističnog spektra; braća i sestre; psihološka adaptacija; odnosi sa braćom i sestrama; odnosi roditelja i deteta; dete; faktori rizika

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by abnormal and/or altered development which manifests before the age of 3, through qualitative anomalies in social interaction and modalities of communication accompanied by a limited, repetitive, and stereotypical repertoire of interests and activities, as well as uneven intellectual development [1]. The prevalence of ASDs has shown a growing trend in the last few years, and according to the literature, the disorder affects one in 68 to 100 children, with 68% of children having some degree of intellectual disability,

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Abbreviations
ASD – autism spectrum disorder
SDQ – Strengths and Difficulties Questionnaire
SPSS – statistical package for the social sciences

while 75% of them require lifelong social and educational support [2]. Although there is no clear explanation for this growth, it is probably due to improvement in diagnostic methods [1]. The child's developmental disorder does not necessarily cause family dysfunction [3], although it involves prolonged active parenting, which can increase the level of stress in the family and affect the stages of a typical life cycle [4, 5]. Characteristics and challenges in the development of a child with ASD, such as sensory sensitivity, repetitive stereotyped behavior, difficulties in social interaction and communication, require adjustment of the whole family, both the parents and the siblings [5]. So far, the efforts of researchers and practitioners have mostly been focused on the children with ASD or their parents, and less often on the experiences and perspectives of siblings living in a family with a child with ASD [6]. A powerful sibling relationship is formed during long term physical and emotional contacts in critical periods of life and it can be a model through which the siblings mature sexually, morally, linguistically, and motorically [7]. Considering these facts, over the last few decades there has been a growing interest in assessing the adjustability of siblings of children with disorders, including siblings of children with ASD [8].

Characteristics of the relationships between siblings of typical development and a child with ASD cannot be generalized [9]. The findings of some research studies suggest that the siblings of children with ASD have a lower level of adjustment when compared to the normative group [10]. The negative effects of autism on typically developing siblings are emphasized, reflecting in the feeling of neglect and loneliness, since the parents must attend to the needs of the child with ASD [11]. Therefore, they are at a higher risk of developing depression when compared to the control group, as well as to the siblings of children with other disorders [12]. Physical and verbal aggressive behavior and the unpredictable nature of a child with ASD may lead to lasting anxiety, pressure and instability in a typically developing child, especially in adolescents [13], who react to judging social interactions in relation to their siblings, with already present intense emotions of anger, rage, disappointment, and frustration [14]. However, other studies point out positive effects, including the siblings of children with ASD, who show more warmth and less conflict than typically developing siblings [15]. The experience of growing up with a sibling with ASD can contribute to the development of empathy [16] altruism and tolerance [17], encouraging behavior such as caring for others and stronger self-esteem [13, 6]. It is argued that typically developing siblings develop great patience, understanding, sensibility and awareness of people from vulnerable groups, often cultivating deep and tender feelings of loyalty and pride towards children from these groups, developing advocacy and self-advocacy skills for them [17].

We can say that the relationship is positive if the siblings understand the disorder, have developed coping skills, and have experienced positive reactions from parents and peers towards children with ASD [18]. The impact of a sibling with a disorder on a typically developing sibling is best seen as a risk factor that may manifest due to sociodemographic factors, individual and family patterns of adjustment and functioning, different sibling characteristics, and the characteristics of the sibling with ASD. Although the literature findings are inconsistent, it seems that it is better when the family is bigger, socioeconomic conditions are better, parents have a positive attitude towards the child with the disability, when the siblings are younger than the child with ASD, when the age difference between the siblings is greater, as well as when the disorder is milder [19].

Researches that focus on the specifics of the sibling relationships and the impact of ASD on these relationships create opportunities for shaping support and reducing possible negative effects. Thus, continuous and organized work with the siblings should be provided, which is still a rarity in the Republic of Serbia. Our research aims to identify problems in the functioning of siblings of children with ASD, and the relationships of these problems with different sociodemographic and family characteristics. In keeping with the objective, we presumed that the siblings of children with ASD exhibit problems in functioning, and that family status, gender, age, and level of functioning of a child with ASD are significantly related to the level of sibling functionality.

Material and Methods

A cross-sectional study included thirty children with a sibling with ASD attending the Schools for Primary and Secondary Education "Milan Petrović" in Novi Sad and "Vule Antić" in Vranje, and the Daycare Center for Individuals with ASD in Novi Sad. The data were collected by using anonymous closed ended questionnaires; written consents of parents/guardians and institutions were obtained; and the approval of the Ethics Committee of the Faculty of Medicine in Novi Sad. The inclusion criterion for participation in the research was a signed consent of the parent/guardian and a sibling of the families of children with ASD.

During the research, the Strength and Difficulties Questionnaire (SDQ) [20] was used to assess the functioning of siblings of children with ASD. It was completed by parents of children without developmental disorders. It consists of 25 items related to the emotional and behavioral problems of children and adolescents in the last six months. The items are divided into five domains (emotional problems, behavioral problems, hyperactivity, peer problems, and prosocial behavior), and the items are scored on a

3-point Likert scale, with the scores in all domains ranging from 0 to 10. There are three versions of the questionnaire: for parents, for preschool teachers/teachers (for children aged 3 – 16), and for adolescents aged 14 – 16. Considering the age of the examinees, the first modality was used in the research. The scale has a good internal consistency ($\alpha = .73$).

The SDQ scoring was performed in two ways. The first provided dimensional values by collecting points within five domains (emotional problems, behavioral problems, hyperactivity, peer problems, and prosocial behavior), and the second established "cut off" scores which may indicate clinically relevant problems. The SDQ scores range from 0 to 40 and point to low risk of clinically significant problems in the functioning of siblings of children with ASD (from 0 to 13), medium risk (from 14 to 16) and a high risk (from 17 to 40).

A sociodemographic questionnaire, created for the purposes of this research, was used to collect data related to family characteristics (family status, gender, age, birth order of a child with/without disorders and parents' assessment of the functioning and performing activities of daily living of their child with ASD).

Data analysis was performed using the Statistical package for the social sciences (SPSS) software for Windows 20, and descriptive methods to describe the sample through descriptive strategic measures (frequencies and percentages for categorical variables, and arithmetic means and standard deviations for dimensional). At the same time, within comparative statistics, a single-factor analysis of variance with repeated measures and Pearson's correlation coefficient were used to check the significance of correlations and influences of the examined variables. In the case of individual variables, the limited sample size allowed the use of nonparametric methods, the Kruskal Wallis test (for testing multiple independent samples) and the Chi-square test (χ^2). The p values below 0.05 and 0.01 were considered statistically significant.

Results

The study included thirty children with siblings with ASD aged 4 to 18, with an average age of 10.90 (SD = 4.05). There was an equal gender distribution in the sample. When it comes to the birth order, in one case the siblings were twins (3.30%), 11 respondents were the firstborn child (36.70%), 16 respondents were the second born (53.30%), while in two cases they were the third born child (6.70%). The family climate, which includes the relationship between parents, showed that in 80% of cases both parents took care of the children, in 10% of cases the parents were divorced, while in the remaining 10% of cases the child was raised by a single mother.

Descriptive statistics of measured traits are presented in **Table 1**. To facilitate the interpretation of parents' assessments of the functionality of children with ASD and the SDQ domain, the values of the scales were divided into categories of low, medium,

Table 1. Descriptive statistics of the measured characteristics
Tabela 1. *Deskriptivna statistika izmerenih karakteristika*

	N	Minimum *Minimum*	Maximum *Maksimum*	Mean *Prosek*	Standard Deviation *Standardna devijacija*
Functioning level/*Stepen funkcionalnosti*	30	2	8	5.77	1.832
Emotional symptoms/*Emocionalni simptomi*	30	0	10	2.57	2.800
Behavioral problems/*Problemi u ponašanju*	30	0	6	1.53	1.570
Hyperactivity/*Hiperaktivnost*	30	0	10	4.57	3.181
Peer problems/*Problemi sa vršnjacima*	30	0	7	2.43	2.501
Prosocial behavior/*Prosocijalno ponašanje*	30	1	10	6.83	2.995
Overall score/*Ukupan skor*	30	0	27	11.30	7.470

Table 2. The severity of the functioning problems in siblings
Tabela 2. *Izraženost problema u fukcionisanju kod braće i sestara*

	Low risk *Nizak rizik*		Medium risk *Srednji rizik*		High risk *Visok rizik*		Total *Ukupno*	
	f	%	f	%	f	%	f	%
Functioning level/*Stepen funkcionalnosti*	5	16.7%	13	43.3%	12	40%	30	100%
Emotional symptoms/*Emocionalni simptomi*	23	76.7%			7	23.3%	30	100%
Behavioral problems/*Problemi u ponašanju*	24	80%	3	10%	3	10%	30	100%
Hyperactivity/*Hiperaktivnost*	18	60%	1	3.3%	11	36.7%	30	100%
Peer problems/*Problemi sa vršnjacima*	18	60%	3	10%	9	30%	30	100%
Prosocial behavior/*Prosocijalno ponašanje*	20	66.7%	1	3.3%	9	30%	30	100%
Overall score/*Ukupan skor*	19	63.3%	3	10%	8	26.7%	30	100%

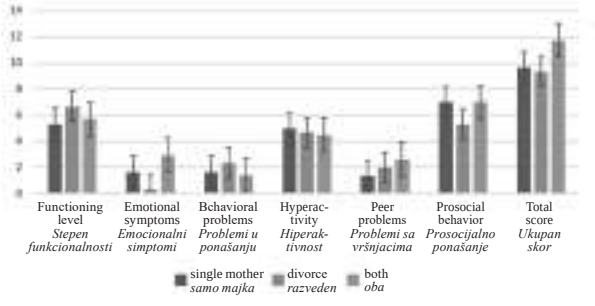
Table 3. The impact of gender on the functioning of siblings of children with autism spectrum disorder
Tabela 3. *Uticaj pola na funkcionisanje braće i sestara dece sa poremećajem iz spektra autizma*

	Gender *Pol*	Low risk *Nizak rizik*		Medium risk *Srednji rizik*		High risk *Visok rizik*		Total *Ukupno*		$\chi^2(2)$	p
Functioning level *Stepen funkcionalnosti*	Male/*Muški*	2	13.30%	7	46.70%	6	40%	15	100%	0.277	0.871
	Female/*Ženski*	3	20%	6	40%	6	40%	15	100%		
Emotional symptoms *Emocionalni simptomi*	Male/*Muški*	13	86.70%			2	13.30%	15	100%	1.677	0.195
	Female/*Ženski*	10	66.70%			5	33.30%	15	100%		
Behavior problems *Problemi u ponašanju*	Male/*Muški*	11	73.30%	2	13.30%	2	13.30%	15	100%	0.833	0.659
	Female/*Ženski*	13	86.70%	1	6.70%	1	6.70%	15	100%		
Hyperactivity *Hiperaktivnost*	Male/*Muški*	7	46.70%	0	0%	8	53.30%	15	100%	4.162	0.125
	Female/*Ženski*	11	73.30%	1	6.70%	3	20%	15	100%		
Peer problems *Problemi sa vršnjacima*	Male/*Muški*	8	53.30%	2	13.30%	5	33.30%	15	100%	0.667	0.717
	Female/*Ženski*	10	66.70%	1	6.70%	4	26.70%	15	100%		
Prosocial behavior *Prosocijalno ponašanje*	Male/*Muški*	8	53.30%	1	6.70%	6	40%	15	100%	2.800	0.247
	Female/*Ženski*	12	80%	0	0%	3	20%	15	100%		
Total score *Ukupan skor*	Male/*Muški*	8	53.30%	3	20%	4	26.70%	15	100%	3.474	0.176
	Female/*Ženski*	11	73.30%	0	0%	4	26.70%	15	100%		

and high degree of functionality of children with ASD that is low, medium, and high risk of clinically significant problems.

Data analysis showed that children with ASD, on average, exhibited medium level of functionality (M = 5.77; SD = 1.83), while siblings of children with ASD showed a low probability of clinically significant problems of this type, both in the overall score (M = 11.3; SD = 7.47) as well as in individual domains. The exceptions were the values on the scale that examined peer problems (M = 2.43; SD = 2.5), which showed that there was an average risk of clinically significant problems. For a better assessment, **Table 2** shows the reference to different risk categories.

To examine the differences in the functioning of siblings of children with ASD in regard to the family status, we used variance analysis and the obtained results showed no differences in the functioning of siblings of children with ASD related to the family status. The overall score showed that children from two parent families were at highest risk of clinically significant functioning problems, although the average scores were in the low-risk category, with no statistically significant difference ($p > 0.05$). Statistical significance was not confirmed within the domain categories, but two parent families were at the highest risk in the domains of emotional problems, behavioral, and peer problems. In the domain of hyperactivity, the highest risk was found in families with a single mother, while in the domain of prosocial behavior, families with divorced parents were at the highest risk. **Graph 1** shows the mean characteristic scores by groups.



Graph 1. Influence of family status on the functioning of children with autism spectrum disorder and their siblings
Grafikon 1. *Uticaj porodičnog statusa na funkcionisanje dece sa poremećajem iz spektra autizma i njihove braće i sestara*

Table 3 shows the influence of gender on the functioning of siblings of children with ASD, risk categories and proportional comparisons using the Chi square test. The total score showed no statistically significant influence of gender on the functioning of brothers and sisters of children with ASD ($p < 0.05$). The average risk of emotional problems was higher in sisters (33%) than in brothers (13%). Within the domains of behavioral issues and peer problems, brothers were at greater risk than sisters. The risk of developing hyperactivity problems was higher in brothers (53%) than in sisters (20%), while in the domain of prosocial behavior the risk was higher in sisters (20%) than in brothers (40%). Statistical significance was not confirmed in any of the above categories.

Pearson's correlation coefficient was used to determine the relationship between age and the problems in functioning of the siblings of children with ASD (**Table 4**, column 1) as well as the relationship between the level of functionality of the child with ASD and the problems in sibling functioning (**Table 4**, column 2). In both cases, the results showed no statistically significant relationship ($p > 0.05$).

As shown in **Table 4**, a statistically significant correlation was found between the risk of emotional problems and peer problems ($r = 0.619$, $p < 0.00$), as

Table 4. Interrelationship of Strengths and Difficulties Questionnaire domains
Tabela 4. *Međusobna povezanost domena Upitnika snaga i teškoća*

		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1. Age/*Uzrast*	r	1							
	p								
2. Functioning level/*Stepen funkcionalnosti*	r	0.187	1						
	p	0.322							
3. Emotional symptoms/*Emocionalni simptomi*	r	-0.201	06	1					
	p	0.286	0.973						
4. Behavior problems/*Problemi sa ponašanjem*	r	0.090	-0.195	0.086	1				
	p	0.636	0.302	0.652					
5. Hyperactivity/*Hiperaktivnost*	r	-0.212	-0.095	0.071	0.524*	1			
	p	0.261	0.618	0.709	0.003				
6. Peer problems/*Problemi sa vršnjacima*	r	-0.312	-0.060	0.619*	0.194	0.506*	1		
	p	0.093	0.753	0.000	0.305	0.004			
7. Prosocial behavior/*Prosocijalno ponašanje*	r	0.109	0.219	0.077	-0.596*	-0.804*	-0.478*	1	
	p	0.565	0.245	0.684	0.001	0.000	0.008		
8. Total score/*Ukupan skor*	r	-0.257	-0.126	0.635*	0.556*	0.771*	0.818*	-0.606**	1
	p	0.170	0.508	0.000	0.001	0.000	0.000	0.000	

well as between the risk of emotional problems and the total score ($r = 0.635$, $p < 0.00$). Furthermore, a statistically significant correlation was found between the risk of behavioral problems and hyperactivity ($r = 0.524$, $p < 0.003$) with the total score – $r = 0.635$, $p < 0.00$, and the risk of prosocial behavior problems – $r = -0.596$, $p < 0.001$. A significant strong positive correlation was noted between hyperactivity and peer problems ($r = 0.506$, $p < 0.004$), negative with prosocial behavior ($r = -0.804$, $p < 0.000$) and positive with the total score ($r = 0.771$, $p < 0.000$). A statistically significant strong and positive correlation was found between the domains of behavioral problems and prosocial behavior ($r = -0.478$, $p < 0.008$), as well as with the total score ($r = 0.818$, $p < 0.000$). Finally, a significant strong negative correlation was found between problems in prosocial behavior and the total score ($r = -0.606$, $p < 0.000$).

Discussion

A constant increase in the number of children with the diagnosis of ASD also increases the number of siblings who are growing up with them. Regardless of the positive or negative experience of the family situation, all family members share the belief that their lives are different from the lives of families with healthy children [1]. The research findings do not clearly indicate why some sibling relationships are warm and supporting, while others are conflicting [21].

Meyer et al. claim that the overall experience of living with a sibling with ASD is quite negative [11], that the siblings are at higher risk of emotional and behavioral problems because they feel either invisible (if the focus is on the child with ASD) or too visible (if the burden of responsibility and/or expectations is on them) [22]. The family conflicts and dysfunctional patterns of coping with stress within the family contribute to the negative perception of relationships [23].

Hastings reports on a lower degree of adjustment of siblings of children with ASD in relation to the normative group [10] even compared to the siblings of children with other disorders [24]. On the other hand, having a sibling with ASD in the family does not necessarily mean a negative experience and facing the new situation is crucial for acceptance [13, 6]. Also, higher levels of compassion, tolerance and understanding are later noted in siblings of children with ASD in relation to their peers [16].

Examination of factors that may be related to sibling adjustment, such as gender, age, level of functionality of the child with ASD, family structure and socioeconomic status of the family, did not confirm the initial assumptions about the strong influence of these variables on sibling adjustment [10], which has also been confirmed by the results of our research. Although the initial assumption was that siblings have huge problems and that all of these variables have a strong impact [25], in the highest percentage of cases there is a low risk of clinically significant problems (63.3%), in 10% of cases the risk is medium, and in 26.7% it is high.

The demands and challenges faced by parents of children with ASD increase marital dissatisfaction, shape the marital and family life, making them perceive it as an unpleasant experience [26]. In such families, all family members experience the burden of emotional worries and stress, especially siblings of the child with ASD [16]. Considering the complexity of such an experience and the fact that it is a

challenge to maintain family unity in the short and long term [27], it was expected that the family status/climate affects the functioning of siblings of children with ASD, which has not been confirmed. In families of single mothers and divorced parents, the risk of clinically significant problems is low (66.7%), high risk does not exist, while in families with both parents, the risk is low in 62.5%, medium in 4%, and high in 33.5%. There are no statistically significant differences between the measured parameters.

In our study, no statistically significant gender differences were found in the functioning of siblings of children with ASD, but brothers proved to be at somewhat higher risk of hyperactivity issues (53%) in relation to sisters (20%). Furthermore, they are at higher risk for peer problems and prosocial behavior problems. The findings of previous researches indicate pronounced behavioral and hyperactivity problems in male siblings of children with ASD [28] compared to the higher level of emotional problems found in female siblings [29], which has also been confirmed by our findings, in which sisters have shown a higher risk for the manifestation and existence of emotional problems (33%) compared to brothers (13%). These findings are interpreted as high expectations of girls to "care for a child with ASD" [29], while boys generally have a tendency to perform worse on psychological adjustment tests [28]. These issues, however, may also be related to extended phenotype of autism in first-degree relatives, especially in male relatives, which may represent the neurological basis of adjustment [27, 30].

The results of our study do not indicate a statistically significant connection between age and problems in the functioning of siblings of children with ASD. We need to take into consideration the fact that our participants, as well as in a great number of studies on this topic, are in a wide range of ages from 4 to 18 years, from childhood to adolescence, that can lead to conflicting results, given that the understanding and expectations from other family members may be different in regard to the life stage they are going through [8]. During their childhood, siblings of children with ASD may not understand the behavior of their siblings due to the "invisible nature" of autism (which can affect the quality of their relationship) just as adolescent siblings may face social inconveniences due to misunderstanding of the disease. Therefore, it is important to study both groups independently [8].

With regard to the age of siblings of children ASD, although our findings do not suggest it, a question arises as to why children younger than their siblings with ASD may have more adjustment problems. One hypothesis relies on the fact that older children had a period of building a relationship with their parents before the child with ASD was born, which may act as a protective factor for later adjustments [10]. In the milieu of contradictory results, the given hypotheses need to be tested by future research.

In their study, Xue et al. claimed that due to worry and discomfort caused by the unpredictability of functioning of the child with ASD, the whole family can be excluded from community activities [31], creating an "autistic family" as a consequence of maladaptation of the child's behavior to the social environment [32], which can undoubtedly affect the sibling's quality of life. In that context, it was to be expected that there was a connection between the level of functionality of the person with ASD and the problems in the sibling functioning, but a statistical significance within these parameters was not found in our research.

Finally, a question arises as to what extent the completed questionnaires reflect the real picture, and to what extent they were shaped by socially desirable norms, given that a small number of parents participated in the survey, compared to the number of parents who were offered to participate. The overall research results, on average, indicate a low risk of problems in the functioning of siblings of children with ASD, which can be interpreted as a possibility that parents who agreed to cooperate have children who, in their opinion have no pronounced issues, and refusal to participate may be associated with the presence of emotional and behavioral problems of siblings of children with ASD. In addition, methodological limitations of the research in terms of sample design, sample size and multidimensionality of diagnoses of children with ASD, can affect the results and prevent the generalization of findings. These assumptions must be the subject of future research in order to determine the factors that influence the existence of clinically relevant problems in the functioning of siblings of children with ASD.

Conclusion

The behavioral, functional and emotional complexities of raising a child with autism spectrum disorder affect all family members. The difficulties and dilemmas faced by the parents of persons with disabilities do not spare their siblings either [33]. Based on the results of our research, we can conclude that the problems in the functioning of siblings of children with autism spectrum disorder do not depend on sociodemographic and family characteristics, which means that all siblings of children with autism spectrum disorder are at the same risk regardless of their gender, age, family status and the level of functioning of the child with autism spectrum disorder. Given the existing models of support to families in Serbia, within which the work with siblings of children with autism spectrum disorder is sporadic and rare, it is necessary to promote this type of research, which will enable modeling the support depending on the needs of siblings, in order to reduce the negative effects these difficulties can cause. Models of support, along with educating the community by raising autism awareness, would enable strengthening the need to maintain quality sibling relationships, but also nurture the need for personal freedom and independence of siblings.

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Rad je primljen 6. XI 2021.
Recenziran 7. XII 2021.
Prihvaćen za štampu 8. XII 2021.
BIBLID.0025-8105:(2021):LXIX:7-8:249-255.

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Professional aricle
Stručni članak
UDK 616.8-009.7-08:615.85
https://doi.org/10.2298/MPNS2108257P

EFFECTS OF MIRROR THERAPY IN THE TREATMENT OF PATIENTS WITH VARIOUS PAIN SYNDROMES – LITERATURE REVIEW

EFEKTI TERAPIJE OGLEDALOM U LEČENJU PACIJENATA SA RAZLIČITIM BOLNIM SINDROMIMA – PREGLED LITERATURE

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Summary

Introduction. Mirror therapy was developed by Vilayanur Subramanian Ramachandran for the treatment of phantom pain after limb amputation. Mirror therapy is a neurorehabilitation technique that helps to relearn the use of affected limbs on many neurological and psychological levels. **Material and Methods.** A literature review was conducted using the following databases: KOBSON, Google Scholar, PubMed and MEDLINE. **Results.** A systematic literature review has shown that there is insufficient evidence of the effectiveness of this treatment in relieving and suppressing phantom limb pain. The results indicate the effectiveness of mirror therapy in relieving pain in people after a stroke. The small amount of evidence and lack of methodology reports have a major impact on the quality of this evidence. After mirror therapy, a significant reduction in pain at rest and during active movement was reported. **Conclusion.** Further research on mirror therapy is needed to help relieve pain. Evidence of the effectiveness of mirror therapy on pain has not been sufficiently investigated so far.

Key words: Mirror Movement Therapy; Feedback, Sensory; Pain; Phantom Limb; Stroke; Complex Regional Pain Syndromes; Rehabilitation

Sažetak

Uvod. Terapiju ogledalom razvio je Ramahandran (*Ramachandran*) sa ciljem lečenja fantomskog bola nakon amputacije ekstremiteta. Terapija ogledalom je neurorehabilitaciona tehnika koja na mnogim neurološkim i psihološkim nivoima pomaže da se ponovo nauči upotreba zahvaćenih ekstremiteta. **Materijal i metode.** Pregled literature je sproveden korišćenjem sledećih baza podataka: *Kobson*, *Google Schoolar*, *PubMed* i *Medline*. **Rezultati.** Sistematski pregled literature pokazao je da nema dovoljno dokaza o efikasnosti ovog tretmana u ublažavanju i suzbijanju fantomskog bola. Rezultati ukazuju na efikasnost terapije ogledalom u ublažavanju bolova kod ljudi nakon moždanog udara. Mala količina dokaza i nedostatak izveštaja o metodologiji imaju veliki uticaj na kvalitet ovih dokaza. Nakon primene terapije ogledalom prijavljeno je značajno smanjenje bolova u mirovanju i tokom aktivnog kretanja. **Zaključak.** Potrebna su dalja istraživanja terapije ogledalom u svrhu kupiranja bola. Dokazi o efikasnosti terapije ogledalom na bol za sada nisu dovoljno ispitani.

Ključne reči: terapija ogledalom; senzorna povratna sprega; bol; fantomski ud; moždani udar; regionalni bolni sindrom; rehabilitacija

Introduction

Mirror therapy (MT) was developed by Vilayanur Subramanian Ramachandran for the treatment of phantom pain after limb amputation, which showed positive results [1]. In the 1990s, Ramachandran used a "mirror box" to "resurrect" phantom limbs and thus to treat the pain that often accompanied them. The experimental success of MT led Ramachandran to see mirrors as a useful model of brain function, a tendency that explains his attraction to work on "mirror neurons". Ramachandran's fascination with and repeated appeal to the mirror can be explained by the way it allowed him to confront a perennial problem in the mind and brain sciences, that of the relationship between a supposedly immaterial mind and a material brain. By producing what Ramachandran called a virtual reality, relating in varied and complex ways to the material world, the mirror reproduced a form of psychophysical parallelism and dualistic ontology, while conforming to the materialist norms of neuroscience today [2]. The MT has been shown to be successful in people with various pain syndromes such as complex regional pain syndrome (CRPS) [3], in children with cerebral palsy [4], and in rehabilitation of the hand and foot after injury or surgery.

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Abbreviations

MT – mirror therapy
PLP – phantom limb pain
CRPS – complex regional pain syndrome
KOBSON – Serbian Library Consortium for Coordinated Acquisition
MEDLINE – Medical Literature Analysis and Retrieval System

The aim of this study was to present the effects and mechanisms of action of MT in the treatment of patients with phantom limb pain (PLP), CRPS and stroke.

Material and Methods

The literature used in this study deals with the application of MT as a form of adjunctive treatment in physical medicine and rehabilitation. Papers in Serbian, Croatian and English were studied. The literature review was conducted using the following databases: Serbian Library Consortium for Coordinated Acquisition (KOBSON), Google Scholar, PubMed and Medical Literature Analysis and Retrieval System (MEDLINE). Search options included the following keywords: mirror therapy, visual feedback, mirror, PLP, CRPS and stroke.

Mirror therapy

The MT is a neurorehabilitation technique that helps to relearn the use of affected limbs on many neurological and psychological levels [5]. The idea of MT is to modulate the brain by performing a series of movements with the healthy limb, which are reflected in the mirror. They create a visual illusion thereby deceiving the brain and making it appear that the movement was performed by the affected limb [6]. Regarding the MT mechanism of action, as one of the possibilities Ramachandran calls "learned paralysis" of the brain, it can be solved by the illusion provided by the reflection in the mirror [7]. Another possibility is a system of mirror neurons, when observing movement in a mirror causes nerve activity in the motor areas of the affected hemisphere, which can ultimately lead to cortical reorganization and progress in motor function. Namely, nerve cells have been discovered that are activated not only by performing a certain action, but also by observing motor activity, which is why they play a major role in the process of relearning motor skills through observation, and are called mirror neurons [8]. The visual illusion, which makes a person appear to have both limbs moving symmetrically, simultaneously activates both hemispheres of the brain and thus increases the activity in the paretic limb. Activation of the cerebral hemispheres then functions as the basis for neurological mechanisms to act on brain plasticity [9]. When talking about the application, the mirror must be placed in front of the central line of the body, so that the affected limb is completely hidden on the other side of the mirror, and the reflection of the unaffected limb is completely visible. The unaffected limb should be placed in a similar position to the affected limb, as this affects the intensity of the illusion [10]. The dimensions of the mirror should be large enough to cover the entire affected limb, and the person can see all movements in front of the mirror. It is recommended to conduct MT at least once a day for at least 10 minutes. The maximum duration of each session depends on the person's cognitive abilities or negative side effects, but usually lasts about 30 minutes [11]. After basic exercises, additional functional tasks with various objects such as wooden cubes or balls may be included in the program.

Results

Effects of mirror therapy in the treatment of phantom limb pain

The PLP, any painful sensation associated with a missing limb, is frequently found among persons who have experienced the loss of a body part through amputation [12]. It is estimated that more than 80% of patients with total or partial loss of a limb develop PLP [13]. It is a kind of neuropathic pain that can be knife-like, needle-like, burning, throbbing, shooting, squeezing, tingling, jabbing, cramping, crushing, itching, and tearing [14–17]. Phantom limb problems affect the amputees with a serious impact on sleep quality, mood, ability to move, activities of daily living, work, and quality of life [18, 19]. There are a few studies on the effectiveness of MT as a pain management intervention for patients with PLP. In the case study of MacLachlan et al., MT was used to treat PLP in a patient with a lower limb amputation who presented PLP at the time of treatment. The authors showed that during the intervention, the patient presented with a significant PLP reduction, increased motor control over the phantom limb and a change in the aspects of the phantom limb that was experienced [20]. In addition, Darnall reported a case in which a 35-year-old man with an acquired above-knee amputation of the left lower limb had success with home-based patient-delivered MT after failing to respond to conventional treatment for PLP; with MT, his phantom pain resolved, and his nerve pain was well managed [21]. In another pilot study of Darnall et al., 40 patients with unilateral amputations and PLP were studied to evaluate the feasibility and preliminary efficacy of self-delivered home-based MT for PLP. Patients received an explanation how to use MT and the treatment lasted for 25 minutes a day. Patients completed the home therapy and posted their answers to sets of outcome questionnaires at 1 and 2 months after treatment**.** The results of the study showed a significant reduction in average phantom pain intensity [22]. A systematic review of the literature on the impact and application of MT on phantom pain has shown that there is insufficient evidence of the effectiveness of this treatment in alleviating and suppressing phantom pain [23]. It is necessary to mention the side effects of this therapy**.** Some studies [24–26] reported adverse effects of MT such as aggravation of the phantom limb sensation and/or PLP, telescopic distortion of the phantom limb, nausea, dizziness, confusion, the sensation of irritation, and grief. Therapists should systematically evaluate such negative effects and sufficiently inform patients before starting MT,

and the treatment should be stopped when the adverse effects are too strong.

Effectiveness of mirror therapy in stroke rehabilitation

Stroke is the third leading cause of disability and premature death of men and women in the world. The psychological, physical and social consequences of stroke may be great [27]. Rehabilitation strategies need to be repetitive, intensive, and task-specific to promote recovery of neuroplasticity [28–30]. It is reported that if therapy begins within 16 hours to 6 months after stroke, there is a significant improvement in activities of daily living with augmented exercise therapy [31]. In contrast to varied therapy approaches which require some degree of voluntary movement, MT can be used even in completely plegic, severely paretic stroke survivors, as MT uses visual rather than somatosensory stimuli for producing a desired response in the affected limb [32].

The effects of MT on the motor functions of the upper limbs are reflected in terms of either improved dexterity, gross and fine motor movements, grip force, decreased movement time, or proximal motor control [33]. As for the lower extremities, the improvement is noticeable in improved walking speed, single limb stance, step and stride lengths, static and dynamic balance, and decreased mediolateral and anteroposterior sway in standing [34]. The results also point to evidence of the effectiveness of MT in relieving pain in people after stroke. The small sample size and lack of methodology reports have a major impact on the quality of this evidence [35]. A future scope for MT is to identify its relation to different presentations of stroke among men and women. Different risk factors, stroke severity, and neurological outcomes between men and women may demand a modified application of MT for rehabilitation in individual genders. Research is also needed on the effects of MT in different subtypes of stroke, be it a pure motor stroke, or a stroke with sensory and other components [36].

Effects of mirror therapy in patients with complex regional pain syndrome

Complex regional pain syndrome is defined as a pain in which, in addition to pain, there are signs of autonomic nervous system disorders. In clinical practice it is most commonly seen as a complication of a wrist fracture. In our country, CRPS is also known as Sudeck atrophy, and it refers to a sore, self-limited, sensitive limb with altered skin color [37]. Many CRPS patients have issues with body perception. The study of Kotiuk et al. found that MT can improve the perception of the body schema as an element of integrated treatment of CRPS [38]. The authors who conducted the research used MT for patients with CRPS and reported significant decreases in self-reported pain at rest, pain with active movement, as well as allodynia one week after treatment with MT [39, 40]. Despite its benefits, MT carries inherent limitations, as it requires the presence of an intact limb. In 2014, Jeon et al. [41] proposed virtual body swapping as a possible alternative. This method aims to induce an illusionary body perception so a patient can identify a virtual body as his/her own. Jeon et al. [41] showed a short video clip (filmed from the first person perspective) that consisted of four body movements (hand opening/closing, elbow flexion/extension, plantar flexion/dorsiflexion, leg flexion/extension). Subjects randomly assigned into the treatment group were instructed to mimic as well as mentally rehearse these movements, whereas those in the control group only watched the video clip. Jeon et al. [41] observed that the treatment group displayed less body disturbance perception (evaluated by the modified Body Perception Disturbance Questionnaire). However, pain scores were similar between the two groups.

Conclusion

Mirror therapy is a useful method that can cause additional improvement in motor functions when performed over a long period of time. It plays an important role in the recovery of manual dexterity, fine motor skills and in speed and range of motion. The evidence of mirror therapy efficacy on pain is so far insufficient to recommend it as a first-line treatment. Randomized controlled trials are still needed to assess the effects of mirror therapy more thoroughly, on both pain and the integration and use of the prosthetic limb, and also to determine the optimal terms for its application.

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Rad je primljen 2. XI 2021.
Recenziran 24. XI 2021.
Prihvaćen za štampu 24. XI 2021.
BIBLID.0025-8105:(2021):LXIX:7-8:257-260.

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CASE REPORTS
PRIKAZI SLUČAJEVA

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Case report
Prikaz slučaja
UDK 616.155.194-02:615.225.065-055.26
https://doi.org/10.2298/MPNS2108261B

DRUG-INDUCED AUTOIMMUNE HEMOLYTIC ANEMIA IN PREGNANCY – A CASE REPORT

AUTOIMUNA HEMOLIZNA ANEMIJA IZAZVANA LEKOVIMA U TRUDNOĆI – PRIKAZ SLUČAJA

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Summary

Introduction. About 10 – 20% of patients taking methyldopa therapy for more than 4 months develop autoantibodies to antigens on the surface of their own red blood cells, while less than 1% develop autoimmune hemolytic anemia. Methyldopa-induced red cell autoantibodies not associated with autoimmune hemolytic anemia are five times more common in pregnant women than in non-pregnant women. **Case Report.** We present a case of methyldopa-induced immune hemolytic anemia in a 23-year-old woman in her first pregnancy with and an estimated gestational age of 30 weeks. The woman presented with obesity and preeclampsia and there was no information that she has ever received a blood transfusion. The hemoglobin concentration was 10.8 g/dL. The woman started taking methyldopa tablets in the twelfth week of pregnancy and 14 weeks later she developed anemia. Warm type autoantibodies were detected in the 30th week of gestation. **Conclusion.** Although methyldopa-induced hemolytic anemia is rare during pregnancy, it is necessary to monitor pregnant women who take this therapy, because only discontinuation of the drug leads to termination of hemolysis and correction of anemia.

Key words: Anemia, Hemolytic, Autoimmune; Pregnancy; Methyldopa; Eclampsia; Autoantibodies; Risk Factors; Serologic Tests; Overweight; Hypertension

Sažetak

Uvod. Od 10 do 20% pacijenata koji uzimaju terapiju metildopom duže od četiri meseca razvije autoantitela na antigene koji se nalaze na membrani eritrocita, dok manje od 1% razvije autoimunu hemoliznu anemiju. Pojava metildopom indukovanih antieritrocitnih autoantitela koja nisu udružena sa autoimunom hemoliznom anemijom je pet puta češća kod trudnica nego kod žena koje nisu trudne. **Prikaz slučaja.** Predstavljamo slučaj imune hemolizne anemije izazvane metildopom kod 23-godišnje žene u prvoj trudnoći, sa procenjenom gestacijskom starosti od 30 nedelja. Kod trudnice je ustanovljena gojaznost i preeklampsija. Nije bilo podataka da je ikada primila transfuziju krvi. Koncentracija hemoglobina bila je 10,8 g/dL. Žena je počela da uzima metildopu oralno u 12. nedelji trudnoće a 14 nedelja kasnije se razvila anemija. Topla autoantitela su otkrivena u 30. nedelji gestacije. **Zaključak.** Iako je hemolizna anemija izazvana metildopom retka tokom trudnoće, potrebno je pratiti trudnice koje uzimaju ovakav vid terapije jer samo prestanak uzimanja leka dovodi do prestanka hemolize i korekcije anemije.

Ključne reči: autoimuna hemolizna anemija; trudnoća; metildopa; eklampsija; autoantitela; faktori rizika; serološki testovi; gojaznost; hipertenzija

Introduction

Drug-induced immune hemolysis has an estimated annual incidence of 1 – 4 cases per million individuals [1]. About 10 – 20% of patients taking methyldopa therapy for more than 4 months develop autoantibodies to antigens on the surface of their own red blood cells (RBCs) [1, 2]. Autoantibodies are usually specific to rhesus (Rh) antigens, but the antibody specificity often cannot be defined. The autoantibody is typically a warm reacting immunoglobulin G (IgG) type and it is detectable in the patient's sera as well as on the RBCs [2]. Less than 1% of patients taking methyldopa develop autoimmune hemolytic anemia (AIHA). The rate of hemolysis and the severity of the anemia vary from mild to severe [3, 4].

Methyldopa is recommended as first-line treatment for gestational hypertension in most countries. Preeclampsia is a serious condition defined as new onset of hypertension (≥ 140/90 mm Hg) in pregnant women who have never had high blood pressure before. It is followed by proteinuria (total protein ≥ 300 mg in a 24-hour urine) and swelling in the feet, legs, and hands. This condition affects 3–4%

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RBCs – red blood cells
Rh – rhesus
IgG – immunoglobulin G
BMI – body mass index
AIHA – autoimmune hemolytic anemia
AHG – anti-human globulin
IAT – indirect antiglobulin test
DAT – direct antiglobulin test

of pregnant women in the world and usually appears after the 20th week, although it can occur earlier [5, 6]. Risk factors include first pregnancies, multiple gestations, previous preeclampsia, high body mass index (BMI) before pregnancy, etc. The goal of the therapy is to prevent cerebrovascular and cardiac complications in pregnant women, as well as to preserve the uteroplacental and fetal circulation. The doses of methyldopa recommended in pregnancy are similar to those used in non-pregnant patients. Methyldopa has no teratogenic effects and does not impair the uteroplacental circulation and consequent fetal growth [7].

The cases of methyldopa-induced AIHA are rarely reported due to a decline in methyldopa use in the general population as well as rarely occurring methyldopa-induced autoantibodies during pregnancy. Consistent with the fact that IgG antibodies cross the placenta, the occurrence of methyldopa IgG antibodies during pregnancy is a risk for both mother and fetus, and it may be low or high [8]. Hemolysis is always extravascular in type.

The objective of this study was to point to the importance of appropriate serologic testing and careful medical history taking in diagnosis of AIHA in pregnant women with methyldopa-induced AIHA.

Case Report

We present a case of methyldopa-induced immune hemolytic anemia in a 23-year-old obese woman in her first pregnancy with the diagnosis of preeclampsia and an estimated gestational age of 30 weeks. There was no information that she has ever received a blood transfusion. The woman was overweight before pregnancy with a BMI = 35.7 (weight 88 kg, height 157 cm). She presented with high blood pressure, protein in the urine, and swollen hands, feet and face in the 9th week of pregnancy. In the 12th week of pregnancy, oral methyldopa therapy was prescribed with a daily dose of 1,500 mg. Methyldopa reduced her blood pressure to 120/80 mm Hg. In the 28th week of pregnancy, the patient was diagnosed with anemia. The complete blood count test performed by automated cell count showed normal white blood cell count (4.9×10^9/L) with a normal differential image, a normal platelet count (195×10^9/L), low RBC count (2.9×10^{12}/L), low hemoglobin concentration (10.8 g/dL), low hematocrit (32.4%), low haptoglobin (< 6), high reticulocyte count (10%), high mean corpuscular volume (111 fL), high mean corpuscular hemoglobin (37 picograms/cell), and high mean corpuscular hemoglobin concentration (33 g/dL). The woman presented with symptoms of anemia such as exhaustion and pale skin two weeks earlier. She did not have jaundice, dark urine or previous history of anemia. The biochemical analyses showed normal indirect bilirubin level of 0.3 mg/dL, aspartate transaminase (29 IU/L) and alanine transaminase (38 IU/L); increased lactate dehydrogenase level (552 IU/L), fibrinogen (4.38 g/L) and D-dimer (1835 mkg/L); normal activated partial thromboplastin time (29.4 s), scintillation proximity assay (112%) and scintillation proximity assay (0.95 Item Unit Value). The urinalysis was normal and serum iron was within normal limits (13μmol/L). When it comes to medication, the patient was receiving methyldopa, diazepam, progesterone and iron tablets (orally).

The woman had O blood type, RhD-positive and her husband had B blood type, RhD-positive.

The woman's serum antibody screening was negative. The standard antibody screening test at 37^0C with antihuman globulin (AHG) phase - indirect antiglobulin test (IAT) was performed on fully automated immunohematology analyzer (IH-500) by agglutination technique in a microtube using a two-cell screening panel (Bio-Rad, ID-DiaCell I-II, DiaMed GmbH, 1785 Cressier, Switzerland). Screening of antibodies at room temperature (22 ± 2^0C) as well as enzyme-treated (with bromelain) panel cells was done by a tube test using a three-cell panel (Reagens Kft, ReaCell I, II, II, Budapest, Hungary).

Direct antiglobulin test (DAT) was found to be positive (2+) on ID-card using polyspecific AHG reagents. The DAT was also found to be positive using monospecific AHG reagents (anti-IgG1, anti-IgA, anti-IgM, anti-C3c, anti-C3d) thereby indicating the presence of IgG on the surface of RBCs (Bio-Rad, DC-Screening I, ID-Card Configuration: IgG, IgA, IgM, C3c, C3d, ctl, DiaMed GmbH, 1785 Cressier, Switzerland).

The RBCs surface bound antibodies were eluted from the cells using a heat technique (10 minutes, 56^0C). Antibody identification was performed by the technique of agglutination in microtubes using a commercial 11-cell RBCs panel (BioRad Set ID-DiaPanel, DiaMed GmbH, 1785 Cressier, Switzerland), with AHG at 37^0C. With this panel, the response was positive with the agglutination strength of 4+ with all cells including auto control.

Methyldopa therapy was discontinued after premature birth in the same week. Atenolol (beta blocker) plus nifedipine (calcium channel blocker) were used postpartum to obtain hypertension control.

The woman remained anemic for 3 weeks after she had stopped taking this therapy and 8 weeks later anemia had completely resolved. A male newborn was born at 30 weeks of gestation with Apgar score of 6.8, birth weight 1,430 g, 41cm long. No hemolytic disease was found in the newborn. The newborn was type B, RhD-positive, DAT negative.

Discussion

We reported a drug-induced immune hemolytic anemia in a pregnant woman who developed anemia

14 weeks after the initiation of alpha-methyldopa therapy. The eluate from the patient's RBCs reacted with normal RBCs without the presence of methyldopa, but the specificity of the autoantibodies was not determined. Methyldopa-induced autoantibody was of a warm antibody type that responded optimally at 37°C.

The AIHA is characterized by the development of antibodies that bind to antigens of their own RBCs. The diagnosis of AIHA can only be made if both clinical and serological findings are present. In general, AIHAs are classified as warm, cold, paroxysmal cold, mixed, and drug-induced [9]. Drug exposure may be the trigger for the production of autoantibodies, or in some cases drug binding to the surface of the RBCs may occur, thereby inducing hemolysis [10, 11]. Drugs that can lead to anemia are penicillin and its derivatives, cephalosporins, tetracycline, erythromycin, nitrofurantoin, nonsteroidal anti-inflammatory drugs etc. The AIHA was first linked with alpha-methyldopa in 1966 by Worlledge and her colleagues at the Royal Postgraduate Medical School [12, 13].

Alpha-methyldopa-induced hemolysis is categorized as true autoimmune and drug-independent antibody [14], meaning that the antibody is in vitro reactive, even in the absence of the drug. In that case, hemolysis is always extravascular in type and IgG-mediated [15]. The mechanism of hemolysis is more complex and heterogeneous with immune dysregulation, autoantibodies via molecular mimicry, and drug adsorption causing altered RBC membrane antigens [14].

Alpha-methyldopa is very effective in the treatment of hypertension with fewer side effects compared to other drugs. Studies have not shown an association between the amount of medication and the intensity of anemia. Cases of alpha-methyldopa-induced AIHA are rarely found in the literature, especially when it comes to pregnancy. In case autoantibodies belong to the IgG class, AIHA can be potentially dangerous for both mother and fetus since IgG antibodies cross the placenta.

In patients who develop methyldopa-induced hemolysis, discontinuation of therapy leads to withdrawal of hemolysis symptoms. The use of corticosteroids can accelerate postpartum recovery in these patients. However, it may take many months before the DAT becomes negative. It takes between 4 weeks and 4 months to normalize RBCs values after discontinuing the use of methyldopa, although DAT remains positive. The period required to stop antibody production varies between 4 and 12 months. An autoimmune phenomenon is the mechanism by which the methyldopa causes a positive DAT [16].

The AIHA can have a wide clinical spectrum, from asymptomatic (fully compensated) to life-threatening. The pathogenic role of antibodies depends on the class, thermal optimum at which they respond, and affinity and efficiency in complement activation. The RBCs autoantibodies are mainly detected via DAT. Positive DAT may be due to autoantibody-induced drugs. However, the presence of RBCs autoantibodies is not always associated with the development of clinical manifestations of AIHA ("silent" antibodies) just as immune hemolysis cannot be ruled out with a negative DAT result. The occurrence of "silent" autoantibodies can be detected during pregnancy and it is five times more frequent in pregnant women than in non-pregnant women.

Conclusion

Our case showed that although potentially dangerous side effects including severe hemolytic anemia are rare after taking methyldopa during pregnancy, it is necessary to diagnose autoimmune hemolytic anemia and to monitor the clinical condition of these patients, because only discontinuation of the drug leads to termination of hemolysis and correction of anemia. Taking an accurate medical history during pregnancy is essential to clarify the diagnosis.

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Rad je primljen 7. XI 2021.
Recenziran 7. XII 2021.
Prihvaćen za štampu 15. XII 2021.
BIBLID.0025-8105:(2021):LXIX:7-8:261-264.

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Case report
Prikaz slučaja
UDK 617.731:616-006.328]-07/-08
https://doi.org/10.2298/MPNS2108270L

OPTICAL COHERENCE TOMOGRAPHY ANALYSIS OF EYES IN PATIENTS WITH CHRONIC CHIASMAL COMPRESSION – A CASE REPORT

OPTIČKA KOHERENTNA TOMOGRAFSKA ANALIZA OČIJU PACIJENTA SA HRONIČNOM HIJAZMALNOM KOMPRESIJOM – PRIKAZ SLUČAJA

Desanka GRKOVIĆ[1, 2], Sava BARIŠIĆ[1], Sofija DAVIDOVIĆ[1, 2], Stefan BRUNET[1, 2] and Svetlana PAVIN[1]

Summary

Introduction. Olfactory groove meningiomas cause progressive compression of the frontal lobes with posterior projection towards the sella turcica. If large enough, these tumors may cause optic nerve compression and optic chiasm. The aim of this study was to determine whether optical coherence tomography, as a method that objectively measures the thickness of retinal nerve fiber layer and retinal ganglion cell complex thickness, provides a realistic assessment of the postoperative visual outcome in chronic chiasmal compression caused by olfactory groove meningioma. **Case Report.** A 55-year-old woman presented with an eight month history of malaise, weakness, frontal headaches, anosmia, and blurred vision in both eyes. Magnetic resonance imaging of the endocranium revealed a large olfactory groove meningioma extending into the prechiasmal portion of the optic nerves and optic chiasm with a marked compressive effect. The mean preoperative retinal nerve fibre layer thickness was 65 μm in the right eye and 63 μm in the left eye. Ten months after surgery, the mean retinal nerve fibre layer thickness was 67 μm in the right eye and 63 μm in the left eye. The mean preoperative ganglion cell complex thickness was 57 μm in the right eye, while it could not be measured in the left eye due to loss of fixation. Ten months after surgery, the mean ganglion cell complex thickness was 56 μm in the right eye and 48 μm in the left eye. The obtained values were significantly lower than the physiologic thickness values. **Conclusion.** Retinal nerve fibre layer thickness and ganglion cell complex thickness measured by optical coherence tomography represent a valid prognostic indicator of visual outcome and recovery after surgical decompression of the optic chiasm.

Key words: Tomography, Optical Coherence; Nerve Fibers; Retina; Retinal Ganglion Cells; Optic Chiasm; Meningioma; Nerve Compression Syndromes; Prognosis; Visual Acuity; Decompression, Surgical

Sažetak

Uvod. Olfaktorni meningeomi uzrokuju progresivnu kompresiju frontalnog režnja sa posteriornom projekcijom do turskog sedla. Ukoliko su dovoljno veliki, ovi tumori mogu uzrokovati kompresiju optičkog nerva i optičke hijazme. Cilj rada je utvrditi da li optička koherentna tomografija, kao metoda koja objektivno meri debljinu retinalnih nervnih vlakana i kompleksa ganglijskih ćelija retine, daje realnu procenu postoperativnog poboljšanja vidne funkcije, u slučaju hronične hijazmalne kompresije uzrokovane olfaktivnim meningeomom. **Prikaz slučaja.** Pacijentkinja stara 55 godina**,** u poslednjih osam meseci oseća nesvesticu, slabost, čeonu glavobolju, gubitak osećaja mirisa i pad vidne oštrine na oba oka. Snimak magnetnom rezonancijom prikazuje veliki olfaktivni meningeom koji doseže do prehijazmalnog dela oba optička nerva i optičke hijazme, sa izraženim kompresivnim efektom. Srednja vrednost debljine retinalnih nervnih vlakana, preoperativno, iznosila je 65 μm na desnom oku i 63 μm na levom oku. Deset meseci nakon operacije srednja vrednost debljine retinalnih nervnih vlakana iznosila je 67 μm na desnom oku i 63 μm na levom oku. Srednja vrednost debljine kompleksa ganglijskih ćelija bila je preoperativno 57 μm na desnom oku, dok se na levom oku zbog gubitka fiksacije nije mogla izmeriti. Deset meseci nakon operacije srednja vrednost debljine bila je 56 μm na desnom oku i 48 μm na levom oku. Dobijene vrednosti su znatno niže od fizioloških vrednosti. **Zaključak.** Debljina retinalnih nervnih vlakana i debljina kompleksa ganglijskih ćelija retine merena optičkom koherentnom tomografijom predstavlja koristan prognostički pokazatelj mogućnosti poboljšanja vidne funkcije nakon hirurške dekompresije optičke hijazme.

Ključne reči: optička koherentna tomografija; nervna vlakna; mrežnjača; ganglijske ćelije mrežnjače; optička hijazma; meningiom; sindrom kompresije nerva; prognoza; oštrina vida; hirurška dekompresija

Introduction

Meningiomas are benign, slow-growing tumors that account for approximately 35% of all primary brain tumors [1, 2]. Olfactory groove meningiomas originate from the anterior cranial base, including the cribriform plate of the ethmoid bone and sphenoid plane. They account for approximately 8 – 13% of intracranial meningiomas. These tumors cause progressive compression of the frontal lobes with posterior projection towards the sella turcica. In addition, if tumors are large enough, the optic nerves and optic chiasm may be compromised. Headaches, anosmia, visual impairment, and personality changes are symptoms that have been commonly reported [1–5].

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Abbreviations

OCT – optical coherent tomography
RNFL – retinal nerve fiber layer
GCC – ganglion cell complex
GCINP – ganglion cell inner plexiform layer
MRI – magnetic resonance imaging
GCIPL – ganglion cell-inner plexiform layer
VF – visual function
VA – visual acuity

Surgical removal of the lesions is an essential aspect of clinical management. One of the primary indications for surgical management of chiasmal compression is a progressive loss of visual function (VF). Surgical treatment enables decompression of optochiasmatic complex, prevents further VF deterioration, and enables visual acuity (VA) improvement at the same time [6–8].

Several predictors for the improvement of VF after decompression of the anterior visual pathway were evaluated in the past with conflicting results. These include duration of symptoms, age, preoperative VA, tumor size, optic disc pallor, and fundoscopic appearance of the retinal nerve fibre layer (RNFL) [9–11].

With the development of optical coherence tomography (OCT), more objective measurements of optic nerve damage and a more accurate visual outcome prediction after treatment have become available. The OCT is a valuable tool for objective and quantitative assessment of the structural and functional damage of the RNFL and retinal ganglion cell complex (GCC) in eyes with optic nerve damages caused by chiasmal compression [12–15].

This case report evaluates whether OCT, providing an objective measurement of the RNFL and GCC thickness, offers a reliable prediction of visual outcome in case of chronic chiasmal compression by an olfactory groove meningioma.

Case Report

We report a case of a 55-year-old woman with an eight month history of malaise, weakness, frontal headaches, anosmia and blurred vision in both eyes. Ophthalmic examination included VA testing (Snellen charts), colour vision test, visual field analysis (Humphrey Field Analyzer, Carl Zeiss Meditec, Inc. Dublin, CA, USA, full-field-120-point suprathreshold testing), oculomotor nerve test, fundus examination and OCT measurements of RNFL and macular GCC thickness.

After pupil dilation (administration of 1% Tropicamide eye drops), OCT imaging was performed using the Cirrus OCT (OCT-3, OCT software version 6.0; Carl Zeiss Meditec Inc. Dublin, CA, USA). The RNFL optic disc cube 200 × 200 and macular cube 512 × 128 scan protocols were used. The ganglion cell analysis algorithm was used to determine macular ganglion cell inner plexiform layer (GCIPL) thickness within the 14.13 mm of 2 elliptical annulus area centered on the fovea. Six sectoral (superior, superonasal, inferonasal, inferior, inferotemporal, and superotemporal) GCIPL thickness values were used for analysis. The Cirrus SD-OCT algorithm calculates the peripapillary RNFL thickness at each point on the circle of 3.14 mm centred on the optic disc. Four quadrants (superior, nasal, inferior, and temporal) RNFL thicknesses were used for analysis.

Ocular position and motility were normal, and pupils were equal in size. Dilated fundus examination revealed a subatrophy of the optic nerve head in the right eye and atrophy of the optic nerve head in the left eye.

Magnetic resonance imaging (MRI) of the endocranium revealed an olfactory groove meningioma measuring 65 x 62 x 55 mm. The tumor extended on the prechiasmal part of both optic nerves and optic chiasm with a pronounced compressive effect **(Figure 1)**.

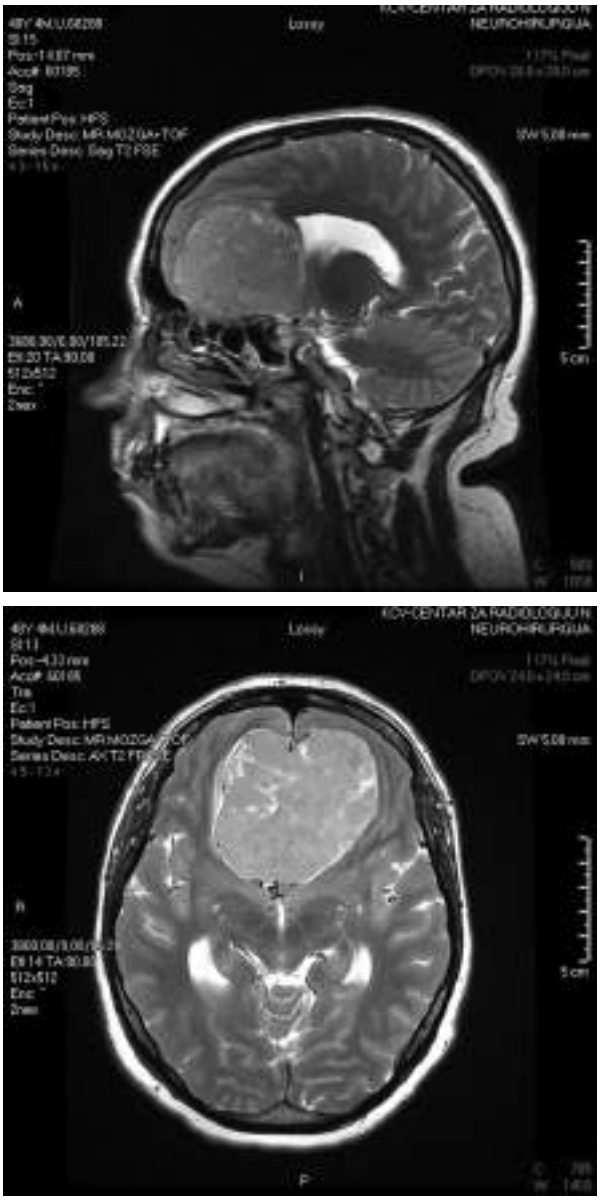

Figure 1. MRI − olfactory groove meningioma with optic chiasm compression
Slika 1. *Snimak magnetne rezonancije – olfaktivni meningeom sa hijazmalnom kompresijom*

Table 1. Rretinal nerve fiber layer thickness
Tabela 1. *Debljina retinalnog sloja nervnih vlakana*

	Preoperative/*Preoperativno*		Postoperative/*Postoperativno*	
	Right eye/*Desno oko* (μm)	Left eye/*Levo oko* (μm)	Right eye/*Desno oko* (μm)	Left eye/*Levo oko* (μm)
Average thickness/*Prosečna debljina*	65	63	67	63
Superior quadrant/*Gornji kvadrant*	74	71	75	61
Inferior quadrant/*Donji kvadrant*	77	80	79	87
Nasal quadrant/*Nazalni kvadrant*	57	52	61	59
Temporal quadrant/*Temporalni kvadrant*	54	49	53	47

Table 2. Thickness of the macular ganglion cell layer
Tabela 2. *Debljina sloja ganglijskih ćelija žute mrlje*

	Preoperative/*Preoperativno*		Postoperative/*Postoperativno*	
	Right eye *Desno oko* (μm)	Left eye *Levo oko* (μm)	Right eye *Desno oko* (μm)	Left eye *Levo oko* (μm)
Average thickness/*Prosečna debljina*	57		56	48
Superior sector/*Gornji sektor*	59		59	50
Inferior sector/*Donji sektor*	50		49	47
Superonasal sector/*Superonazalni sektor*	67		66	50
Inferonasal sector/*Inferonazalni sektor*	62		59	57
Superotemporal sector/*Superotemporalni sektor*	52		51	44
Inferotemporal sector/*Inferotemporalni sektor*	53		54	42

Preoperative average RNFL thickness was 65 μm in the right eye and 63 μm in the left eye **(Table 1)**. Ten months after surgery, the average RNFL thickness was 67 μm in the right eye and 63 μm in the left eye **(Table 1)**. The average preoperative GCC thickness was 57 μm in the right eye, while it could not be

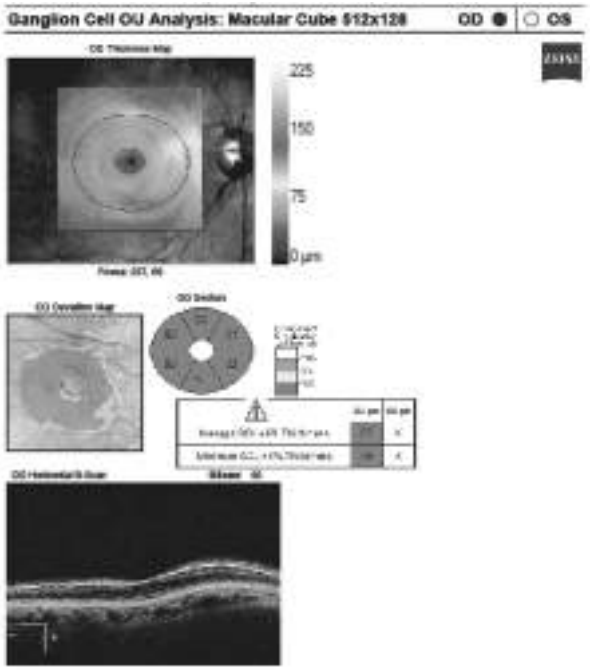

Figure 2. Optical coherent tomography analysis of the ganglion cell layer in the right eye preoperatively
Slika 2. *Optička koherentna tomografska analiza sloja ganglijskih ćelija desnog oka preoperativno*

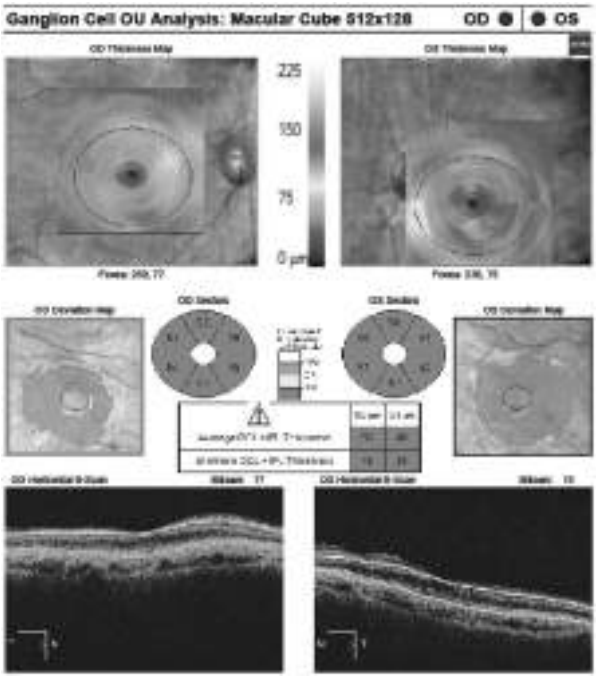

Figure 3. Postoperative optical coherent tomography of the ganglion cell complex in both eyes
Slika 3. *Postoperativna optička koherentna tomografija kompleksa ganglijskih ćelija retine oba oka*

measured in the left eye due to fixation loss **(Table 2, Figure 2)**. Ten months after surgery, the average GCC thickness was 56 μm in the right eye and 48 μm in the left eye **(Table 2, Figure 3)**.

The VA (Snellen) was 0.6 in the right eye and 0.03 (2/60) in the left eye. The visual field in the right eye showed 12/120 points and 0/120 points in the left eye.

Discussion

Olfactory groove meningiomas can grow insidiously large before clinical presentation due to subtle symptoms. Visual symptoms occur after an olfactory groove meningioma has extended posteriorly causing compression on one or both optic nerves or the optic chiasm. Damage to the retinal ganglion cell axons is due to downward pressure on the nerve and chiasm from above, causing stretching and chronic ischemia of retinal ganglion cell axons resulting in bitemporal hemianopsia and optic atrophy [1–4].

Individual variations of chiasmal position and the inclination of its oblique plain determine the duration of the silent stage of the olfactory groove meningioma. This stage is followed by the gradual, slow deterioration of VF with an uncertain prognosis for visual recovery. After surgical treatment of chiasmal compression, visual recovery occurs after tumor removal, secondary remyelination and restoration of axoplasmic flow over months to years [15, 16].

In this day and age, OCT assessment of RNFL and GCC thicknesses is recommended in the preoperative evaluation of patients with sellar/suprasellar masses. Numerous authors use OCT analysis as an objective indicator of visual outcome and recovery after surgical decompression of the chiasm [12–17]. In the evaluation of compressive optic neuropathies, OCT of the RNFL and GCC thicknesses have been established as the most important visual prognostic factors [14–16].

In recent years, it has been established that patients who have measurable RNFL and GCC thickness loss at the time of surgery of chiasmal compressive lesions are less likely to experience recovery of VA and VF after surgery [13, 16, 18]. Lower preoperative RNFL and macular GCC thickness were associated with worse postoperative VA and VF parameters. At the same time, preserved OCT of the RNFL and macular GCC thickness confers a good visual outcome [19].

In the case of our patient, chronic chiasmal compression caused not only conduction block but also significant atrophy of retinal ganglion cells that were confirmed with OCT parameters that mainly remained decreased. Ten months after surgery, the average RNFL thickness was 67 μm in the right eye and 63 μm in the left eye **(Table 1)** and the average GCC thickness was 56 μm in the right eye and 48 μm in the left eye **(Table 2, Figure 3)**. This is significantly lower than physiological values of RNFL thickness published by Loo et al. [8] evaluating it at 95.5 μm ± 11.0 and in a study by Tieger et al. [15] finding normal GCC thickness to be 86 ± 5 μm.

Postoperative VA was unchanged, and VF in the right eye showed a mild improvement, while it remained unchanged in the left eye.

Results in our patient showed to be comparable with the findings of Danesh-Meyer et al. [17, 18] who studied a series of 40 cases with chiasmatic compressive lesions using OCT and VF analysis; the pre and post decompression treatment results in patients with thin RNFL did not demonstrate significant improvement in VA and VF, just like in our patient. Jing Zhang et al. [19] as well Laowanapiban et al. [20] used preoperative and postoperative RNFL thickness analysis and found that eyes with VF defects but normal preoperative RNFL thickness showed a more significant improvement in postoperative VF than those with low preoperative RNFL thickness. Similarly, Jacob et al. [21] and Loo et al. [8] demonstrated that reduction in preoperative circumpapillary RNFL thickness indicates poor chances of recovery of initial visual field defect three or nine months after the treatment.

Recent data suggest that the thickness of macular GCC may be a more accurate and reliable predictor of VA recovery than RLNF thickness [14–16]. According to numerous authors, GCC thinning remained relatively unchanged before and after decompression [14–16]. We found a similar outcome in our patient, as well. Consequently, patients with GCC loss before decompression have decreased chances of postoperative VF recovery [17–20], a fact that we confirmed in our case report.

Conclusion

Retinal nerve fiber layer and ganglion cell complex thickness measurement by optical coherent tomography is a valuable prognostic indicator in the preoperative assessment of chiasmal compression and has a potential to become an essential tool in the pre-treatment evaluation of opticochiasmal tumors. Optical coherent tomography analysis may be an objective method in the diagnosis and follow-up of patients with chiasmal lesions.

In the patient reported in our study, chronic chiasmal compression led to pronounced axonal damage that manifested in significant retinal nerve fiber layer and ganglion cell complex thinning and poor postoperative recovery of the visual function. The ganglion cell layer of the macula proved to be a more accurate and reliable indicator of postoperative visual outcome.

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Rad je primljen 10. IX 2021.
Recenziran 22. XII 2021.
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Case report
Prikaz slučaja
UDK 616.61:616.94]-085
https://doi.org/10.2298/MPNS2108265G

USE OF CYTOSORB HEMOADSORPTION IN A PATIENT WITH SEPSIS AND ACUTE KIDNEY INJURY − A CASE REPORT

UPOTREBA CYTOSORB HEMADSORPCIJE KOD PACIJENTA SA SEPSOM I AKUTNIM OŠTEĆENJEM BUBREGA − PRIKAZ SLUČAJA

Bojana LJUBIČIĆ[1], Violeta KNEŽEVIĆ[2, 3], Tijana AZAŠEVAC[2, 3], Dejan ĆELIĆ[2, 3], Marija ŠIBALIĆ SIMIN[4] and Ana LAZAREVIĆ[1]

Summary

Introduction. Continuous renal replacement therapy is one of the methods that proved to be successful in the treatment of sepsis and its complications such as acute kidney injury. One of the adsorbers tested is CytoSorb, which contains polystyrene-divinylbenzene pores with a biocompatible polyvinylpyrrolidone coating and that is also a highly adsorptive and biocompatible filter that facilitates selective removal of medium molecular weight molecules (10 – 15 kDa). **Case Report.** A 51-year-old male patient was hospitalized at the Department of Anesthesia, Resuscitation and Intensive Care due to multiple traumas caused by a fall from the third floor. After admission, laboratory and diagnostic methods were performed, the injuries were surgically treated and the patient received infusion, antibiotic and preventive anti-ulcer therapy along with measures of intensive treatment and monitoring of vital parameters. Progression of the pulmonary lesions, prolonged refractory hypotension unresponsive to therapeutic protocols, predisposed the onset of acute renal impairment and continuous venovenous hemodialysis with CytoSorb was applied. The procedure lasted 21 hours and 39 minutes, during which the urine output increased, and the need for vasoactive support was significantly reduced, while the lactate levels declined. Upon completion of the procedure, hemodynamic stability was achieved, with gradual improvement of consciousness, regression of pulmonary changes and reduction of inflammatory parameters and parameters of renal function. **Conclusion.** We can say that control of the patient's hyperinflammatory response was the key factor in achieving stabilization of the patient, as well as organ recovery and survival. Future research should provide additional information on the contribution of CytoSorb in the treatment of patients with sepsis.

Key words: Acute Kidney Injury; Continuous Renal Replacement Therapy; Sepsis; Shock, Septic; Hemofiltration; Inflammation Mediators; Cytokines; Treatment Outcome; Critical Illness; Intensive Care Units

Sažetak

Uvod. Kontinuirana terapija bubrežne funkcije jedna je od metoda koja se pokazala uspešnom u lečenju i sepse i njenih komplikacija u obliku akutnog oštećenja bubrega. Jedan od adsorbera čija se primena testira je *CytoSorb* koji sadrži pore polistiren-divinilbenzena s biokompatibilnim polivinilpirolidinskim omotačem, a takođe je i visokoadsorptivni i biokompatibilan filter koji olakšava selektivnu metodu uklanjanja molekula srednje molekularne mase (10−15 kDa). **Prikaz slučaja.** Pacijent, star 51 godinu, hospitalizovan je na Odeljenju za anesteziju, reanimaciju i intenzivnu negu zbog politraume uzrokovane padom sa trećeg sprata. Nakon prijema, sprovedenih laboratorijskih i dijagnostičkih metoda, bolesnik je zbrinut hirurški, primenjene su infuziona, antibiotska i antiulkusna terapija, uz mere intenzivnog lečenja i praćenja vitalnih parametara. Pogoršanje plućnog oštećenja, protrahovana hipotenzija, refratkterna na primenjenu terapiju, predisponirali su početak akutnog oštećenja bubrega i primenjena je kontinuirana veno-venska hemodijaliza s hemadsorberom − *CytoSorb.* Postupak je trajao 21 sat i 39 min, tokom kog je došlo do povećanja diureze, a potreba za vazoaktivnom podrškom značajno je smanjena, dok je nivo laktata bio u opadanju. Po završetku postupka postignuta je hemodinamička stabilnost, s postepenim poboljšanjem svesti, regresijom plućnih promena i smanjenjem inflamatornih parametara i parametara bubrežne funkcije. **Zaključak.** Kontrola nad bolesnikovim hiperinflamatornim odgovorom bila je ključni element u postizanju kliničke stabilizacije pacijenta, oporavka organa i preživljavanja. Buduća istraživanja trebalo bi da pruže dodatne informacije o doprinosu *CytoSorb*-a u lečenju bolesnika sa sepsom.

Ključne reči: akutno oštećenje bubrega; kontinuirana terapija bubrežne funkcije; sepsa; septični šok; hemofiltracija; medijatori inflamacije; citokini; ishod lečenja; kritična bolest; jedinice intenzivne nege

Introduction

Sepsis and sepsis treatment represent one of the main challenges in intensive care units. Despite the development of new therapies, morbidity and mortality due to sepsis is still very high [1]. Continuous renal replacement therapy (CRRT) is one of the methods that proved to be successful in treating

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Abbreviations
CRRT – continuous renal replacement therapy
kDa – kilo Dalton
CVVHD – continuous venovenous hemodialysis
PTT – partial thromboplastin time
SIRS – systemic inflammatory response syndrome

both sepsis and its complications such as acute kidney injury. There are several types of high cut-off membranes in the test that has 2 – 3 times larger pores and cut-off greater than 60 – 70 kilodaltons (kDa) compared to high-flux membranes, which allows the elimination of cytokines, pleiotropic immune response regulators that play a key role in complex pathophysiology of sepsis [2, 3]. One of the adsorbers tested is CytoSorb hemoadsorber [4]. CytoSorb contains polystyrene-divinylbenzene pores with biocompatible polyvinylpolypyrrolidone coating. One of the most significant features of this filter is its large surface area of 8,500 m^2, unlike conventional filters with a membrane surface area of 1.5 m^2, which provides a high level of cytokine adsorption - tumor necrosis factor, interleukin 6, chemokine, chemokine ligand 1, and chemokine ligand 2 [5–7]. CytoSorb is also a highly adsorptive and biocompatible filter, facilitating a concentration-dependent but selective method of removing medium molecular weight molecules (10 – 15 kDa) [8]. The main characteristics of CytoSorb are the control of the systemic inflammation, modulation of the immune response, improvement of hemodynamics and fluid balance, and prevention of organ failure [6, 9–11]. This was the main therapy goal in our patient. The immunomodulatory role of CytoSorb is presented by its ability to effectively reduce excessive cytokine levels [6, 9], decrease de novo synthesis of inflammatory mediators [5, 10], control attenuation of the overshooting immune response, and re-targeting of the cellular immune defense to the focus of infection [9, 12]. CytoSorb is approved in the European Union by the Conformitè Europëenne and International Organization for Standardization as a certified hemoadsorption device which helps in reduction of excess inflammatory cytokines in the blood [3, 11]. This unique therapy can eliminate bacterial exotoxins, myoglobin, free hemoglobin, bilirubin, activated complement and hosts of other inflammatory agents which can lead to fatal systemic inflammatory response syndrome (SIRS) [5]. Its clinical utility is also observed in various other clinical conditions including cardiac surgeries, liver failure, respiratory failure, and various autoimmune diseases and infections [11–13]. We presented a case of a patient with sepsis and multiple traumas treated with CytoSorb hemoadsorber during CRRT treatment.

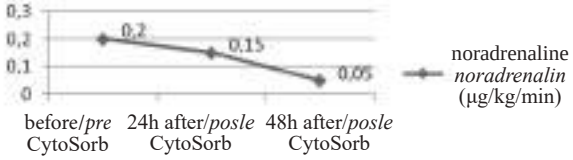
Case Report

A 51-year-old male patient was hospitalized at the Department of Anesthesia, Resuscitation and Intensive Care due to multiple traumas caused by a fall from the third floor. He was conscious, slightly hypotensive (TA 96/50 mmHg), tachycardic (pulse rate 105/min), afebrile (36.1°C), with normal respiratory rate (16/min), Acute Physiology and Chronic Health Enquiry II score was 3. Upon admission, after relevant clinical, laboratory and radiological diagnostics, the injuries were surgically treated and the patient received antibiotics (cefuroxime/metronidazole) and preventive anti-ulcer therapy (pantoprazole), while measures of intensive treatment and invasive monitoring of vital parameters were performed. On the same day, the patient developed a left-sided hemithorax and a thoracic drain was placed with active drainage and due to hemodynamic instability, dual vasoactive therapy (norepinephrine/dopamine) was initiated. On the next day, the patient developed a fever and the laboratory test results showed that the inflammatory markers were increased. A control lung scan was done and multilobar pneumonia was diagnosed caused by methicillin-resistant Staphylococcus aureus, confirmed in blood culture, and antibiotic therapy was corrected to linezolid and colistin, and mechanical ventilation was included due to respiratory failure. Laboratory findings are shown in **Table 1**. The patient was also

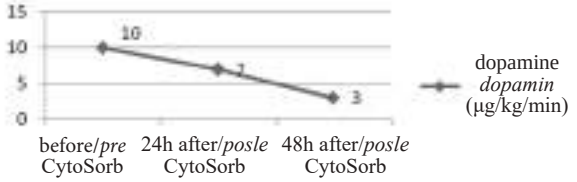
Table 1. Patient's laboratory findings during hospitalization
Tabela 1. *Laboratorijski nalazi bolesnika tokom hospitalizacije*

Laboratory parameters *Laboratorijski parametri*	On admission to hospital *Na prijemu u bolnicu*	Hospital day 1 *Bolnički dan 1*	Hospital day 3 *Bolnički dan 3*	Hospital day 6 *Bolnički dan 6*	Hospital day 8 *Bolnički dan 8*	Hospital day 11 *Bolnički dan 11*	Discharge from the hospital *Otpust iz bolnice*
Blood urea nitrogen *Urea u krvi*	9.8 mmol/l	10.2 mmol/l	12.3 mmol/l	16.7 mmol/l	13.9 mmol/l	8.2 mmol/l	6.9 mmol/l
Creatinine/*Kreatinin*	97 umol/l	122 umol/l	237 umol/l	422 umol/l	281 umol/l	193 umol/l	89 umol/l
K+/*kalijum*	4.1 mmol/l	4.0 mmol/l	4.9 mmol/l	6.1 mmol/l	5.1 mmol/l	4.2 mmol/l	3.9 mmol/l
pH/*pH*	7.32	7.36	7.30	7.29	7.33	7.39	7.39
CRP/*CRP*	89.6 mg/l	209.9 mg/l	255.6 mg/l	310.9 mg/l	mg/l	mg/l	56 mg/l
PCT/*PCT*	0.8 ng/ml	1.17 ng/ml	1.36 ng/ml	2.04 ng/ml	ng/ml	ng/ml	0.56 ng/ml
24h diuresis *24-časovni urin*		1100 ml	960 ml	400 ml	1440 ml	1900 ml	2150 ml

Legend: pH - potential of hydrogen, CRP – C-reactive protein, PCT – procalcitonin
Legenda: pH – potencijal vodonika, CRP – C-reaktivni protein, PCT – prokalcitonin



Graph 1. Norepinephrine usage before and after CytoSorb hemoadsorption
Grafikon 1. *Upotreba norepinefrina pre i nakon primene CitoSorb hemadsorbera*



Graph 2. Dopamine usage before and after CytoSorb hemoadsorption
Grafikon 2. *Upotreba dopamina pre i nakon primene CitoSorb hemadsorbera*

examined by a general surgeon, orthopedic surgeon, and a pulmonologist. In the further course, on the 6th day of hospitalization, the patient still had persistent hypotension, despite vasoactive support, hypervolemia (central venous pressure was + 19), and oliguria developed (24 hours diuresis up to 400 ml) with laboratory signs of acute kidney injury. Sepsis and septic shock due to progression of pulmonary lesions were contributing factors for the occurrence of acute renal failure, and because the clinical stabilization of the patient was not achieved with standard medical procedures, we decided to perform continuous venovenous hemodialysis (CVVHD) with a CytoSorb hemoadsorber. CytoSorb was used in CVVHD mode (Multifiltrate, Fresenius Medical Care), blood flow rate 100 – 150 ml/min, anticoagulation was achieved using heparin, targeting a partial thromboplastin time (PTT) of 60 to 80, monitored every 4 h. The procedure took 21 hours and 39 minutes and there was 2,830 ml of unbalanced fluid. During the procedure, the state of consciousness gradually improved, the patient was becoming hemodynamically more stable with a significant reduction of vasoactive infusions, lactate level decreased and hourly urine output increased up to 60 ml, so the procedure was discontinued. Over the first 8h after CytoSorb procedure, vasoactive support was discontinued. The usage of vasoactive support before and after CytoSorb therapy is shown in the **Graph 1** and **Graph 2**. In the following days, the patient was extubated, and diuresis also improved with a significant decrease in parameters of kidney function. On the 4th day after the procedure, significant decrease in the parameters of renal function and inflammation was noted, as well as normalization of acid base and electrolyte status values. The lung scan showed a regression of inflammatory changes and the patient was respiratory stable with oxygen therapy applied over a mask. On the 11th day of hospitalization, the patient was transferred to the Department of Urgent Surgery, where the conservative treatment was continued. The patient was hemodynamically stable, normotensive, normocardic, afebrile, with satisfactory diuresis. The patient recovered well and he was discharged on the 16th day of hospitalization, with the laboratory results shown in **Table 1**.

Discussion

Hemoadsorption, using a new polymer, CytoSorb, as an extracorporeal cytokine hemoadsorber, is a promising alternative in supportive treatment of patients with a systemic inflammatory response in sepsis or septic shock. A recently introduced extracorporeal cytokine hemoadsorption device called CytoSorb has gained interest in the field of critical care and cardiac surgery as a strategy to help control severe SIRS. Unlike metabolic approaches to anti-inflammation, CytoSorb is designed to directly capture and reduce mid-molecular weight inflammatory mediators (~10 – 60 kDa) in blood including pro- and anti-inflammatory cytokines, chemokines, and bacterial exotoxins. The reasons why we decided to apply CytoSorb hemoadsorber in our patient were the lack of clinical stabilization during the standard medical treatment, persistently elevated markers of inflammation, and development of multiorgan failure. The available literature data on the indications for CytoSorb hemoadsorber also supported our reasons for administration of this therapy [3, 12, 13]. Bonavia et al. have pointed to the potentially beneficial impact of the use of hemoadsorption methods in terms of better survival as well as the reduction of inflammation in hemodynamically unstable septic patients, as well as the gradual withdrawal of vasoactive support following the procedure of hemoadsorption with CytoSorb [5]. In our patient, hemodynamic stability was achieved after completion of the CRRT procedure with a CytoSorb, which corresponds to the previously reported literature data. Recent studies and previous clinical experience point to survival benefits if CytoSorb therapy is started early, in cases where the patients do not respond well to guideline therapeutic recommendations [6, 7, 10, 14]. Results similar to ours were noted in the paper of Morris C et al., where two patients with pneumonia developed multi organ failure with prominent hemodynamic instability, and after applying the CytoSorb, vasoactive doses were reduced and discontinued, and hemodynamic stability was achieved [15]. In our case, the procedure lasted for 21 hours and 39 minutes, during which the urine output increased, the need for vasoactive support significantly reduced, and lactate level decreased. Little data is available on the discontinuation of anti-infective drugs during treatment with CytoSorb. Besides a published in vitro attempt by König et al., who investigated the adsorptive capacity of various commonly used antibiotics and antimycotics, Schneider et al. also investigated this important topic [16, 17]. In our patient, we used short-

er intervals in antibiotic administration during CytoSorb treatment as recommended in the paper of Zoller M et al. [18]. Comparing to other adsorptive membranes, since CytoSorb has originally been marketed for the removal of excess cytokine blood levels, its effects seem to reach far beyond this and there is growing evidence that the adsorption properties also enable the removal of pathogen-associated molecular pattern molecules, such as bacterial exotoxins, as well as damage-associated molecular pattern molecules [19]. In this regard, CytoSorb may have also facilitated to reduce the pathogenic burden by removing bacterial exotoxins in addition to other inflammatory relevant parameters resulting in attenuation of the inflammatory response in our patient. Although a causal relationship cannot be proven, we presume that controlling the excessive inflammatory response helped to stabilize the patient.

Conclusion

In conclusion, we can say that control of the patient's initial hyperinflammatory response was the key factor in achieving clinical stabilization of the patient, as well as organ recovery and survival. Our case report showed that the CytoSorb is a safe and well tolerated rescue therapy option in patients with severe septic shock. Further prospective randomized controlled trials should be performed to provide additional information on the contribution of CytoSorb hemoadsorbers in the treatment of patients with sepsis.

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Rad je primljen 15. XII 2021.
Recenziran 25. XII 2021.
Prihvaćen za štampu 9. I 2022.
BIBLID.0025-8105:(2021):LXIX:7-8:270-273.

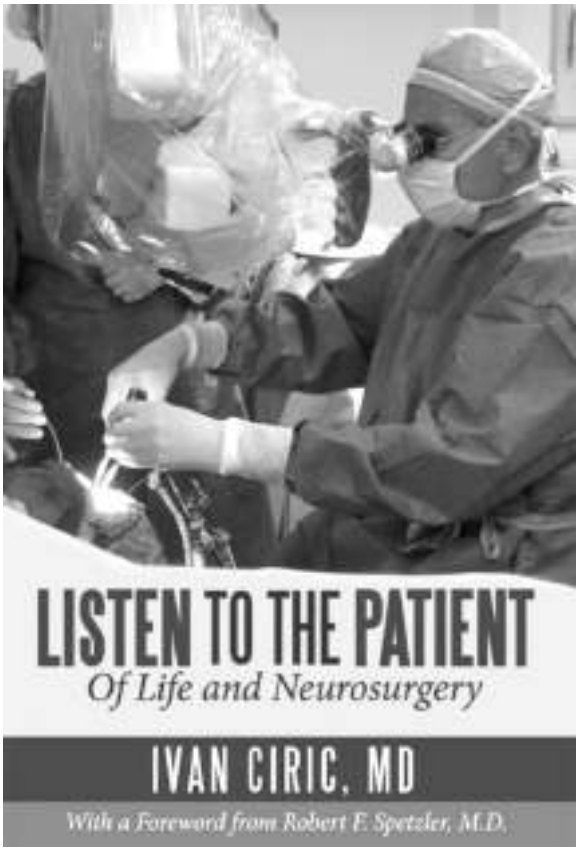
IN MEMORIAM
IN MEMORIAM

Prof. dr IVAN S. ĆIRIĆ
(1933–2021)

Profesor dr Ivan Ćirić je nakon duže bolesti preminuo 12. novembra 2021. godine u svom domu u Čikagu, SAD, u 87. godini. Izgubili smo predanog neurohirurga, vernog prijatelja, mentora mnogim neurohirurzima, profesora po pozivu Medicinskog fakulteta u Novom Sadu, Kragujevcu i Beogradu i počasnog člana Udruženja neurohirurga Srbije.

Ivan Ćirić je rođen 15. decembra 1933. godine u Beču, Austrija. Odrastao je u Sremskim Karlovcima, Srbija, Jugoslavija, gde je završio osnovnu školu i gimnaziju. Diplomirao je na Medicinskom fakultetu Univerziteta u Beogradu 1958. godine kao student generacije sa prosekom 10,00. Doktorirao je na Univerzitetu u Kelnu, Nemačka, gde je završio i specijalizaciju iz neurohirurgije. Sa suprugom Anom 1967. godine odselio se u Čikago, SAD, gde je i započe rad u Evanston bolnici i Univerzitetu Nortvestern. Svojim predanim radom profesor Ćirić je postigao da bude profesor neurohirurgije na Medicinskom fakultetu Univerziteta Nortvestern, Univerzitetu u Čikagu, zamenik predsednika Odeljenja za neurološke hirurgije i načelnik Službe za neurohirurgiju u bolnici Evanston. Kao priznanje profesoru Ćiriću za doprinos u radu, na Univerzitetu Nortvestern ustanovili su nagradu nazvanu po njemu – *Ivan Ciric Distinguished Educator Award* – koja se dodeljuje najboljim nastavnicima.

U svom kliničkom radu profesor Ćirić je razvio poseban interes za mikrohirurgiju tumora hipofize, prvi je izveo mikroskopsku transsfenoidnu operaciju tumora hipofize u SAD, 1968. godine. Od velikog značaja su bili i njegovi radovi o mikrohirurgiji vestibularnih švanoma i meningioma. U oblasti neuroonkologije posebno se bavio uticajem obima resekcije zloćudnih moždanih glioma na rezultate hirurškog lečenja i ishod kod pacijenata sa ovim tumorima. Njegova klinička istraživanja su obuhvatala i studije o degenerativnim bolestima kičme i proučavanje sten-



oze lateralnog recesa kao komponente sindroma stenoze kičmenog kanala. Rad i hirurško umeće profesora Ćirića kao jednog od vodećih svetskih neurohirurga svoga vremena su bili presudni za stručni i konceptualni napredak u neurohirurgiji u čitavom svetu.

Veliku pažnju posvećivao je razvoju neurohirurgije u Srbiji. Posebnu bliskost je razvio sa kolegama na Klinici za neurohirurgiju, Kliničkog centra Vojvodine u Novom Sadu gde je periodično dolazio, operisao sa nama i nesebično delio svoje znanje i iskustvo. Zajedno sa kolegama neurohirurzima iz Srbije učestvovao je na međunarodnim kongresima, bio nam velika podrška, kako u SAD tako i u Srbiji. Njegova posvećenost stručnom i naučnom radu ali pre svega pacijentima, kao i umerenost, dobrota i osećaj odgovornosti učinili su da ga svi cene i vole.

Profesor Ćirić se penzionisao 2011. godine ali je nastavio da bude aktivan kako u neurohirurgiji tako i kao pisac i pesnik. Godine 2016. objavio je knjigu „Slušaj pacijenta: Život i neurohirurgija“. U ovim memoarima Ćirić prepliće priču o svom životu koji ga je odveo iz Srbije, preko Nemačke, daleko u SAD sa značenjem, tajnama i etičkim problemima neurohirurgije. Kolege lekari, ali i oni koji se ne bave medicinom, mogu u ovoj knjizi naći mudre pouke izvučene iz sudara prošlosti i sadašnjosti, uspeha i neuspeha, nade i očaja. U ovoj knjizi se kroz seriju priča i operacija odabranih pacijenata opisuje jedinstvena privilegija i zastrašujuća odgovornost neurohirurga u borbi sa bolestima ljudskog mozga.

Pored medicine, interesovanja dr Ćirića su obuhvatala i druge oblasti kao što su atletika, čitanje i putovanja.

Iza Ivana Ćirića ostala je voljena supruga Ana, troje dece Sendi, Keti i Stiven, unuci Hadson i Emil, kao i njegove sestre koje žive u Srbiji Evelina i Katarina.

Za nas koji smo ga poznavali malo bliže – zauvek će ostati upamćen kao neurohirurg visokog profila i kao nežna i velikodušna, inspirativna osoba i prijatelj. Krasile su ga brojne vrline. Već samo svojom pojavom, prijatnim nastupom i predanošću poslu bio je primer brojnim mladim lekarima kojima je nesebično i nenametljivo prenosio svoje znanje i iskustvo. Njegovo nasleđe kao muža, oca, dede, revolucionarnog hirurga i čoveka mudrosti i saosećanja zauvek će čuvati oni čije je živote dotakao.

Prof. dr Petar Vuleković

UPUTSTVO ZA AUTORE

Časopis ***Medicinski pregled*** objavljuje radove koji prethodno nisu objavljeni niti poslati u drugi časopis. U Časopisu mogu biti objavljeni radovi iz različitih oblasti biomedicine, koji su namenjeni lekarima različitih specijalnosti.

Od 1. januara 2013. godine *Medicinski pregled* je počeo da koristi usluge *e-Ur* − Elektronskog uređivanja časopisa. Svi korisnici sistema − autori, recenzenti i urednici, moraju biti registrovani korisnici sa jednom elektronskom adresom.

Korisnici časopisa treba da se registruju na adresi:
http://aseestant.ceon.rs/index.php/medpreg/user/register

Prijava rada treba da se učini na adresi:
http://aseestant.ceon.rs/index.php/medpreg/

U postupku prijave neophodno je da se pošalje saglasnost i izjava autora i svih koautora da rad nije delimično ili u celini objavljen ili prihvaćen za štampu u drugom časopisu.

Elektronsko uređivanje časopisa obezbeđuje korišćenje sistema *CrossCheck*, koji prijavljene radove automatski proverava na plagijarizam i autoplagijarizam. Autori ne bi smeli da pošalju isti rad u više časopisa istovremeno. Ukoliko se to desi, glavni urednik časopisa *Medicinski pregled* ima pravo da rad vrati autorima bez prethodnog slanja rada na recenziju; da odbije štampanje rada; da se obrati urednicima drugih časopisa u koje je rad poslat ili da se obrati direktoru ustanove u kojoj su autori rada zaposleni.

Primaju se samo radovi koji su napisani na engleskom jeziku, uz sažetak rada i naslov rada koji treba da budu napisani na engleskom i srpskom jeziku.

Radove koji su pristigli u časopis *Medicinski pregled* pregleda jedan ili više članova Uređivačkog odbora Časopisa. Oni radovi koji su napisani prema pravilima Časopisa šalju se na anonimnu recenziju kod najmanje dva recenzenta, stručnjaka iz odgovarajuće oblasti biomedicine. Načinjene recenzije radova pregleda glavni urednik ili članovi Uređivačkog odbora i one nisu garancija da će rad biti prihvaćen za štampu. Materijal koji je pristigao u časopis ostaje poverljiv dok se rad nalazi na recenziji, a identitet autora i recenzenata su zaštićeni, osim u slučaju ako oni odluče drugačije.

U časopisu *Medicinski pregled* objavljuju se: uvodnici, originalni članci, prethodna ili kratka saopštenja, pregledni članci, stručni članci, prikazi slučajeva, članci iz istorije medicine i drugi članci.

1. Uvodnici – do 5 strana. Sadrže mišljenja ili diskusiju o posebno značajnoj temi za Časopis, kao i o podacima koji su štampani u ovom ili nekom drugom časopisu. Obično ih piše jedan autor po pozivu.

2. Originalni članci – do 12 strana. Predstavljaju rezultate istraživanja autora rada i njihovo tumačenje. Istraživanje treba da bude obrađeno i izloženo na način da se može ponoviti, a analiza rezultata i zaključci jasni da bi se mogli proveriti.

3. Pregledni članci – do 10 strana. Predstavljaju sistematsko, sveobuhvatno i kritičko izlaganje problema na osnovu analiziranih i diskutovanih podataka iz literature, a koji oslikavaju postojeću situaciju u određenom području istraživanja. Literatura koja se koristi u radu mora da sadrži najmanje 5 radova autora članka iz uže naučne oblasti koja je opisana u radu.

4. Prethodna ili kratka saopštenja – do 4 strane. Sadrže izuzetno važne naučne rezultate koje bi trebalo objaviti u što kraćem vremenu. Ne moraju da sadrže detaljan opis metodologije rada i rezultata, ali moraju da imaju sva poglavlja kao originalni članci u sažetoj formi.

5. Stručni članci – do 10 strana. Odnose se na proveru ili prikaz prethodnog istraživanja i predstavljaju koristan izvor za širenje znanja i prilagođavanja originalnog istraživanja potrebama postojeće nauke i prakse.

6. Prikazi slučajeva – do 6 strana. Opisuju retke slučajeve iz prakse. Slični su stručnim člancima. U ovim radovima prikazuju se neuobičajeni oblici i tokovi oboljenja, neočekivane reakcije na primenjenu terapiju, primene novih dijagnostičkih procedura ili retke i nove bolesti.

7. Članci iz istorije medicine – do 10 strana. Ovi članci opisuju događaje iz prošlosti sa ciljem da omoguće očuvanje medicinske i zdravstvene kulture. Imaju karakter stručnih članaka.

8. Ostali članci – U časopisu Medicinski pregled objavljuju se feljtoni, prikazi knjiga, izvodi iz strane literature, izveštaji sa kongresa i stručnih sastanaka, saopštenja o radu pojedinih zdravstvenih organizacija, podružnica i sekcija, saopštenja Uredništva, pisma Uredništvu, novosti u medicini, pitanja i odgovori, stručne i staleške vesti i članci napisani u znak sećanja (*In memoriam).*

Priprema rukopisa

Kompletan rukopis, uključujući tekst rada, sve priloge i propratno pismo, treba poslati na elektronsku adresu koja je prethodno navedena.

Propratno pismo:

– mora da sadrži izjavu svih autora da se radi o originalnom radu koji prethodno nije objavljen niti prihvaćen za štampu u drugim časopisima;

– autori svojim potpisom preuzimaju odgovornost da rad ispunjava sve postavljene uslove i da ne postoji sukob interesa i

– autor mora navesti kategoriju članka (originalni rad, pregleni rad, prethodno saopštenje, stručni rad, prikaz slučaja, rad iz istorije medicine, itd.).

Rukopis

Opšta uputstva

Tekst rada treba da bude napisan u programu *Microsoft Word* za *Windows*, na A4 formatu stranice (sve četiri margine 2,5 cm), proreda 1,5 (isto važi i za tabele), fontom *Times New Roman*, veličinom slova 12 *pt*. Neophodno je koristiti međunarodni sistem mernih jedinica (*SI*), uz izuzetak temperature (° *C*) i krvnog pritiska (*mmHg*).

Rukopis treba da sadrži sledeće elemente:

1. Naslovna strana

Naslovna strana treba da sadrži: kratak i sažet naslov rada, bez skraćenica, skraćeni naslov rada (do 40 karaktera), imena i prezimena autora (ne više od 6) i afilijacije svih autora. Na dnu strane treba da piše ime, prezime i titula autora zaduženog za korespondenciju, njena/njegova adresa, elektronska adresa, broj telefona i faksa.

2. Sažetak

Sažetak ne može da sadrži više od 250 reči niti skraćenice. Treba da bude strukturisan, kratak i sažet, sa jasnim pregledom problema istraživanja, ciljevima, metodama, značajnim rezultatima i zaključcima.

Sažetak originalnih i stručnih članaka treba da sadrži uvod (sa ciljevima istraživanja), materijale i metode, rezultate i zaključak.

Sažetak prikaza slučaja treba da sadrži uvod, prikaz slučaja i zaključak.

Sažetak preglednih članaka treba da sadrži Uvod, podnaslove koji odgovaraju istima u tekstu i Zaključak.

Navesti do 10 ključnih reči ispod sažetka. One su pomoć prilikom indeksiranja, ali autorove ključne reči mogu biti izmenjene u skladu sa odgovarajućim deskriptorima, odnosno terminima iz *Medical Subject Headings, MeSH.*

Sažetak treba da bude napisan na srpskom i engleskom jeziku. Sažetak na srpskom jeziku trebalo bi da predstavlja prevod sažetka na engleskom, što podrazumeva da sadrži jednake delove.

3. Tekst članka

Originalni rad treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima istraživanja), Materijal i metode, Rezultati, Diskusija, Zaključak, spisak skraćenica (ukoliko su

korišćene u tekstu). Nije neophodno da se u posebnom poglavlju rada napiše zahvalnica onima koji su pomogli da se istraživanje uradi, kao i da se rad napiše.

Prikaz slučaja treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima), Prikaz slučaja, Diskusija i Zaključak.

Uvod

U poglavlju Uvod potrebno je jasno definisati predmet istraživanja (prirodu i značaj istraživanja), navesti značajne navode literature i jasno definisati ciljeve istraživanja i hipoteze.

Materijal i metode

Materijal i metode rada treba da sadrže podatke o vrsti studije (prospektivna/retrospektivna, uslove za uključivanje i ograničenja studije, trajanje istraživanja, demografske podatke, period praćenja). Detaljno treba opisati statističke metode da bi čitaoci rada mogli da provere iznesene rezultate.

Rezultati

Rezultati predstavljaju detaljan prikaz podataka koji su dobijeni istraživanjem. Sve tabele, grafikoni, sheme i slike moraju biti citirani u tekstu rada i označeni brojevima po redosledu njihovog navođenja.

Diskusija

Diskusija treba da bude koncizna, jasna i da predstavlja tumačenje i poređenje rezultata studije sa relevantnim studijama koje su objavljene u domaćoj i međunarodnoj literaturi. U poglavlju Diskusija potrebno je naglasiti da li su postavljene hipoteze potvrđene ili nisu, kao i istaknuti značaj i nedostatke istraživanja.

Zaključak

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

4. Literatura

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

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

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

∗ Organizacija kao autor

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

∗ Bez autora

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

∗ Volumen sa suplementom

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

∗ Sveska sa suplementom

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

∗ Sažetak u časopisu

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

Knjige i druge monografije

∗ Jedan ili više autora

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

∗ Urednik (urednici) kao autor (autori)

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

∗ Poglavlje u knjizi

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

∗ Zbornik radova sa kongresa

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

∗ Disertacija

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

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

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

∗ Monografija u elektronskom formatu

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

∗ Kompjuterska datoteka

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

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

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

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

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

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

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

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

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

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

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

6. Dodatne obaveze

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

INFORMATION FOR AUTHORS

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

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

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

Manuscript submission should be made on the web address:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Preparation of the manuscript

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

The covering letter:

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

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

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

The manuscript:

General instructions.

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

The manuscript should contain the following elements:

1. The title page.

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

2. Summary.

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

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

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

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

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

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

3. The text of the paper.

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

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

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

Materials and methods should contain data on design of the study (prospective/retrospective, eligibility and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

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

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

Conclusion must deny or confirm the attitude towards the 0based solely on the author's own results, corroborating them. Avoid generalized and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

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

Articles in journals

∗ *A standard article*

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

∗ *An organization as the author*

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

∗ *No author given*

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

∗ *A volume with supplement*

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

∗ *An issue with supplement*

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

∗ *A summary in a journal*

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

Books and other monographs

∗ *One or more authors*

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

∗ *Editor(s) as author(s)*

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

∗ *A chapter in a book*

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THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

– Tables, graphs, schemes and photographs are to be submitted as separate documents, on separate pages.

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