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Letter from the Editor-in-Chief
Medical Research Journal
Faculty of Medicine, University of Belgrade

Dear Readers,

It is my great pleasure to welcome you to the *Medical Research Journal*, a peer-reviewed scientific journal published by the Faculty of Medicine, University of Belgrade. Established in 1971, the journal has proudly maintained a tradition of academic excellence for over five decades.

Published four times annually, the *Medical Research Journal* is committed to advancing biomedical science by publishing high-quality research from both members and affiliates of the Belgrade Faculty of Medicine, as well as from scientists and professionals engaged in biomedical research worldwide. Our mission is to serve as a platform for the dissemination of innovative and impactful research that contributes to the development of medical science and clinical practice.

The journal publishes original scientific articles, review papers, case reports, and other types of contributions submitted in English. We welcome submissions that present previously unpublished results of original research, offer critical and comprehensive analyses of current topics, or describe unique and particularly illustrative clinical cases. Our journal encompasses research in basic biomedical sciences, clinical medicine, and preventive healthcare. Each manuscript undergoes statistical review to ensure methodological and analytical rigor. In line with our open-access policy, all published content is freely available to the public, supporting transparency, accessibility, and the global exchange of knowledge.

Looking ahead, our goal is to further elevate the journal's visibility and academic influence. We are fully committed to transitioning from our current national categorization (M53) to recognition as an international journal (M23). We are actively pursuing inclusion in prominent international indexing databases, aiming to increase the visibility and impact of the research we publish. Our long - term vision includes strategic efforts to enhance the quality, diversity, and global reach of our submissions, with a focus on fostering international collaboration and multidisciplinary research. While this journey is demanding, it is essential—and entirely possible through the joint commitment of our editorial team, authors, reviewers, and readers.

On behalf of the entire Editorial Board, I invite you to explore the latest contributions in this issue, and I warmly encourage researchers to consider the *Medical Research Journal* as a venue for publishing their work. Together, we will continue to promote excellence in medical science.

With sincere appreciation,
Prof. Dragana Protic, MD, PhD
Editor-in-Chief

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ORIGINAL ARTICLE

Reduction clitoroplasty for congenital adrenal hyperplasia: our experience

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Summary

Introduction: Feminizing genitoplasty for congenital adrenal hyperplasia (CAH) includes clitoroplasty, vaginoplasty, urethroplasty and labioplasty. The aim is to achieve normal female anatomy without compromising sexual function. We present our technique and outcomes of reduction clitoroplasty.

Methods: A total of 21 patients with CAH, aged from 13 months to 24 years (median 16 months), underwent feminizing genital reconstruction in our center between January 2011 and December 2020. Surgical treatment included reduction clitoroplasty and labioplasty, with or without urethroplasty and vaginoplasty. Clitoroplasty was performed by complete disassembly of the clitoris into glans with neurovascular bundle and cavernosal bodies. Glans cap was separated from the tips of the cavernosal bodies, avoiding injury of the arteries that run lateroventrally. Cavernosal bodies were completely removed, followed by glans reconstruction and reassembly of all entities, to attain normal clitoral morphology. For hypothesis testing, we used the Mann-Whitney test and Fisher's exact test.

Results: Follow-up ranged from 44 to 162 months (median 84 months). An excellent aesthetic outcome with normal appearance of the genitalia was achieved in all cases. Five patients (24%) reached puberty, and all have regular menstrual cycle. Postoperative complications were observed in 5 (24%) cases. No correlation was found between complications and age at the time of surgery (p=0.131) or duration of surgery (p=0.136).

Conclusion: Reduction clitoroplasty by clitoral disassembly presents a good choice for clitoromegaly repair in patients with CAH. This approach leaves the neurovascular bundle intact and completely preserves the glans cap and urethral plate blood supply. Long-term follow-up is necessary for the evaluation of sensation and sexual function.

Keywords: Congenital adrenal hyperplasia, feminizing genitoplasty, clitoroplasty

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INTRODUCTION

Congenital adrenal hyperplasia (CAH) is diagnosed in one of 10000 newborns, and its treatment requires multidisciplinary approach. The role of surgery is to achieve anatomy and function of female external genitalia, enabling normal psychosocial and psychosexual development. Feminizing genital reconstruction in virilized girls with CAH involves clitoroplasty, labioplasty, urethroplasty and vaginoplasty. The type of surgical reconstruction depends on the severity of the virilization and may include several stages. Appreciation for female genital anatomy and individualized approach are necessary for successful outcome (1-3).

Clitoral hypertrophy is the important feature of CAH, and different treatment approaches have been used throughout history (4). The total amputation of the clitoris used to be the single option because the clitoris was considered unnecessary for female sexual maturation (5). As understanding of clitoral anatomy and sexual function has advanced, more refined surgical techniques have been developed since the initial introduction of reduction clitoroplasty in the 1960s (6,7). Preservation of the neurovascular bundle has become the main goal, and therefore less invasive procedures have been proposed lately. However, loss of sensation, aesthetic mutilation and ultimately compromising sexual function are still relevant issues, making clitoral reconstructive surgery quite challenging. Additionally, data about long-term effects of these procedures are not available (4).

Due to experience in penile disassembly procedure for various congenital and acquired penile anomalies, reduction clitoroplasty based on clitoral disassembly was introduced in our center as a standard part of feminizing genitoplasty. In this study we evaluated surgical outcomes of the procedure for clitoromegaly in CAH.

MATERIALS AND METHODS

A retrospective, single-center study included 21 patients with CAH, aged from 13 months to 24 years (median 16 months), who underwent primary surgical repair between January 2011 and December 2020. The study protocol was approved by the Institutional Ethics Committee at University Children's Hospital (number 12/262, date 11/2024). Patients who underwent re-do clitoroplasty after primary repair elsewhere were excluded from the study. Complete multidisciplinary evaluation is carried out preoperatively, and all patients underwent echosonography and voiding cystourethrography (VCUG). Hormonal therapy was administered preoperatively. Complete one-stage genital reconstruction was performed in 17 patients, and included reduction of hypertrophied clitoris, labioplasty, urethroplasty and vaginoplasty. In 4 cases with severe urogenital sinus, a two-stage surgery was planned, leaving vaginal reconstruction for later age.

Surgical technique

Surgery starts with subcoronal circumferential incision and clitoral skin degloving **(Figures 1A and B)**.

Wide suspensory ligament is dissected in the right plane and preserved, to maintain its role - to prevent the clitoris from straightening, while keeping its stability during sexual activity. Urethral plate is mobilized with Buck's fascia. Dissection is continued towards glans cap, and neurovascular bundle is dissected under Buck's fascia in order to preserve its' structures. Paired clitoral neurovascular bundle originates from pudendal neurovascular bundle, ascending to the upper part of the clitoral body where the crura unite. Glans cap is separated from the tips of the cavernosal bodies, avoiding the injury of the arteries running lateroventrally, as well as dorsal clitoral nerves.

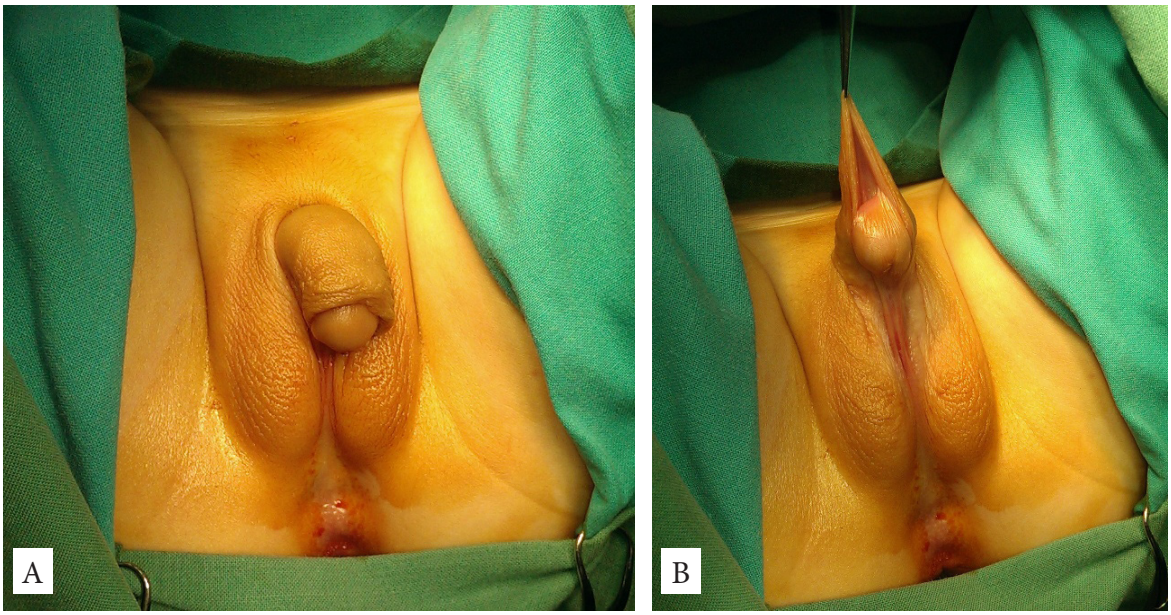

Figure 1. Preoperative appearance of virilized genitalia in an 18-month-old female infant. A. Clitoromegaly with abundant skin. B. Genitalia have hypospadiac aspect. Authors obtained written informed permission to publish these images in scientific/medical journal.

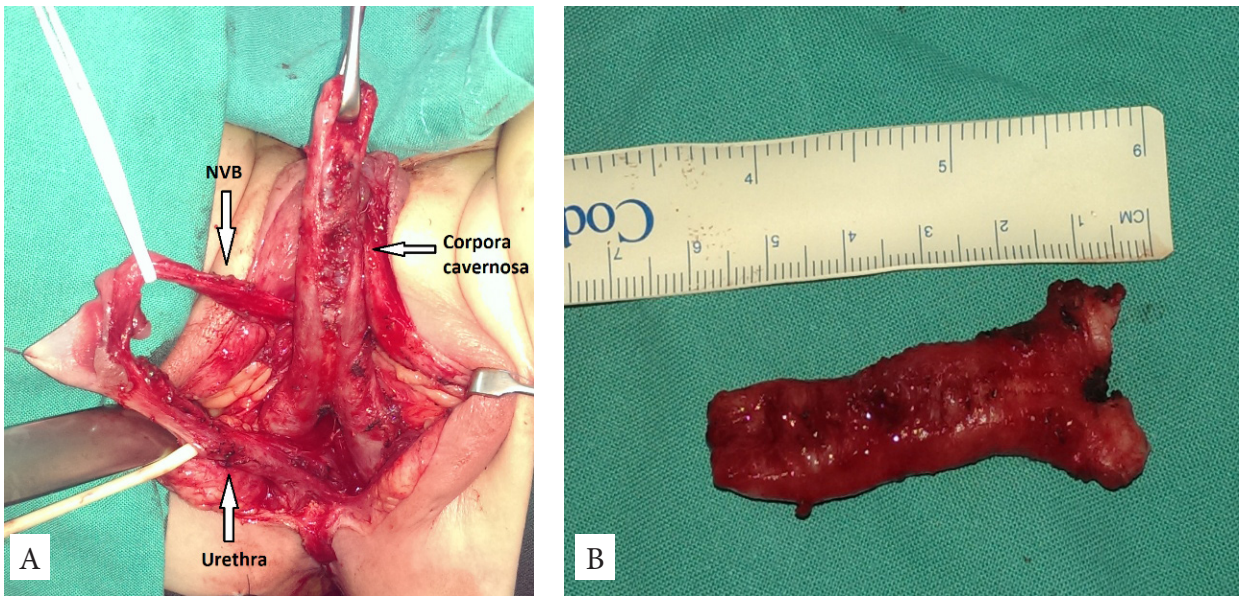

Figure 2. Complete disassembly of the clitoris is done. A. Enlarged cavernosal bodies are totally separated from the glans cap, neurovascular bundle and urethra. B. A 6-cm long cavernosal bodies are removed. Authors obtained written informed permission to publish these images in scientific/medical journal.

The dorsal clitoral nerves pass in large fibers to enter the deep layers of the glans, whose innervation is rich, particularly in its dorsal part. Finally, complete disassembly of the clitoris into glans with neurovascular bundle, urethral plate and corpora cavernosa is done **(Figure 2A)**. This allows adequate removal of corpora cavernosa by complete excision below bifurcation, and suturing the remaining parts limited to the attachment only **(Figure 2B)**.

The glans is then resected, if needed, to achieve normal size, with preserving its' dorsal part that has rich innervation. Reconstruction of the glans and reassembly of all entities is then performed, in order to attain normal clitoral morphology **(Figure 3)**. Labioplasty is then performed, when labia minora and majora are created **(Figure 4)**.

Reconstruction of urogenital sinus follows. A simple cut-back procedure in mild cases, vaginoplasty with U-flap in more severe, or complete urogenital sinus mobilization with complex urethral and vaginal reconstruction in most severe cases and may be performed in one- or two-stages.. Foley catheter is left indwelling for 7 days. Postoperative vaginal dilations are performed in most patients **(Figure 5)**.

Statistical analysis

Depending on the type of variables and the normality of the distribution, data are described as n (%), arithmetic

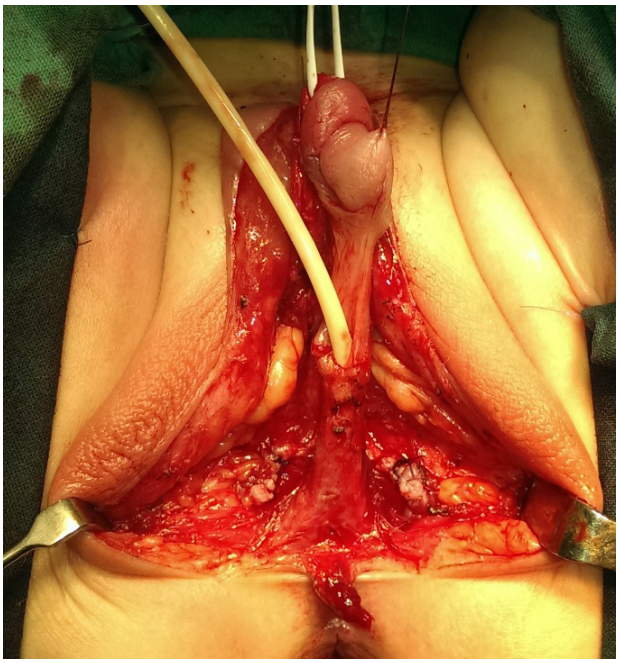

Figure 3. Reassembly of all entities is anatomically planned. Authors obtained written informed permission to publish these images in scientific/medical journal.

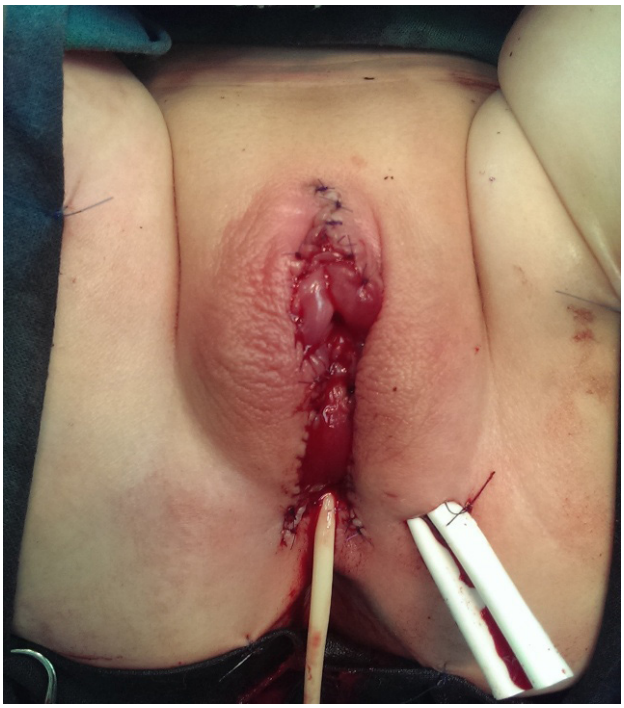

Figure 4. Appearance after surgery. Labioplasty, urethroplasty and introitoplasty are performed. Authors obtained written informed permission to publish these images in scientific/medical journal.

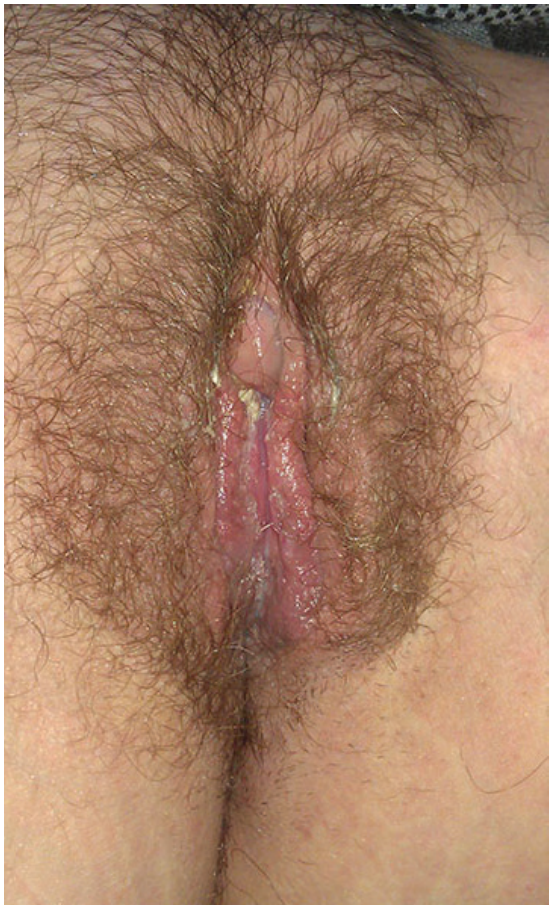

Figure 5. Good aesthetic outcome 10 years after surgery. Authors obtained written informed permission to publish these images in scientific/medical journal.

mean ± standard deviation, or median (min-max). For hypothesis testing, we used the Mann-Whitney test and Fisher's exact test. Statistical hypotheses were tested at a significance level (alpha level) of 0.05.

RESULTS

Follow-up ranged from 44 to 162 months (median 84 months). The median hospital stay was 3 days, ranged from 2 to 7 days. There was no significant intraoperative or early postoperative bleeding. Median length of the removed cavernosal bodies was 4cm, ranging from 3.5cm to 8cm **(Table 1)**.

Good aesthetic outcome with female appearance of vulva was achieved in 20 (95%) patients. Complications were observed in 5 (24%) patients. One case resulted in asymmetry of the glans and labia minora, and partial necrosis of the clitoral skin, which required minor revision 6 months later. One patient presented with stenosis of the vaginal introitus 3 years after surgery and underwent successful repair. Three patients (14%) had postoperative recurrent urinary tract infections and transient urinary leakage, and there were no cases of incontinence. Five patients (24%) reached puberty, and all have regular menstrual cycle. Sensitivity of the reduced clitoris seemed preserved in all cases, according to self-reports. One adult patient reported good sensitivity, penetrative sexual intercourse and satisfaction with surgical outcome. Still, patients' age do not allow complete functional and psychosexual assessment, which is expected after puberty.

There was no correlation between complications and age at the time of surgery (p=0.131) or duration of surgery (p=0.136). Complications occurred in 2 (12%) cases with a low confluence and 3 (60%) with a high confluence, which was not a statistically significant difference (p=0.063). Complications were more frequent among participants who underwent clitoroplasty and labioplasty (100%) compared to those who underwent complete one-stage reconstruction (6%) (p=0.001). Due to the limited sample size and low incidence of potential outcomes of interest - revision surgery (10%), unsatisfactory aesthetic result (5%), transfusions (0%), and clitoral sensitivity disturbance (0%), it was not possible to perform an analysis regarding these outcomes. A limitation of the study is that multivariable analyses could not be performed due to the limited sample size.

DISCUSSION

The treatment for genital ambiguity in CAH is still a subject of debates. Current guidelines suggest a multi-disciplinary approach and a shared decision model, including gender assignment and genital surgery in accordance with national regulations. The type of feminizing genitoplasty depends on the degree of virilization, and current standard of care is to perform reconstructive surgery at an early age rather than wait for adolescence (1,8). A survey among pediatric surgeons and urologists showed that early surgery, before the age of two years, is preferred by 78% of the surgeons and most of them would include clitoroplasty, vaginoplasty and labioplasty (9).

Clitoral reconstruction has evolved from clitoral amputation to less invasive procedures including plication,

Table 1. Summary of case parameters

	Age at surgery (months)	Follow-up period (months)	Hospital stay (days)	Duration of surgery (minutes)	Length of removed cavernosal bodies (cm)
Mean	30.3	91.6	3.2	143.3	4.1
SD	57.4	36.8	1.2	29.8	1.1
Median (range)	16 (13-280)	84 (44-162)	3 (2-7)	140 (90-200)	4 (3.5-8)

* SD – Standard deviation

concealing and reduction (2-7). Recent improvements are based on new insights in clitoral anatomical features and components, including location of the clitoral nerves along the shaft of the clitoris and glans, clitoral body and ligaments (10). Reduction clitoroplasty where part of the shaft is excised with glans preservation is currently the most accepted and used technique. However, this may result in various problems and poor long-term outcomes, including loss of sensation, glans atrophy and sexual dysfunction (11).

Yankovic et al. reported partial excision of the corpora cavernosa as the most frequent surgical technique used for the clitoroplasty (9). A corporal preserving approach is preferred in 20% of surgeons, including surgeons that bury the corpora and those who perform a split-dismembered clitoroplasty. The self-reported outcomes from the participants that perform clitoral surgery are very good in 57%, good in 26% and 16% poor (9). Gupta et al. evaluated long-term cosmetic, functional and psychosexual outcomes in 50 patients with CAH who underwent feminizing genitoplasty (12). They report clitoral reduction with preservation of the glans, without description of the surgical procedure. After the mean follow-up of 6 years, they reported poor cosmetic outcome in 3 out of 50 cases, due to atrophic clitoris in 2 and clitoromegaly in 1 case. The case of recurrent clitoromegaly was explained by non-compliance to hormonal therapy for a period of 3 years, and required re-do surgery. Functional and psychosexual outcomes were satisfactory in the majority of cases (12). One study reports 100% success in terms of size of the clitoris and cosmesis after partial excision of the cavernosal bodies in 82 cases, but without data regarding clitoral sensation or patient satisfaction after surgery (13).

Ventral approach has also been used to preserve neurovascular bundle, removing cavernosal bodies with tunica albuginea, but only distal to bifurcation (14). The authors suggest that by leaving some erectile tissue distal to the bifurcation, clitoral erection is maintained, as well as allowing support and elevation of the clitoris beneath the pubis. However, the long-term results of these partial excisions are not available. A recent comprehensive retrospective study reports favorable long-term outcomes in 40 patients with CAH who underwent genitourinary reconstruction. They also used ventral approach with partial excision and preservation of the bundle. In a median follow-up of 7 years (1-19 years), clitoral glans size was normal and hidden by labia minora in the vestibule in 37 (92.5%) patients. The clitoris was atrophic in one (2.5%), and too large in two cases (5%). (15) Still, concerns related to remaining erectile tissue remain, as the potential need for additional procedures, long-term effects on psychosocial and sexual function, and cosmetic and functional outcomes (16).

We report good surgical outcomes and high level of patients' satisfaction in 21 cases who underwent feminizing genitoplasty, with a mean follow-up of 7 years. Our technique includes clitoral disassembly, which enables complete removal of erectile tissue to the attachments of cavernosal bodies and preservation of all neurovascular elements, allowing anatomical reassembly of entities. In this way, pain and difficulties during sexual intercourse in the future due to remaining erectile tissue is definitely avoided. This approach is based on vast experience in penile disassembly technique as well as clitoral reconstruction in many congenital anomalies and gender affirmation surgeries (10,17). In a long-term follow-up, in this cohort there were no complications such as clitoral atrophy, clitoromegaly recurrence or patients' reports on psychosexual impairment. The statistical analysis revealed no correlation between complications and age at the time of surgery or duration of surgery, as well as significant difference in complication rate regarding level of confluence. However, analysis of other outcome parameters was not possible due to a small number of cases.

The limitations of our study are inconsistent functional and psychosexual evaluation, which can be expected when all patients reach late adolescence or adulthood. The importance of transitional care, especially in these severe cases, is crucial. Also, a limited sample size did not allow for multivariable statistical analysis.

CONCLUSION

Reduction clitoroplasty based on clitoral disassembly in CAH enables complete removal of cavernosal bodies with preservation of the neurovascular bundle, glans cap and urethral plate blood supply. This approach has good long-term surgical outcomes, without compromising the sensation and potential sexual function. Transitional care into adulthood is necessary for complete evaluation.

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Conflict of Interest Statement: No conflict of interest to report.

Author Contributions: BS: preparing the draft of the manuscript; the acquisition, analysis, and interpretation of data; MBiz: the acquisition, analysis, and interpretation of data; MBen: analysis and interpretation of data; supervision of content; MDj: the conception and design of the work; supervision of content

Ethical approval: The study protocol was approved by the Institutional Ethics Committee at University Children's Hospital (number 12/262, date 11/2024).

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REDUKCIONA KLITOROPLASTIKA KOD KONGENITALNE ADRENALNE HIPERPLAZIJE: NAŠA ISKUSTVA

Borko Stojanović[1,2], Marta Bižić[1,2], Marko Benčić[1,2], Miroslav L. Đorđević[1,2]

Sažetak

Uvod: Feminizirajuća genitoplastika kod kongenitalne adrenalne hiperplazije (KAH) uključuje klitoroplastiku, vaginoplastiku, uretroplastiku i labioplastiku. Cilj je postići izgled i normalnu anatomiju ženskih genitalija, bez oštećenja seksualne funkcije. Predstavljamo našu tehniku i rezultate redukcione klitoroplastike.

Metode: Feminizirajuća genitoplastika je u našem centru urađena kod 21 pacijenta sa KAH, uzrasta od 13 meseci do 24 godine (medijana 16 meseci), u periodu od januara 2011. do decembra 2020. godine. Operacija je uključivala redukcionu klitoroplastiku i labioplastiku, sa ili bez uretroplastike i vaginoplastike. Klitoroplastika je podrazumevala kompletno rastavljanje klitorisa na glans sa neurovaskularnom peteljkom i kavernozna tela. Glans je odvojen od vrhova kavernoznih tela uz prezervaciju arterija. Kavernozna tela su kompletno uklonjena, a potom je učinjena rekonstrukcija glansa i svih struktura, kako bi se postigla normalna morfologija klitorisa. Od metoda za testiranje statističkih hipoteza koristili smo Mann-Whitney test i Fisherov test tačne verovatnoće.

Rezultati: Period praćenja je od 44 do 162 meseca (medijana 84 meseca). Odličan estetski rezultat je postignut u svim slučajevima. Pet pacijenata (24%) je prošlo adolescenciju i ima uredne menstrualne cikluse. Komplikacije operacije su se javile u 5 (24%) slučajeva. Sa komplikacijama nisu povezani: uzrast u vreme operacije (p=0,131) i trajanje operacije (p=0,136).

Zaključak: Redukciona klitoroplastika zasnovana na kompletnom rastavljanju klitorisa daje dobre rezultate kod pacijenata sa KAH. Ovom tehnikom se čuva neurovaskularna peteljka, kao i vaskularizacija glansa i uretralne ploče. Dugoročno praćenje je neophodno za kompletnu evaluaciju osetljivosti i seksualne funkcije.

Ključne reči: kongenitalna adrenalna hiperplazija, feminizirajuća genitoplastika, klitoroplastika

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ORIGINAL ARTICLE

Older-age-related one-year mortality in patients with acute myocardial infarction with ST elevation treated with percutaneous coronary angiography

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Summary

Introduction: The rise in life expectancy has resulted in a greater prevalence of elderly patients presenting with acute myocardial infarction with ST elevation (STEMI).

The Aim: Investigating the association between advanced age and one-year mortality in STEMI patients treated with primary PCI.

Material: The study involved 395 STEMI patients who underwent primary PCI and were admitted to the Coronary Care Unit between June and December 2019. The patients were categorized into three age groups: ≤64 years, 65-74 years, and ≥75 years, with the ≤64-year age group as the comparison reference. All-cause mortality was analyzed over a one-year period.

Results: The mean age of the patients was 62 years; 27.6% were aged between 65 and 74, while 15.7% were 75 years or older. Women accounted for 28.7% of the total, with higher representation in older age groups. Older patients exhibited elevated rates of diabetes, chronic renal insufficiency, anemia, and heart failure (Killip 2-4). Primary PCI rates were notably high across all age groups at 93.3%, 93.6%, and 87.1%, respectively, primarily using a radial approach. The one-year mortality risk was twice as high for those aged 64 to 75 years and seven times higher for those aged 75 and over, with age 75 and above being an independent predictor of all-cause mortality.

Conclusion: Elderly patients with STEMI, particularly those aged 75 and older, show a significantly higher one-year mortality rate compared to their younger counterparts aged 64 and younger due to the considerable burden of comorbidities, even when receiving guideline-directed therapies.

Keywords: the elderly, STEMI, primary PCI, mortality

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INTRODUCTION

An increase in life expectancy has led to a growing number of elderly patients presenting with acute myocardial infarction with ST elevation (STEMI). Patients aged 75 years and older represent approximately 14–28% of all STEMI cases (1-3).

Diagnosing and managing STEMI in this population presents unique challenges that significantly influence mortality rates. The primary challenge involves an increased prevalence of atypical clinical presentations among older adults, which may delay prompt diagnosis and timely intervention (4). Furthermore, older patients often demonstrated a higher prevalence of coronary risk factors, including hypertension, diabetes mellitus, and chronic kidney disease (CKD), complicating both clinical assessment and therapeutic decision-making processes. Frailty also emerges as a significant concern in this age group (5) as it correlates with diminished physiological reserves, ultimately rendering these patients more susceptible to adverse outcomes during hospitalization (6). Finally, there is an elevated risk of in-hospital complications, including bleeding, contrast-induced nephropathy, and cardiogenic shock. All of this makes this population significantly associated with a high mortality rate.

The current standard of care for STEMI, which incorporates primary percutaneous coronary intervention (PCI) alongside dual antiplatelet therapy (DAPT), has significantly improved the outcomes for patients of all ages enrolled (7). These therapies reduce mortality, limit myocardial damage, and enhance long-term prognosis. However, older adults, particularly those aged 75 and over, are often underrepresented or excluded from numerous clinical trials (8, 9). In contrast, a limited number of studies involving elderly patients have demonstrated that PCI offers more benefits than fibrinolytic therapy (10-12) or medication therapy alone in terms of mortality in this age group (13-15). This has led to insufficient evidence concerning the effectiveness of PCI specific to this population, thereby complicating the formulation of optimal treatment strategies.

The aim is to explore the link between STEMI patients aged 65 and older and one-year mortality after primary PCI.

METHODS

Study design and patient population. This single-center observational study included 395 consecutive patients with ST-elevation myocardial infarction (STEMI) who were admitted to the Coronary Care Unit between June and December 2019 and referred for primary percutaneous coronary intervention (PCI). The patients were divided into three age groups for analysis: ≤64 years, 65–74 years, and ≥75 years, with the ≤64-year age group as the comparison reference.

The diagnosis of STEMI was established based on the Fourth Universal Definition of Myocardial Infarction (16). All patients received a loading dose of dual antiplatelet therapy, consisting of aspirin and either ticagrelor (180 mg), clopidogrel (600 mg), or prasugrel (60 mg), followed by maintenance doses of aspirin (100 mg daily) combined with either clopidogrel (75 mg daily), ticagrelor (90 mg twice daily), or prasugrel (10 mg daily). Anti-ischemic therapy was administered according to current guidelines and tailored to each patient's clinical status.

All patients included in the study underwent coronary angiography and PCI. The interventional strategy, including decisions regarding stenting, vascular access (via the radial or femoral artery), and the use of glycoprotein IIb/IIIa inhibitors during or after the intervention, was determined at the discretion of the interventional cardiologist. Transthoracic echocardiography was conducted within the first three days following PCI, and the left ventricular ejection fraction was classified as reduced if it fell below 40%.

Anemia was defined as the hemoglobin level below 12 g/dL in women and below 13 g/dL in men. Baseline kidney function was assessed using the Cockcroft-Gault equation, with an estimated glomerular filtration rate (eGFR) of <60 mL/min/m^2 considered indicative of reduced kidney function.

Data collection. Patient demographic information, including age, sex, and cardiovascular risk factors (hypertension, hyperlipidemia, diabetes mellitus, and smoking status), was collected. Medical histories, such as chronic kidney disease (CKD), anemia, and prior cardiovascular conditions (e.g., myocardial infarction, stroke, previous percutaneous revascularization, and vascular disease), were also documented. Furthermore, key laboratory parameters, angiographic findings, and procedural characteristics were recorded, including details of drug therapy prescribed at discharge. A one-year follow-up was conducted through telephone interviews or outpatient visits to gather data on clinical outcomes and patient status.

Outcomes. The primary endpoint was one-year all-cause mortality.

Ethics. This study has been conducted in accordance with all ethical principles of the Declaration of Helsinki and in accordance with and with approval from all national and institutional ethical standards.

Statistical analysis. The collected data were analyzed using standard descriptive and analytical statistical methods. Continuous variables were expressed as the mean ($\bar{x}$) ± standard deviation (SD) for normally distributed data and assessed using the Student's t-test. The median and interquartile range (IQR) were reported and compared for non-normally distributed data using the Mann-Whitney U test. Categorical variables were presented as absolute frequencies (n) and percentages, with comparisons

conducted using the chi-square test. Cumulative survival curves over a one-year follow-up period for the compared age groups were generated using the Kaplan-Meier method and analyzed with the Log-Rank test.

Univariable and multivariable analyses were performed using the Cox proportional hazards model, with patients aged ≤64 as the reference group to identify predictors of all-cause mortality. Statistical significance was determined using two-tailed p-values of < 0.05. All analyses were conducted with IBM SPSS Statistics version 21 (SPSS Inc., Chicago, IL, USA).

RESULTS

From June to December 2019, 404 STEMI patients were hospitalized, and 395 were referred for primary PCI, which led to their inclusion in the statistical analysis.

The baseline clinical characteristics are presented in **Table 1**. The average age of the patients was 62 years; 27.6% were between 65 and 74 years, while 15.7% were 75 years and older. Women represented 28.7% of the patient population and displayed progressively higher representation in the older age groups, with 35.8% in the 65-74 age group and 50% among individuals aged 75 years and above. Older patients exhibited a greater prevalence of conditions, including arterial hypertension, diabetes mellitus, chronic kidney disease, anemia, and prior cerebrovascular events, but a lower incidence of active smokers. Furthermore, patients aged 75 years and above had more frequent prior revascularization procedures, including PCI or CABG, compared to those aged 64 years and younger. Heart failure, classified as Killip class II-IV, was frequently observed in elderly patients, particularly those aged 65 to 74 years and those aged 75 years and older, as well as with a reduced ejection fraction (EF 40%). Older patients often presented with new-onset atrial fibrillation. Moreover, there was a significant difference in the medications prescribed upon hospital discharge; older patients more commonly received the less potent P2Y12 inhibitor clopidogrel and diuretics **(Table 1)**.

Regarding coronary angiograms and procedural characteristics, the findings indicated that multivessel disease was more prevalent among older patients, specifically those aged 65 to 74 and those aged 75 and older. Moreover, incomplete revascularization was more common among older patient groups. Additionally, in patients aged 75 and above, the presence of the left main artery or graft as an infarct artery was significantly more frequent compared to patients aged 65 years or younger. Neverthe-

Table 1. Baseline clinical characteristics and discharge therapy

Characteristics	≤ 64 yrs N= 224	65-74 yrs * N=109	≥75 yrs ** N=62	P*	P**
Female, n (%)	44 (19.6%)	39 (35.8%)	31 (50.0%)	**0.001**	**<0.001**
Hypertension n(%)	140 (62%)	77 (70.6%)	54 (87.1%)	0.157	**<0.001**
Diabetes mellitus n (%)	47(21.1%)	43 (39.4%)	23 (37.1%)	**<0.001**	**0.010**
HLP n (%)	136 (62.1%)	46 (43.0%)	24 (40.0)	**0.001**	**0.002**
Active smokers n (%)	136 (59.6%)	37 (34.3%)	1 (1.6%)	**<0.001**	**<0.001**
Previous MI n (%)	30 (13.5%)	23 (21.1%)	13 (21.0%)	0.074	0.144
Previous CVI n (%)	5 (2.2%)	9 (8.3%)	8 (12.9%)	**0.010**	**<0.001**
Previous PCI n (%)	28 (12.6%)	19 (17.4%)	15 (24.2%)	0.231	**0.024**
Previous CABGn (%)	5 (2.2%)	2(1.8%)	5 (8.1%)	0.808	**0.028**
CKD n (%)	22 (10.0%)	31(28.7%)	35 (56.6%)	**<0.001**	**<0.001**
Anemia n (%)	25 (11.2%)	34 (31.2%)	36 (58.1%)	**<0.001**	**<0.001**
Killip class 2-4 n (%)	42 (18.8%)	31 (28.7%)	24 (38.7%)	**0.040**	**0.001**
LVEF<40% n (%)	46 (21.8%)	38 (37.3%)	20 (35.7%)	**0.004**	**0.032**
New-onset AF n (%)	3 (1.3%)	9 (8.3%)	9 (14.5%)	**0.001**	**<0.001**
LDL cholesterol med (IQR)	3.10(2.54-3.79)	2.77(2.14-3.40)	2.38(1.81-3.28)	**0.001**	**<0.001**
Ticagrelor n (%)	155 (73.5%)	65 (65.7%)	18 (40.9%)	0.064	**<0.001**
Prasugrel n (%)	11 (5.2%)	2 (2.0%)	1 (2.3%)		
Clopidogrel n (%)	45 (21.3%)	32 (32.3%)	25 (56.8%)		
Beta-blocker n (%)	183 (86.3%)	88 (88.9%)	39 (83.0%)	0.529	0.554
ACE inhibitor n (%)	161 (75.9%)	71 (71.7%)	37 (78.7%)	0.425	0.684
Statin n (%)	204 (96.2%)	96 (97.0%)	42 (89.4%)	0.741	0.051
Diuretic n (%)	55 (26.1%)	47(47.5%)	24 (51.1%)	**<0.001**	**0.001**

DM: Diabetes mellitus; IM: myocardial infarction; CVI: Cerebrovascular insult; PCI: percutaneous coronary intervention; CABG: coronary artery bypass graft; HKD: Chronic kidney disease, ACE-angiotensin-converting enzyme *Comparison between ≤ 64 yrs and 65-74 yrs; ** Comparison between 65-74 yrs and ≥75 yrs

Table 2. Baseline angiographic and procedural characteristics

Characteristics	≤ 64 yrs N=224	65-74 yrs * N=109	≥75 yrs ** N=62	P*	P**
pPCI n (%)	209 (93.3%)	102 (93.6%)	54 (87.1%)	0.925	0.112
Stent implanted n (%)	202 (90.6%)	98 (90.7%)	49 (80.3%)	0.963	0.027
Radial approach n (%)	199 (89.2%)	97 (89.0%)	52 (83.9%)	0.946	0.249
GpIIb/IIIa n (%)	19 (8.5%)	15 (13.8%)	8 (12.9%)	0.135	0.292
One-vessel disease n (%)	111 (49.6%)	36 (33.0%)	16 (25.8%)	**0.004**	**0.001**
Complete revascularization n (%)	127 (58.3%)	51 (47.2%)	21 (34.4%)	0.060	**0.001**
LAD n (%)	105 (47.7%)	44 (40.4%)	18 (29.5%)		
LCx n (%)	33 (15.0%)	13 (11.9%)	8 (13.1%)		
RCA n (%)	76 (34.5%)	50 (45.9%)	28 (45.9%)	0.206	0.006
LM n (%)	2 (0.9%)	0 (0.0%)	4 (6.6%)		
graft n (%)	4 (1.8%)	1 (0.9%)	3 (4.9%)		

LAD-left anterior artery, LCx-left circumflex artery, LM-left main artery, RCA-right coronary artery, *Comparison between ≤ 64 yrs and 65-74 yrs; ** Comparison between 65-74 yrs and ≥75 yrs

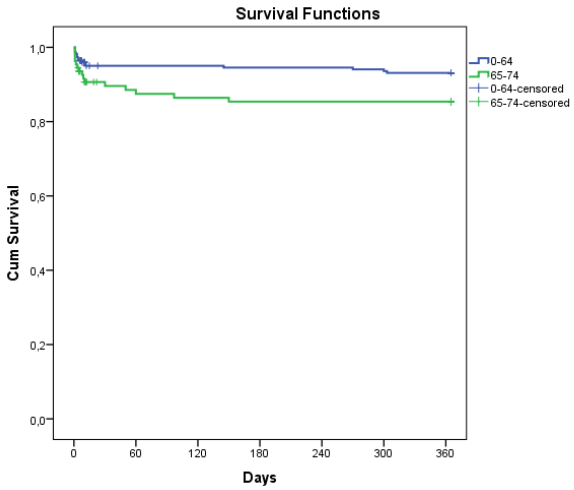
Table 3. All-cause mortality on 30-day and 1-year

	≤ 64 yrs N=224	65-74 yrs * N=109	≥75 yrs ** N=62	P*	P**
30 days n(%)	11 (4.9%)	11 (10.1%)	17 (27.4%)	0.074	**<0.001**
1 year n (%)	15 (6.7%)	15 (13.8%)	24 (38.7%)	**0.035**	**<0.001**

less, primary PCI was performed at a high rate across all three age groups, with rates of 93.3%, 93.6%, and 87.1%, predominantly using a radial approach. Stent implantation was performed at high rates across all age groups, although significantly less in patients aged 75 and older, with no notable differences observed in the use of Gp IIb/IIIa inhibitors among the different age groups (Table 2).

Table 3 presents the one-year and 30-day all-cause mortality rates. At the one-year follow-up, mortality was recorded in 55 patients, accounting for 13.6% of the total, while the 30-day mortality rate was noted in 39 patients, which is 9.9%. It was observed that patients aged 75 years and older had significantly higher mortality rates at both the 30-day and one-year marks compared to those aged 64 and younger.

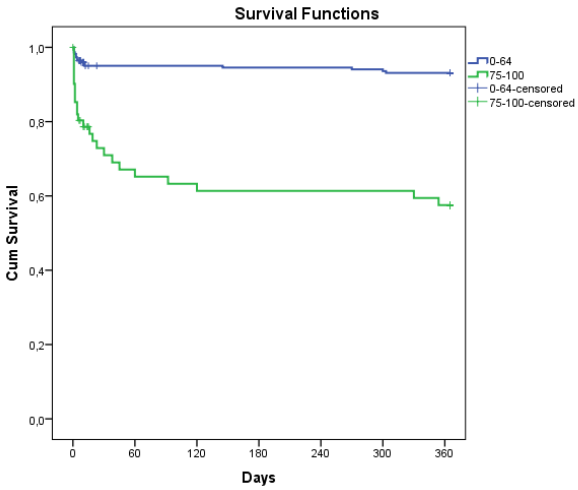
Kaplan-Meier curves illustrate one-year survival rates for patients aged 65–74 (Figure 1) and those aged 75 and older (Figure 2). Both age groups showed statistically significantly lower one-year survival compared to patients 64 and younger, as determined by log-rank tests ($p = 0.027$ and $p < 0.001$, respectively).



Log Rank – 0.027

No ≤ 64 yrs	224	213	213	212	211	220	199
No 65-75 yrs	109	96	95	94	94	94	94

Figure 1. Kaplan-Meier curves showing one-year survival rate in patients 64-75 years



Log Rank <0.001

No ≤ 64 yrs	224	213	213	212	211	220	199
No ≥75 yrs	62	42	40	40	40	40	38

Figure 2. Kaplan-Meier curves showing the one-year survival rate in patients 75 years and older

Table 4. Predictor of all-cause mortality at 30 days and 1 year

	Univariable Analysis		Multivariable Analysis	
	HR (95%CI)	p value	HR (95%CI)	p Value
		30 days		
65-74 yrs	2.16(0.93-4.99)	0.070	1.39(0.55-3.53)	0.484
≥75 yrs	6.41 (3.00-13.70)	**0.000**	3.35 (1.28-8.77)	**0.013**
DM	2.13 (1.12-4.03)	**0.021**	1.06 (0.52-2.15)	**0.872**
CKD	4.53 (2.41-8.51)	**<0.001**	2.00 (0.93-4.30)	**0.076**
Killip 2-4	2.62 (1.38-4.98)	**0.003**	1.81 (0.91-3.59)	**0.091**
Complete revascularization	0.14 (0.05-0.35)	**<0.001**	0.16 (0.06-0.46)	**0.001**
		1 year		
65-74 yrs	2.19 (1.07-4.48)	0.032	1.45 (0.64-3.13)	0.37
≥75 yrs	7.36 (3.85-14.07)	**0.000**	4.57 (2.05-10.18)	**0.000**
DM	**2.23 (1.30-3.82)**	**0.004**	1.10 (0.60-2.00)	**0.764**
CKD	**3.38 (2.24-6.54)**	**<0.001**	1.63 (0.86-3.10)	**0.137**
Killip 2-4	**3.32 (1.94-5.69)**	**<0.001**	2.43 (1.36-4.32)	**0.003**
Complete revascularization	**0.22 (0.11-0.44)**	**<0.001**	0.30 (0.15-0.61)	**0.001**

DM-Diabetes mellitus, CKD -Chronic kidney disease

Univariate Cox proportional hazards analysis showed that older patients, particularly those aged 65–74 and ≥75 years, had higher one-year mortality rates compared to patients aged ≤64. However, after adjusting for confounding factors including gender, diabetes mellitus, chronic kidney disease, Killip class II–IV, and incomplete revascularization, age ≥75 remained an independent predictor of all-cause mortality at both 30 days and one year **(Table 4)**.

DISCUSSION

The results of this observational study indicate that STEMI patients aged 75 and older, as well as those aged 65 to 74 who undergo primary PCI, have significantly higher one-year and 30-day mortality rates compared to individuals aged 64 and younger. Furthermore, the one-year mortality risk doubles for those aged 65 to 74 and increases sevenfold for those aged 75 and older. Additionally, being 75 or older is recognized as an independent prognostic factor for one-year mortality.

Our patients' demographic and baseline characteristics align with data from the literature, showing that the percentage of individuals aged 75 years and older with STEMI treated with primary PCI is comparable to that observed in our study, at approximately 18%. (3, 17, 18).

While the increased adoption of the recommended management strategies for treating STEMI (such as invasive therapy, DAPT) has significantly reduced the mortality rate among older patients over the past 15 years (19), even in the cases of cardiogenic shock (14), the mortality rate remains significantly higher compared to their younger counterparts. Topaz et al. demonstrated that among STEMI patients undergoing primary PCI from the TAPAS registry, those aged 75 years and older had both short- and long-term mortality rates more than four times higher than those under 75 years (17). Analysis of 5745 STEMI patients undergoing primary PCI from the APEX-AMI trial revealed that patients 65 years and older had an increased 90-day mortality rate (2.3% vs. 4.8% vs. 13.1%; >65 vs. 65-74 vs. ≤75 years, respectively). Furthermore, with every decade increase in age, there was a two-fold increase in the hazard of 90-day mortality (3). Results from the ULTIMASTER registry indicate that among STEMI patients treated with PCI using the Ultimaster stent, those aged ≥ 80 years (7.2%) experienced significantly higher one-year all-cause mortality compared to the younger group (10.1% vs 2.3%) (20).

The increased mortality rate associated with advancing age in STEMI patients, even when managed according to current guideline recommendations, may be attributed to the specific clinical profile of these individuals.

Firstly, patients aged 65 and over often have numerous comorbidities, particularly hypertension, diabetes mellitus, chronic kidney disease, and anemia. Each of these, both individually and synergistically, significantly affects the mortality rate, regardless of treatment with (21-23). Secondly, the proportion of women with acute coronary disease increased with advancing age. In our study, approximately 50% of patients aged 75 and older are female, consistent with observations from existing clinical research (3, 17). It is important to note that during menopause, there is a substantial increase in the incidence of cardiovascular disease among women (24) due to the decline of the protective effect of circulating on the endothelium of blood vessels (25). Additionally, it's well-documented that women tend to experience higher mortality rates than men following STEMI, both

during hospital stays and at the one-year mark after PCI (26). Thirdly, in our study, older patients experienced more frequent symptoms and signs of heart failure, as well as atrial fibrillation, conditions that are closely associated with an adverse prognosis (27, 28). Fourthly, as our study observed, the literary data confirmed that patients aged 65 and above exhibited significantly higher rates of multivessel coronary disease, leading to incomplete revascularization and subsequently contributing to poorer outcomes (27). In contrast, primary PCI was extensively performed across all age groups, predominantly using a radial approach. The administration of GpIIb/IIIa inhibitors was consistent across age groups, suggesting uniform treatment protocols despite variations in patient age and complexity. As a result, the older patients in our study received treatment that adhered to contemporary standards, akin to that of their younger counterparts.

Clinical status variations have led to significant differences in medical therapy among age groups at hospital discharge. Specifically, patients aged 75 years and older were prescribed more potent P2Y12 inhibitors less frequently, consistent with their safety profiles, particularly given the higher incidence of chronic kidney disease and anemia in this demographic. Moreover, diuretics were more commonly prescribed to older patients, especially those aged 75 and older, due to the frequent presence of reduced ejection fraction (EF) in this age group.

CONCLUSION

In recent years, the number of STEMI patients aged 75 and older has been increasing, along with a greater burden of comorbidities that significantly impact mortality rates. Although these older patients receive optimal invasive and pharmacological treatments, they do not benefit from standard treatment protocols to the same degree as younger populations. This complexity highlights the necessity for tailored approaches when managing acute coronary events in elderly patients to address their specific clinical and individual needs effectively.

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Author Contributions:

The conception or design of the work: A. M, M. A.

The acquisition, analysis or interpretation of data: L. S, I. J, S. G, Ž. I, A. M.

Preparing the draft of the manuscript: A. M, D. M.

Ethical Approval: This study has been conducted in accordance with all ethical principles of the Declaration of Helsinki and in accordance with and with approval from all national and institutional ethical standards.

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POVEZANOST STARIJEG ŽIVOTNOG DOBA SA JEDNOGODIŠNJIM MORTALITETOM KOD BOLESNIKA SA AKUTNIM INFARKTOM MIOKARDA SA ST ELEVACIJOM LEČENIH PRIMARNOM PERKUTANOM KORONARNOM INTERVENCIJOM

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Sažetak

Uvod: Povećanje očekivane dužine životnog veka je rezultiralo većom prevalencom bolesnika s akutnim infarktom miokarda sa ST elevacijom (STEMI).

Cilj: Ispitivanje veze između starije životne dobi i jednogodišnjeg mortaliteta kod bolensika sa STEMI lečenih primarnom PCI.

Metodologija: Studija je obuhvatila 395 bolesnika sa STEMI lečenih primarnom PCI u Koronarnoj jedinici u periodu jun-decembar 2019. godine. Bolesnici su kategorisani u tri starosne grupe : ≤ 64godine, 65-74 godina i ≥75 god, pri ćemu grupa ≤ 64godine služi kao referentna grupa za poređenje.

Rezultati: Prosečna starost pacijenata uključenih u studiju je bila 62 godine; 27,6% je bilo starosne dobi od 65 do 74 godine, dok je 15,7% imalo 75 godina ili više. Žene su činile 28,7% od ukupnog broja, sa većom zastupljenošću u starijim starosnim grupama. Pacijenti starosti od 65 do 74 godine i oni stariji od 75 godina su pokazali povećanu stopu dijabetesa, hronične bubrežne insuficijencije, anemije i srčane insuficijencije (Killip 2-4). Primena primarne PCI je bila značajno visoka u svim starosnim grupama i iznosila je 93,3%, 93,6% i 87,1%, izvedena je uglavnom radijalnim pristupom. Rizik od jednogodišnjeg mortaliteta bio je dvostruko veći za one od 64 do 75 godina i čak sedam puta veći za bolesnike od 75 i više godina, pri čemu je starost od 75 godina i više bila nezavisni prediktor ukupnog mortaliteta.

Zaključak: Stariji pacijenti sa STEMI, posebno oni od 75 godine i stariji, imaju znatno veću jednogodišnju stopu mortaliteta u poređenju sa onima od 64 godine i mlađim zbog značajnog opterećenja komorbiditetima uprkos primeni smernicama preporučenoj terapiji.

Ključne reči: starije osobe, STEMI, primarni PCI, mortalitet

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ORIGINAL ARTICLE

Association between coronary microvascular dysfunction indices and infarction size following primary percutaneous coronary intervention

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Summary

Introduction: Coronary microvascular dysfunction (CMD) has been associated with impaired prognosis in patients with ST-elevation myocardial infarction (STEMI) despite timely and successful primary percutaneous coronary intervention (PCI). Our aim was to compare the ability of two different CMD indices, coronary flow reserve (CFR) and hyperemic microvascular resistance (HMR), to predict infarct size.

Methods: The analysis included 31 patients with STEMI in whom valid invasive measurements of coronary blood flow velocity with the Doppler-tipped coronary wire had been performed at the end of primary PCI, and in whom infarct size was determined by cardiac magnetic resonance (CMR) after at least 3 months. Receiver operating (ROC) curves were used to comparatively assess the capacity of HMR and CFR to predict large infarction, defined as the highest quartile of the included population (≥16% of the left ventricular mass).

Results: Invasive measurements at the end of primary PCI revealed an average HMR value of 2.72±0.86 mmHg/cm/s, and that of CFR to be 1.65± 0.36. Both indices of microvascular function significantly correlated with infarct size (rho=0.569, p<0.01, for HMR, and rho= -0.391, p=0.029, for CFR). Comparative assessment of ROC curves revealed a similar capacity of HMR and CFR to predict large myocardial infarction (AUC=0.669, 95%CI 0.472-0.866 for HMR vs. AUC=0.712, 95%CI 0.517-0.907 for CFR; p=0.718 for comparison).

Conclusion: Doppler wire-derived HMR and CFR, measured simultaneously at the end of primary PCI, exhibited similar capacities for predicting large myocardial infarction.

Keywords: coronary microcirculation, infarct size, primary PCI

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INTRODUCTION

Primary percutaneous coronary intervention (PCI) with the resulting recanalization of the infarct-related artery (IRA) is the recommended standard of care for patients presenting with ST-elevation myocardial infarction (STEMI) (1). Early research during the emergence of recanalization therapies in the 1990s revealed that, in a substantial proportion of patients, the myocardium within the risk area remained inadequately perfused despite timely and successful epicardial artery recanalization (2). Contemporary research has confirmed that in around 50% of patients undergoing primary PCI, there is residual coronary microvascular injury leading to impaired reperfusion at the level of cardiac myocytes, which is ultimately associated with increased mortality (3, 4). The injury to the coronary microcirculation post primary PCI is nowadays most frequently expressed as microvascular obstruction (MVO) on cardiac magnetic resonance (CMR) imaging or as coronary microvascular dysfunction (CMD) estimated by an invasive measurement of coronary flow velocity either directly by a Doppler-tipped coronary wire or indirectly by the thermodilution method (5). Both MVO and invasive indices of CMD, such as coronary flow reserve (CFR) or microvascular resistance, have been associated with increased mortality following primary PCI (6-9). As microvascular injury has been found to be an independent predictor of mortality after STEMI (10), it becomes a therapeutic target, with ongoing research efforts to define optimal treatment strategies to decrease the degree of microvascular damage post-primary PCI (11, 12). Infrastructural constraints associated with the inability to routinely perform CMR in STEMI have shaped the current research paradigm towards stratifying patients at the end of primary PCI according to the presence of CMD as assessed by invasive indices of microvascular function, such as the index of microcirculatory resistance (IMR) (13, 14). This approach is based on a series of studies demonstrating that different indices of microvascular function, such as IMR, hyperemic microvascular resistance (HMR), and CFR, have been associated with mortality and heart failure following primary PCI (6, 7, 14). Several comparative studies indicated that resistance indices (IMR or HMR) may have been more closely related to MVO or infarct size than CFR (15). Pressure at zero flow (PzF), an index considering extravascular compression, had the best capacity to predict structural myocardial damage post MI (6, 16). However, the invasive nature of indices of microvascular resistance and PzF constrains their use in daily practice. Given the correlation of non-invasive, transthoracic doppler echocardiography (TTDE)-derived CFR with the invasive, Doppler wire-derived CFR (17), similar predictive ability of invasive CFR and HMR may open the door towards a more widespread use of CFR to risk-stratify patients after STEMI according to their risk of mortality and heart failure. Hence, our aim was to comparatively assess the ability of HMR and CFR, measured simultaneously at the end of a timely and successful primary PCI, to predict large infarct size, as a surrogate of an increased risk of future mortality and heart failure (18).

METHODS

Patients

This was a prospective, observational study, which included 31 patients with valid simultaneous invasive measurements of coronary blood flow velocity and distal coronary pressure with a Doppler-tipped intracoronary guidewire in the recanalized IRA immediately post primary PCI, for whom infarct size assessment on CMR was also available, at least 3 months after the acute event (CMR was available for 32 patients). Patients who presented with STEMI, within 12 hours of chest pain onset, were included if undergoing a successful primary PCI, with TIMI 3 flow and residual stenosis <20-30% post-PCI. Exclusion criteria were acute heart failure or a known history of chronic heart failure, a known prior coronary artery disease and the presence of a critical stenosis in a non-IRA territory. All patients were treated with a loading dose of aspirin (300mg) and either ticagrelor 180mg or clopidogrel 600mg, prior to primary PCI, and with standard maintenance doses afterwards. Use of Glycoprotein IIb/IIIa inhibitors at any point of the procedure or post-procedurally, and manual thromboaspiration were at the discretion of the operator. Ethics Committee of the University Clinical Center of Serbia (protocol code 747/11, 19 July 2018) approved the protocol, and the research was conducted in accordance with the Declaration of Helsinki.

Study procedures

Immediately after a successful primary PCI, patients underwent simultaneous coronary flow velocity and distal coronary pressure measurements by positioning a Doppler-tipped coronary guide wire (ComboWire, Philips Volcano, San Diego, California) in the distal segment of the recanalized IRA. The simultaneous coronary pressure and flow velocity measurements were performed at rest and again after inducing hyperemia by the intracoronary injection of adenosine, at least 200 mcg for the left anterior descending (LAD) and 100 mcg for the right coronary artery (RCA). Blood flow velocity was expressed as average peak velocity (APV) over three heart cycles. From those measurements, CFR and HMR were automatically calculated by the ComboMap system (Philips Volcano, San Diego, California), which processed the data obtained by the ComboWire together with the simultaneous ECG and aortic pressure tracings. Fractional flow reserve (FFR) was also calculated from the available data, as the ratio of distal coronary pressure (Pd) over the aortic pressure (Pa).

After 3 months (median 7.5 months), patients underwent cardiac magnetic resonance (CMR) imaging with a 1.5 Tesla MR-scanner (Magnetom Avanto, Siemens, Erlangen, Germany). Infarct size was measured as a proportion of the LV mass that is infarcted, as delineated by late gadolinium enhancement (LGE) after the administration of 0.2 mmol/kg of Gadobutrol (Gadovist, Bayer Inc, Canada). In addition, CMR measurements included the assessment of left ventricular size and function, as expressed by left ventricular ejection fraction (LVEF), end-diastolic volume (EDV) and end-systolic volume (ESV).

Statistics

After testing for the normality of their distribution with the Shapiro-Wilk test, variables with normal distribution were presented as means with standard deviation and compared with the student t test, whereas variables with non-normal distribution were presented as median with interquartile range and compared with the Mann-Whitney U test. Counts were used to present categorical variables, which were compared with the chi-squared test. If necessary, correlations between continuous variables were assessed using the Pearson (r) or Spearman (rho) coefficient. We constructed receiver-operating characteristic (ROC) curves to assess the ability of HMR and CFR to predict large infarct size, representing the highest quartile of the included population (≥16% of the mass of the left ventricle). Optimal HMR and CFR cut-off values for the prediction of a large infarction were determined using the Youden index. The Delong method was used to compare the ROC curves constructed for HMR and CFR. The degree of association of HMR and CFR with infarct size was explored by constructing multivariable regression models with the inclusion of other key predictor variables known at the time of the primary PCI procedure. P-value <0.05 was adopted as the marker of statistical significance. SPSS 26.0 (IBM, Chicago, Illinois) and GraphPad Prism (La Jola, California) were used for all statistical analyses and graph constructions.

RESULTS

Table 1 lists baseline characteristics of the included patients. The study population consisted predominantly of patients with STEMI who underwent primary PCI early after chest pain onset (median time to reperfusion was just under 3 hours). Median age was 59 and 20% were females. The procedure was performed via transradial route in all cases, and thromboaspiration was utilized in 16%, whereas Glycoprotein inhibitors (GPI) were administered in 13% of cases. The culprit artery was the LAD in two-thirds of patients. In almost all of the included patients (90%), the culprit artery was occluded at the start of the procedure. In-hospital echocardiography, several days following primary PCI, revealed an average LVEF of 45%. Finally, post-PCI ECG established the presence of incomplete ST-segment resolution in close to 50% of patients, which has been an accepted surrogate for coronary microvascular impairment.

Table 1. Baseline characteristics.

Baseline clinical and angiographic variables	n=31
Age, years, median (IQR)	59 (53-62)
Male, n (%)	26 (84)
Diabetes mellitus, n (%)	6 (19)
Hyperlipidemia, n (%)	24 (77)
Hypertension, n (%)	18 (58)
Time, symptom onset to reperfusion, min, median, (IQR)	175 (125-270)
Infarct-related artery LAD, n (%)	21 (68)
TIMI flow grade 0 before primary PCI, n (%)	28 (90)
Trans-radial access, n (%)	31 (100)
Manual aspiration thrombectomy, n (%)	5 (16)
Glycoprotein IIb/IIIa inhibitors, n (%)	4 (13)
Echocardiography during index hospitalization	
Left ventricular ejection fraction, %, mean ± SD	45 ±11
End-systolic diameter, mm, mean ± SD	54 ± 5
End-diastolic diameter, mm, mean ± SD	38 ± 6
Electrocardiography after 90 minutes of primary PCI	
Incomplete ST-segment resolution, n (%)	15 (48)

IQR – interquartile range, LAD – left anterior descending, PCI – percutaneous coronary intervention, SD – standard deviation.

Table 2 shows the average parameters of intracoronary physiology at the end of the primary PCI procedure. Mean HMR was 2.72±0.86 mmHg/cm/s, above the earlier threshold of 2.50 mmHg/cm/s, implying the presence of CMD in an average patient undergoing primary PCI. Similarly, the mean CFR was 1.65±0.36, which is below the established threshold of 2.50, also implying the presence of CMD. It should be noted that the average value of FFR was 0.92, which likely suggested no significant residual disease, confirming that the obtained CFR values primarily reflected microvascular function.

Table 2. Invasive measurements of coronary pressure and flow at the end of primary PCI.

Coronary physiology by Doppler wire	n=31
APV resting, cm/s, mean ± SD	21.00 ± 6.05
APV hyperemic, cm/s, mean ± SD	34.58 ± 12.48
Pa, resting, mmHg, mean ± SD	93.48 ± 11.71
Pd, resting, mmHg, mean ± SD	89.45 ± 12.29
Pa, hyperemic, mmHg, mean ± SD	88.71 ± 12.52
Pd, hyperemic, mmHg, mean ± SD	81.90 ± 11.64
CFR, mean ± SD	1.65 ± 0.36
HMR, mmHg/cm/s, mean ± SD	2.72 ± 0.86
FFR, mean ± SD	0.92 ± 0.04

APV – average peak velocity, Pa – pressure aorta, Pd – pressure distal, CFR – coronary flow reserve, HMR – hyperemic microvascular resistance, FFR – fractional flow reserve, SD – standard deviation.

Table 3. Invasive coronary physiology in the culprit artery at the end of primary PCI and parameters of left ventricular structure and function on CMR after 3 months according to the infarct size.

	Small infarct size (<16% of the LV) n=22	Large infarct size (≥16% of the LV) n=9	p-value
Invasive coronary physiology at the end of primary PCI			
APV resting, cm/s, mean ± SD	21.55 ±6.89	19.67 ±3.16	0.442
APV hyperemic, cm/s, mean ± SD	36.77 ±13.73	29.22 ±6.59	0.128
Pa, resting, mmHg, mean ± SD	93.32 ±11.14	94.80 ±13.26	0.745
Pd, resting, mmHg, mean ± SD	90.00 ±11.86	88.90 ±13.38	0.817
Pa, hyperemic, mmHg, mean ± SD	88.59 ±11.93	89.00 ±14.64	0.936
Pd, hyperemic, mmHg, mean ± SD	81.64 ±11.75	82.56 ±12.04	0.846
CFR, mean ± SD	1.73 ±0.38	1.48 ±0.25	0.084
HMR, mmHg/cm/s, mean ± SD	2.58 ±0.84	3.04 ±0.84	0.177
FFR, mean ± SD	0.92 ±0.05	0.91 ±0.03	0.508
Left ventricular structure and function at follow-up			
Ejection fraction, %, mean ± SD	55.00 ±6.22	36.89 ±7.90	<0.001
End-diastolic volume, ml, mean ± SD	150.86 ±33.14	220.33 ±59.77	<0.001
End-systolic volume, ml, mean ± SD	71.14 ±22.13	142.33 ±57.57	<0.001
Infarct size, %, median, IQR	10.50, 3.75-12.25	19.50, 16.00-23.75	<0.001

APV – average peak velocity, Pa – pressure aorta, Pd – pressure distal, CFR – coronary flow reserve, HMR – hyperemic microvascular resistance, FFR – fractional flow reserve, SD – standard deviation, IQR – interquartile range.

When intracoronary physiology parameters were compared between patients with large and small infarct size (**Table 3**), a tendency was observed towards higher HMR values and lower CFR values in patients with large infarcts, pointing towards an association of CMD with the size of infarction (2.58 ±0.84 mmHg/cm/s vs. 3.04 ±0.84 mmHg/cm/s, p=0.177, for HMR and 1.73 ±0.38 vs. 1.48 ±0.25, p=0.084, for CFR, in small and large infarctions, respectively).

This tendency was confirmed by a finding of moderate but significant correlation between both HMR (rho=0.569, p<0.01) and CFR (rho=-0.391, p=0.029) with infarct size expressed as a continuous variable (**Figure 1**).

Both HMR (AUC 0.669, 95%CI 0.472-0.866) and CFR (AUC 0.712, 95%CI 0.517-0.907) had similar capacity (p=0.718 for the comparison of ROC curves) to predict large infarct size (defined as ≥16% of the LV mass) (**Figure 2**).

Optimal cut-off values to predict large infarct size were 2.25 mmHg/cm/s for HMR and 1.45 for CFR. Median infarct size in the overall population was 11.50% (6.75-16.00) and it was significantly larger in patients with HMR ≥2.25mmHg/cm/s (15.00% vs. 4.00%, p<0.001). Similarly, patients with CFR <1.45 had significantly larger median infarct size compared with those with values above this cut-off (15.50% vs. 9.00%, p=0.007). In the univariable regression analysis, both HMR≥2.25 mmHg/cm/s and CFR≤1.45 were significantly associated with the infarct size as a continuous variable. However, when adjusted for clinical variables known at the time of primary PCI, HMR≥2.25 mmHg/cm/s remained an independent predictor, whereas CFR≤1.45 was not (**Table 4**).

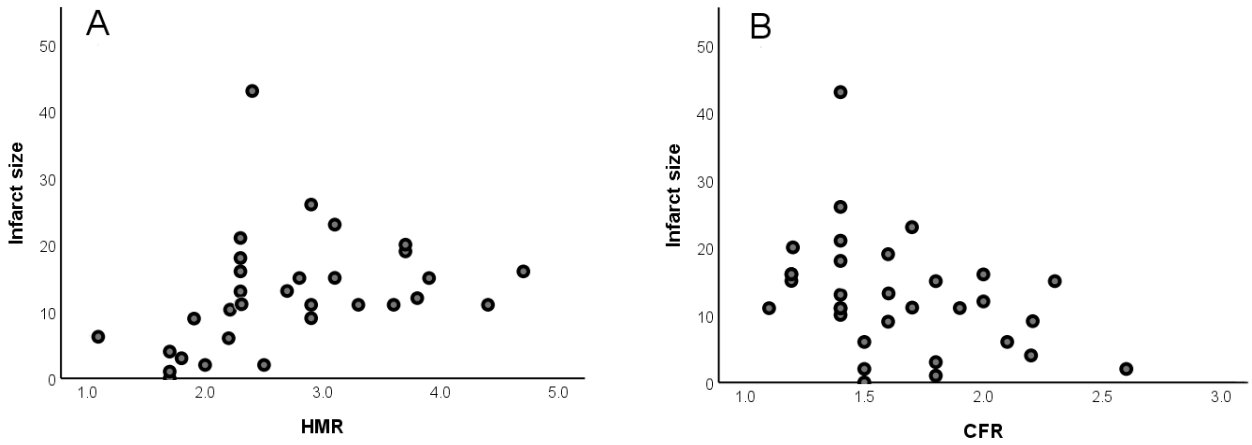

Figure 1. Significant correlation between coronary microvascular dysfunction and infarct size. **Panel A.** Increase in HMR values correlates with an increase in infarct size. **Panel B.** Decrease in CFR values correlates with an increase in infarct size.

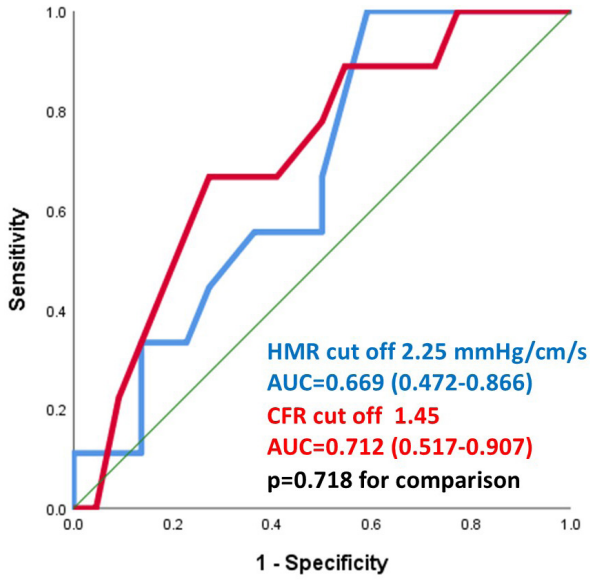

Figure 2. ROC curves comparison for the capacity of HMR vs. CFR to predict large infarct size.
Comparison of ROC curves showing similar capacity (p=0.718) of HMR (blue curve) and CFR (red curve) to predict large infarct size (≥16% of the left ventricular mass).

DISCUSSION

The main findings of our study are the following. First, both HMR and CFR, when measured at the end of a successful primary PCI, correlate significantly with infarct size. Second, HMR and CFR showed similar ability to predict large infarct size, defined as the highest quartile of the study population. Those findings need to be interpreted in the context of previous research in this field, which has both evaluated the prognostic value of different indices of microvascular function and explored its practical application as a means of patient stratification and therapy guidance to achieve better outcomes post primary PCI (5, 12, 13, 15, 16, 19, 20).

Teunissen et al. established the ability of HMR to predict microvascular injury (MVI) in a contemporary primary PCI setting, with values ≥2.5 mmHg/cm/s independently predicting MVI on CMR (19). MVI was defined akin to MVO, as hypointense areas within the infarcted, hyperintense myocardium on late gadolinium enhancement, assessed 4-6 days after primary PCI. The average HMR in the IRA immediately after recanalization was 2.87 ± 1.45 mmHg/cm/s, closely aligning with our findings of 2.72 ± 0.86 mmHg/cm/s. Post-primary PCI CFR was 1.80±0.80, also similar to our findings (1.65± 0.36). Although the primary result of Teunissen et al. was to demonstrate the ability of HMR, unlike CFR, to predict MVI, it also reported a significant correlation of HMR with infarct size on CMR on day 4-6 (r=0.41, p<0.01) and the significantly larger infarcts at 3 months in patients with HMR≥2.5 mmHg/cm/s (15.1% vs. 9.9%) (19). In our study, the correlation between HMR and infarct size on CMR at least 3 months after primary PCI was even stronger (rho=0.569, p<0.01; **Figure 1**). More-

Table 4. Predictors of infarct size*.

	Univariable** analysis		Multivariable analysis	
	Beta (95% CI)	p	Beta (95% CI)	p
Age	-0.045 (-0.685 – 0.594)	0.885		
Sex	2.000 (-9.492 – 13.492)	0.724		
Diabetes	-7.000 (-15.559 – 1.559)	0.105		
Hypertension	-2.000 (-11.136 – 7.136)	0.658		
Hyperlipidemia	0.000 (-10.109 – 10.109)	1.000		
Time to reperfusion	0.008 (-0.022 – 0.038)	0.573		
Infarct-related artery LAD	6.000 (2.369 – 15.631)	0.010	3.455 (0.967 – 11.033)	0.021
Incomplete ST-segment resolution	5.000 (-2.330 – 12.330)	0.174		
HMR≥2.25mmHg/cm/s	11.000 (5.413 – 16.587)	<0.001	8.000 (2.638 – 13.362)	0.005
CF≤1.45	-7.000 (-13.942 – 0.058)	0.048	-3.000 (-8.110 – 2.110)	0.239

*The reported results are from the quantile regression analysis with a quantile value set at 0.5.
**Baseline variables routinely obtained at the start of primary PCI or in the immediate periprocedural period are tested as predictors of infarct size, as measuring microvascular function during primary PCI may be actionable if it is of added value above and beyond the already known parameters. Those variables with p<0.100 from the univariable regression analysis are included into the multivariable regression analysis.

over, the ability to classify patients with large final infarct size (after at least 3 months) according to the study-specific HMR cut offs was very similar in both studies. In our study, patients with HMR≥2.25 mmHg/cm/s had average infarct size of 16% of the left ventricle mass, whereas patients with HMR≥2.5 mmHg/cm/s had average infarct size of 15.1% in the study by Teunissen et al (6). On the other hand, our study did demonstrate similar ability to predict infarct size by CFR, whereas no such data were reported by Teunissen et al. Our results reinforce earlier studies, which showed the ability of CFR measured with the Doppler wire in the IRA immediately after recanalization, as in our study, to predict LV structural and functional recovery after STEMI (21). At the same time, CFR was shown to predict MVO on CMR in other studies utilizing the thermodilution method, but with a very low specificity (34% and sensitivity of 79%) (22), likely explaining why in the study by Teunissen et al. HMR was a better predictor, especially of the high-extent MVO (specificity 65% and sensitivity 93%) (19). A possible explanation is that different indices, such as microvascular resistance (IMR or HMR) or flow reserve (CFR), may reflect different pathways of microvascular destruction (4), resulting in a variable association of individual indices with MVO or infarct size on CMR.

In practical terms, if invasive indices of microvascular function, which are available immediately after primary PCI, are associated with clinical outcomes, they can be used to guide adjunctive therapeutic strategies (13). Although previous studies suggested that CFR, HMR and IMR are associated with mortality and heart failure after primary PCI (6, 7, 22), recent comparisons indicated better ability of HMR (Doppler-derived hyperemic microvascular resistance) (6) and IMR (thermodilution-derived index of microcirculatory resistance) (15) over CFR to stratify patients according to the risk of mortality and heart failure. Recent efforts to standardize risk stratification after primary PCI favored IMR due to the wealth of studies utilizing this thermodilution-derived index, which has been seen as a more practical alternative to Doppler wire-based measurements that may require more time and effort to obtain (5, 13). However, the quest for an optimal index of microvascular injury to guide any adjunctive therapies targeting coronary microcirculation after STEMI is still on-going, as more information become available about the multifactorial process of microvascular injury and its impact on subsequent clinical events (4, 23, 24). The injury to coronary microvasculature may be associated with myocardial edema and eventually lead to intramyocardial hemorrhage, which has been more closely associated with increased mortality and heart failure than MVO (25). Intracardial edema and hemorrhage may exert extravascular pressure on the microvasculature (26), which may be best captured by pressure at zero flow (PzF), i.e., pressure at which antegrade coronary flow would cease, an index derived from simultaneous coronary pressure and flow tracing of a Doppler-tipped coronary guide wire. In a study by Patel et al., PzF (AUC=0.94) has been more closely related to large infarct size, defined as ≥24% of the LV mass, than HMR (AUC=0.74) or IMR (AUC=0.54) (16).

From the above-described studies comparing different indices of microvascular function, it seems to follow that a) PzF has the strongest capacity to predict infarct size (16), b) MVO on CMR can be predicted by both HMR (6) and IMR (15) better than CFR, and c) HMR and IMR seem to be superior to CFR in predicting mortality and heart failure (6, 22). Our study adds that CFR may have comparable ability to predict large infarct size as HMR. As large infarct size is associated with mortality and heart failure (18), our results support some of the previous studies showing the association of post-primary-PCI CFR with clinical outcomes (7). But the weaker association of CFR with MVO and intramyocardial hemorrhage, which both have been shown to predict mortality and HF independently of infarct size (10, 25), may explain the inferiority of CFR when compared to HMR and IMR in other studies.

In summary, our study did show comparable ability of invasively obtained HMR and CFR to predict large infarct size, which may be of practical relevance given the possibility of non-invasive echocardiography-derived CFR assessment (17) and the ability of CFR to provide prognostic information across a broad spectrum of cardiac diseases (27). However, our results should be interpreted with caution and in the aforementioned context of previous comparative analyses demonstrating better microvascular resistance indices (HMR and IMR) ability to predict MVO on CMR and clinical outcomes in the follow-up. Given the heterogeneity of post-STEMI changes in the myocardial structure and function, with infarct size, MVO and intramyocardial hemorrhage all being associated with mortality and heart failure, any one index of microvascular function may only represent a partial assessment of the damage conferred by the sequence of IRA occlusion and recanalization. Future research is needed to demonstrate which of the available invasive indices of coronary microcirculation may provide optimal patient stratification and therapeutic guidance at the end of the primary PCI.

The presented results need to be interpreted bearing in mind several important limitations. First, a limited number of patients. However, most of the original research that assessed indices of microvascular function in STEMI included a comparable patient population size (n=30-60). Second, no detailed MVO data were available for this analysis. Although our analysis did show a comparable predictive capacity between HMR and CFR in terms of predicting large infarct size, the correlation of CFR with infarct size was weaker, and CFR, unlike HMR, did not remain independently associated with infarct size after adjusting for other baseline predictors. Third, PzF was not available for this analysis. Given previous data on the supremacy of PzF over HMR and IMR, comparing PzF with HMR and CFR would have provid-

ed additional value to our analysis. Fourth, the known practical difficulty of routinely obtaining adequate intracoronary Doppler signals limits the applicability of our findings to everyday practice.

CONCLUSION

Invasive CFR and HMR, obtained by a Doppler-tipped coronary guide wire in the recanalized infarct-related artery, have similar capacity to predict large infarct size. Further research is needed to understand which index of microvascular function can be effectively used at the end of primary PCI to guide adjunctive therapies aimed at ameliorating microvascular injury and improving overall prognosis post-STEMI.

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POVEZANOST RAZLIČITIH INDEKSA DISFUNKCIJE KORONARNE MIKROCIRKULACIJE SA VELIČINOM INFARKTA NAKON PRIMARNE PERKUTANE KORONARNE INTERVENCIJE

Dejan Milašinović[1]

Sažetak

Uvod: Ranija istraživanja pokazala su da je kod bolesnika sa infarktom miokarda sa elevacijom ST-segmenta (STEMI), stepen disfunkcije koronarne mikrocirkulacije nakon primarne perkutane koronarne intervencije (PCI) povezan sa lošijom prognozom. Cilj ovog rada bio je da uporedi sposobnost dva različita indeksa disfunkcije koronarne mikrocirkulacije, koronarne rezerve protoka (CFR) i hiperemijske mikrovaskularne rezistencije (HMR), da predvide veličinu infarkta nakon primarne PCI.

Metode: Analiza je obuhvatila 31 bolesnika sa STEMI i validnim invazivnim merenjima brzine koronarnog protoka Dopler metodom na kraju primarne PCI, a kod kojih je magnetnom rezonancom srca (CMR) nakon najmanje 3 meseca procenjena veličina infarkta. Poređenjem ROC krivih ispitana je sposobnost CFR i HMR da predvide veliki infarkt miokarda, koji je bio definisan kao najviši kvartil analizirane populacije (≥16% mase leve komore).

Rezultati: Merenjem na kraju primarne PCI utvrđena je srednja vrednost HMR od 2.72±0.86 mmHg/cm/s, dok je u isto vreme srednja vrednost CFR bila 1.65± 0.36. Oba indeksa mikrovaskularne disfunkcije značajno su korelisala sa veličinom infarkta (rho=0.569, p<0.01, za HMR, i rho= -0.391, p=0.029, za CFR). Uporedna analiza ROC krivih pokazala je sličan kapacitet HMR i CFR da klasifikuju bolesnike prema riziku od velikog infarkta miokarda (AUC=0.669, 95%CI 0.472-0.866 za HMR vs. AUC=0.712, 95%CI 0.517-0.907 za CFR; p=0.718 za poređenje dva indeksa).

Zaključak: Vrednosti HMR i CFR dobijene invazivnim merenjem Dopler žicom nakon rekanalizacije infarktne arterije imaju sličnu sposobnost da predvide veliki infarkt nakon primarne PCI.

Ključne reči: koronarna mikrocirkulacija, veličina infarkta, primarna PCI

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ORIGINAL ARTICLE

Micromorphological features of mastocytes in the trigeminal and human sympathetic superior cervical ganglions

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Summary

Introduction: Mastocytes (Ms) are usually localized close to microvessels and terminals of the nerves innervating meninges and visceral organs. The granules of Ms contain numerous mediators that affect nerve cells and modify their reactions.

Aim: Our study was conducted with the aim of analyzing the presence and localization of Ms, and mastocytes density (MsD) in three segments of the human trigeminal ganglions (TGs): ophthalmic, maxillary and mandibular, as well as in two parts: upper and lower, of the sympathetic superior cervical ganglions (SSCGs), and establishing the correlation between the studied parameters.

Material and Methods: Five TGs and five SSCGs of adult individuals were processed for this histochemical and immunohistochemical analysis. The sections are serially sliced and stained with hematoxylin and eosin and the trichrome method of Masson, as well as for the mastocyte tryptase immunostaining.

Results: The average number of tryptase-positive (Tryp+) Ms in the marked fields on the microscope, the MsD, was 1.4 in the ophthalmic parts, 1.45 in the maxillary parts, and 1.5 in the mandibular parts of TGs. The median of numbers of Tryp+ Ms in fields on the microscope was 4.5 (from 2 to 6 Ms) in the upper segments, and 5 (from 3 to 6 Ms) in the lower segments of SSCGs. The distributions of Ms in three parts of the human TGs and two segments of the SSCGs were evenly distributed with no special morphological peculiarities. This is the first micromorphological analysis of the Tryp+Ms and MsD in human TGs and SSCGs.

Conclusion: We did not find a statistically significant difference between the means of MsD in the three segments of the TG ($p = 0.883$). We did not find a statistically significant differences between the MsD groups in two segments of the SSCGs ($p = 0.899$). We found statistically significant differences in MsD of Tryp+ Ms between TG and SSCG ($p<0.001$). MsD in two parts of the SSCG was significantly higher than MsD in three parts of TG.

Keywords: micromorphological analysis, mastocytes, mastocytes density, sympathetic superior cervical ganglion, trigeminal ganglion

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INTRODUCTION

The story of mast cells dates back to 1878, when Paul Ehrlich, in his doctoral thesis on the properties of histological stains, introduced the medical community to this previously unknown cell type (1). While doing experiments, Ehrlich noticed a new type of aniline reactive cells in connective tissue, as granular cells with spherical shape and dimensions bigger than known leukocytes (1). He named these cells "mastzellen", suggesting that they have characteristics of fixed cells of connective tissue (1).

Mastocytes derive from the bone marrow, from the stem cells of myeloid lineage (2). Their expansion and maturation happen in bone marrow by the influence of many cytokines, such as colony stimulating factor (CSF) and nerve growth factor (NGF) (2, 3). The specific characteristic of mastocytes is their tissue residence and not presence in the bloodstream (which is a property of most of the myeloid lineage cells) (2). Stem cells of myeloid lineage differentiate into mastocyte precursor cells, which migrate through the blood to the tissues. In tissues, mastocyte precursors differentiate into mastocytes (4). The differentiation of mastocytes is a very complex and specific process in which many other cells, neuropeptides and cytokines take part (2, 4-6). These substances work under strictly controlled conditions, which is essential for the differentiation and maturation of mastocytes (6).

Mastocytes are cells of the immune system. Their diameter varies from 10 to 20 μm and they are usually oval in shape (6). In the cytoplasm of mastocytes numerous granules are detected. These granules are basophilic, with a diameter of 0.3-2 μm and they exhibit metachromatic staining characteristics (1, 6). These granules contain many classic mediators such as: proteases (most common in mastocytes are chymase and tryptase), growth factors (transforming growth factor, nerve growth factor, vascular endothelial growth factor, etc.), proinflammatory cytokines (leukotrienes, prostaglandins) and chemokines, tumor necrosis factor (TNF), proteoglycans (most common heparin), biogenic amins (serotonin, histamine), neuropeptides (SP, CGRP, VIP, CRH and many others) (4-7).

Mastocytes are well-known participants in the inflammatory process and as of today more research has proven the orchestrating function of mastocytes in inflammatory reactions (2). Antibody-dependent mechanisms can stimulate mastocytes to release their numerous substances of inflammation (2). Mastocytes are usually localized close to microvessels and terminals of the nerves innervating meninges and visceral organs (2, 8). It is believed that the membranes of mastocytes and nerve cells are in such a close contact that they even exchange the contents of granules. Many experiments approved the influence of mastocytes on neurogenic inflammation (2, 4, 6-9). Neurogenic inflammation is a very complex process that involves interactions between the nervous and vascular systems (2). The process starts with peripheral nerve sensitization when sensitized terminals of peripheral nerves release proinflammatory mediators, like SP (substance P) and CGRP (calcitonin gene-related peptide) (2, 8). This release provokes plasma extravasation and vascular dilatation (2). At the same time, CGRP and SP have effect on mastocytes, causing their activation (2). Activated and degranulated mastocytes release mediators from their granules which affect nerve terminals to release even more proinflammatory substances (2, 8). These actions form a vicious cycle in which more and more mastocytes are activated, more nerve terminals are sensitized, which causes the amplification of neurogenic inflammation (2).

Mastocytes degranulation and activation are important factors in pathogenesis of many medical conditions (2). Their most known role is in allergies and anaphylaxis, but in the past few decades their contribution to the persistent state of pain (such as in migraine) has been reported (2, 8). It is believed that mastocyte – sensory nerve interactions take a big part in the pathophysiology of migraine (8). According to some studies, mastocytes contribute to neuropathic pain, but also pelvic pain and osteoarthritis (8). The latest studies show the function of mastocytes in sickle cell disease, atopic dermatitis, psoriasis and cancer (2, 10-13).

Our study was conducted with the aim of analyzing the presence of mastocytes and mastocyte density (MsD) in three segments of the human trigeminal ganglions (TGs): ophthalmic, maxillary and mandibular, as well as in two segments: upper and lower of the sympathetic superior cervical ganglions (SSCGs), and establishing the correlation between the studied parameters.

MATERIAL AND METHODS

Histological and immunohistochemical study

Five human trigeminal ganglions (TGs) and five sympathetic superior cervical ganglions (SSCGs) of adult individuals were processed for this histochemical and immunohistochemical study. Each ganglion was removed after specific dissection of the Meckel's trigeminal cave, for the TG, and the highest part of the posterior neck, for the SSCG. We used the isotonic saline solution for the immersion of obtained ganglions, and fixed them in 4% buffered formalin. After dehydratation, specimens were embedded in paraffin and serially sliced using the Leica RM2235 rotary microtome. The sections were serially stained with hematoxylin and eosin, the next with the trichrome method of Masson, as well as the next with the mastocyte tryptase immunostaining. We prepared sets of plates from every specimen of ganglion. We treated all specimens for the immunohistochemical staining for antigen retrieval after deparaffinization. The incubation of the slices with 3% hydrogen peroxide solution

was performed to block the endogenous peroxidases. The immunostaining occured by the treatment of slices with the following mouse monoclonal primary antibody anti-mastocyte tryptase (DAKO A/S, M 7052, Denmark, 1:100). We visualized the bound antibodies after staining with a secondary antibody, after use a Mouse/Rabbit PolyDetector DAB HRP Brown (Bio SB) detection system. We counterstained the sections with Mayer's hematoxylin, dehydrated them and covered with glass slips. Two independent researchers semi-quantitatively assessed the intensity of staining. The intensity of immune reaction has been identified as very positive (+++). The quality of immunostaining was tested using the negative control, by incubating the slices with non-immune serum without primary antibody. The colored plates were examined under a light microscope (Leica DMLS) and photographed using a digital camera (Leica DFC295). Morphometric analyses were performed using image analysis software (Leica Interactive Measurements). This research protocol and examination have been approved by the authorities of the Institute of Histology and Embryology, the Institute of Anatomy, the Institute of Pathology and the Ethics Committee of the Faculty of Medicine (No. 1322/VII-23, Date 07.07.2022).

Morphometric examination

Mastocytes density (MsD) was defined as the average number of immunostained mastocytes identified in microscopic fields of analyzed ganglionic tissue. The number of mastocytes was counted in three microscopic fields of the TG parts (ophthalmic, maxillary and mandibular) in 10 slices per TG at x400 magnification (objective lens 40× and ocular lens 10×), and in two microscopic areas of the SSCG parts (upper and lower). The arithmetic mean of the 20 fields of each of the 5 ganglions, measuring 341.7 μm × 250.0 μm of each size, with a corresponding area of 85425 μm^2 (0.085 mm^2) per field, was calculated for the number of mastocyte count.

Statistical analysis

For statistical analysis we used the IBM SPSS Statistics 25.0 statistical software package (SPSS, Inc., Chicago, IL, USA). We performed statistical procedures of descriptive statistics (median, mean) of the measured data, the Mann-Whitney test for independent samples and Kruskal-Wallis test with Bonferroni's correction. The normality of distribution was tested applying the Kolmogorov-Smirnov test. The probability level of $p<0.05$ was considered as a statistically significant difference.

RESULTS

The trigeminal ganglion (TG) is the largest sensory ganglion as a part of the trigeminal nerve. It is made up of pseudounipolar nerve cells. It can be subdivided into three segments in relation to the three branches that originate from the TG. Our study of the micromorphological description of mastocytes also showed the exact morphometric characteristics of tryptase-positive mastocytes in the TGs **(Figure 1A and Figure 1B)**. The mastocytes were oval in shape **(Figure 2)**. The results presented in **Table 1** clearly show that each microscopic area of the ophthalmic segment of the TG contained from 0 to 6 (median 1, mean 1.4) mastocytes **(Figure 2A)**. The maxillary segment of the TG showed from 0 to 5 Ms (median

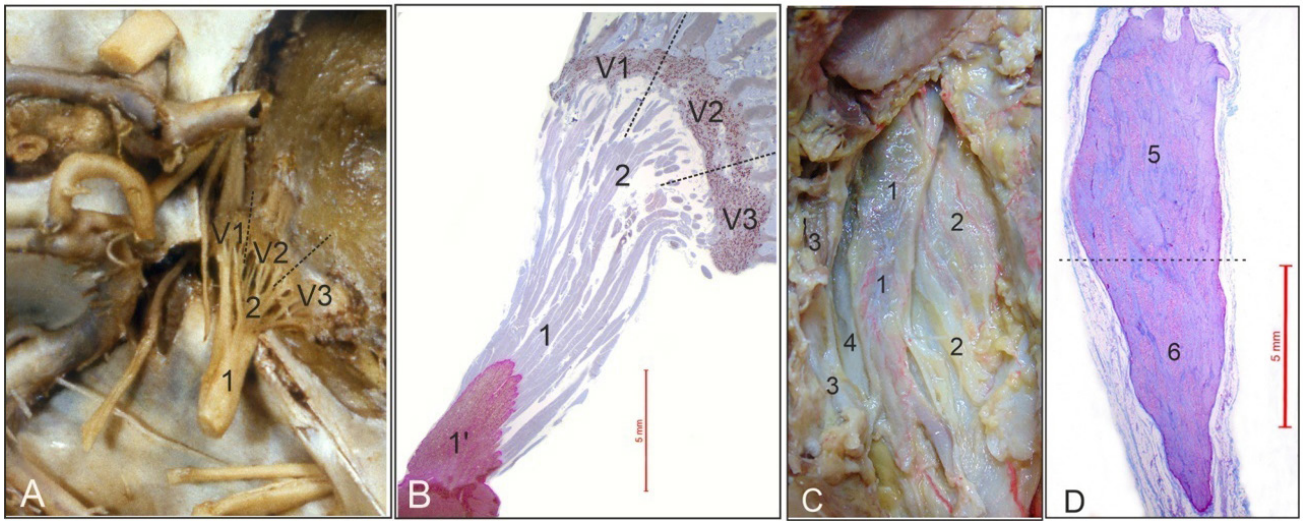

Figure 1. Microanatomical description of the trigeminal nerve (TN) and human trigeminal ganglion (TG) unite: **A** – anatomical dissection (view from above to the base of skull); **B** – histological TN-TG specimen stained with the Masson trichrome method. The intracranial part of TN (1), close to the pons composed of myelin originated from oligodendrocytes (1'). Intracaval plexiform part of TN (2) forms the TG, with ganglionic nerve cells in the ophthalmic (V1), maxillary (V2) and mandibular (V3) segments. Microanatomical description of the sympathetic superior cervical ganglion (SSCG): **C** – anatomical dissection (view from behind after removal of the vertebral column); **D** – SSCG section stained with the trichrome staining method of Masson. SSCG (1), posterior to the internal carotid artery (2), internal jugular vein (3) and vagus nerve (4). Upper (5) and lower (6) parts of the SSCG.

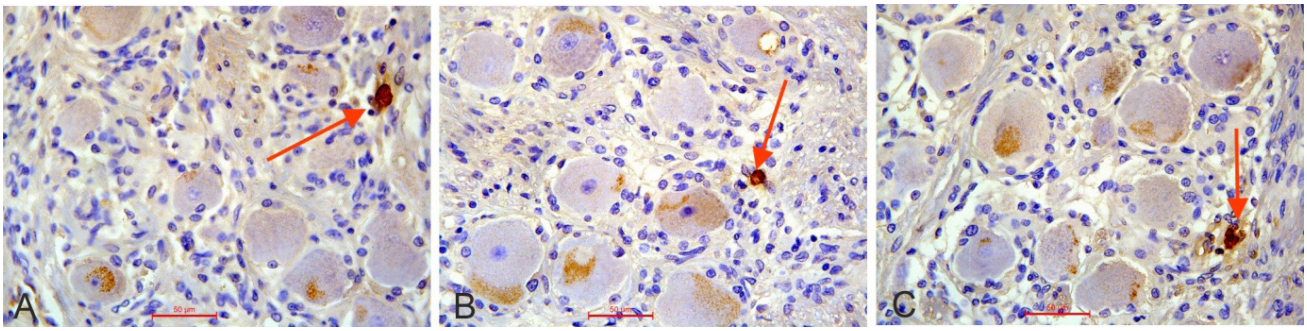

Figure 2. Nerve cells of T Ginophthalmic, V1 (**A**), maxillary, V2 (**B**) and mandibular, V3 (**C**) segments of the TG with satellite glial cells. Rare tryptase-positive mastocytes close to the nerve cells are labeled with arrows (mastocytes tryptase immunostaining, MsTryp).

1, mean 1.45) **(Table 1) (Figure 2B)**. The MsD presented in the mandibular segment had from 0 to 5 mastocytes per microscopic field of the TG (median 1, mean 1.5) **(Table 1) (Figure 2C)**. Using the Kruskal-Wallis test, we did not find a statistically significant difference in MsD between the three segments of the TG (p = 0.883) **(Figure 4)**.

Our sections showed the rare presence of the tryptase positive mastocytes (Ms) between the TN axons themselves, always near the longitudinal microvessels that accompany the nerve fibres. The numerous populations of Ms were identified in the subdural connective tissue surrounding the TG.

The sympathetic superior cervical ganglion (SSCG) is located deep in the lateropharyngeal space, posterior to internal jugular vein and the internal carotid artery. It contains multipolar sympathetic neurons. Topographically, it can be subdivided into an upper part, which sends axons to the internal carotid nerves, and a lower part, from which axons for external carotid nerves originate. Our analysis also included the exact morphometric characteristics of Ms in the SSCGs and surrounding area **(Figure 1C and Figure 1D)**. The tryptase-positive brownish mastocytes (Ms), oval in shape, had a different mean radius **(Figure 3)**. As shown in **Table 1** each microscopic area of the proximal segments of the SSCGs contained from 2 to 6 (median 4.5, mean 4.35) mastocytes **(Figure 3A and Figure 3B)**. The mastocytes density (MsD) of the distal segments of the SSCGs had from 3 to 6 (median 5, mean 4.45) Ms **(Table 1) (Figure 3C and Figure 3D)**. We did not find a statistically significant differences between the MsD groups in two segments of the SSCGs ($p = 0.899$) **(Figure 4)**.

Tryptase-positive mastocytes (Tryp+ Ms) were very often located among the nerve fibers of sympathetic nerves, usually close to microvessels, and in the perigan-glionic fibrous tissue.

Kruskal-Wallis test showed overall statistically significant differences in MsD between the three segments of trigeminal ganglions: ophthalmic, maxillary and mandibular, and two microscopic segments of the SSCG: proximal and distal ($p<0.001$) **(Table 1) (Figure 4)**.

DISCUSSION

The most common localization of mastocytes is in parts of the body that communicate with outer environment, such as mucosa of the digestive system, mucosa and submucosa of the respiratory system, brain, skin, hair follicles and dura mater (6). Some studies explain that mastocytes are connected to endothelial cells of blood vessels owing to the connections between their membranes and that they take part in forming the so-called perivascular environment (6). Our research demonstrated the homogenous and modest presence of Tryp+ Ms in all three parts of the TG. According to our research the mean number of

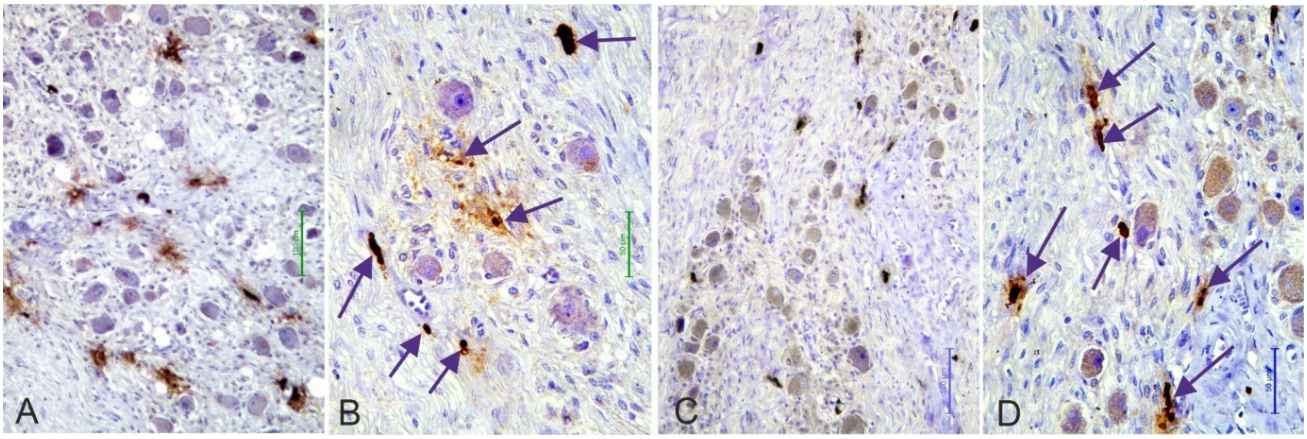

Figure 3. (**A**) Proximal segment of SSCG section and (**B**) a detail of closer view with tryptase-positive mastocytes (arrows) close to the ganglionic nerve cells (Ms tryptase immunostaining, MsTryp). (**C**) Distal segment of SSCG section and (**D**) a detail of closer view with tryptase-positive mastocytes (arrows) close to the ganglionic nerve cells (mastocytes tryptase immunostaining, MsTryp).

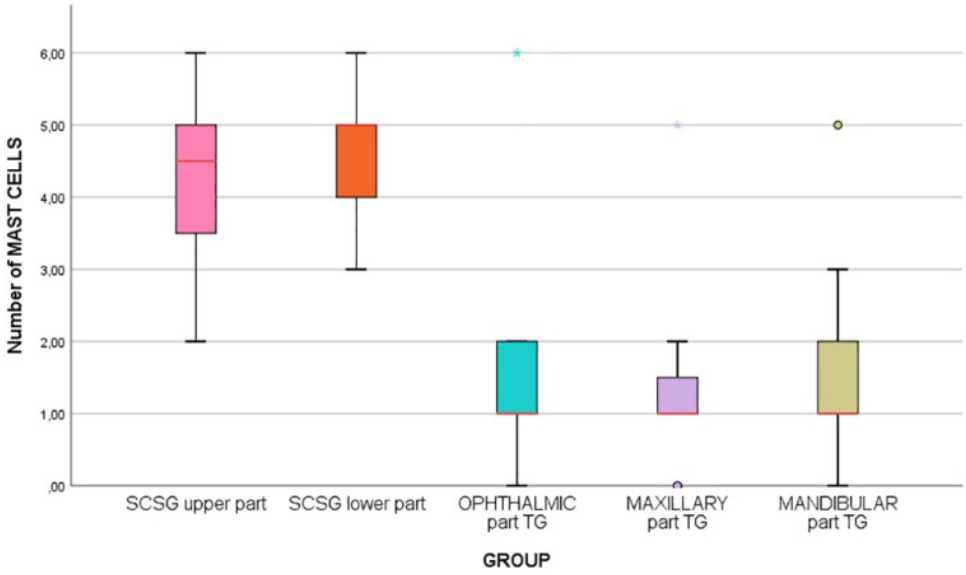

Figure 4. Comparison of mastocytes density (MsD) defined by MsTryp in microscopic areas of the SSCG parts: proximal and distal; no statistically significant differences were found between two groups ($p = 0.899$). Comparison of mastocytes density (MsD) in three segments of TGs: ophthalmic, maxillary and mandibular; no statistically significant differences were found among three segments ($p = 0.883$). Quantitative analysis revealed that MsD in two parts of the SSCG was significantly higher than MsD in three parts of TG ($p<0.001$), Kruskal-Wallis test and Bonferroni multiple comparison test.

Table 1. Mastocytes density (MsD) in three segments of the trigeminal ganglions (TGs): ophthalmic, maxillary and mandibular, and in two parts of the sympathetic superior cervical ganglions (SSCGs): proximal and distal.

Number of mastocytes/area		median (range) mean	p-value	p-value overall
Trigeminal ganglions	Ophthalmic segment	1 (0-6) 1.4	0.883	<0.001
	Maxillary segment	1 (0-5) 1.45		
	Mandibular segment	1 (0-5) 1.5		
Sympathetic superior cervical ganglions	Proximal part	4.5 (2-6) 4.35	0.899	
	Distal part	5 (3-6) 4.45		

Tryp+ Ms found in the three segments of TG was 1.4 in the ophthalmic, 1.45 in the maxillary and 1.5 in the mandibular segment. Our earlier preliminary study of microvascularization, also referred to MsD in TGs, identified, among other findings, very similar results to the current values: the mean number of Tryp+ Ms in TGs was 1.34 in the ophthalmic, 1.26 in the maxillary and 1.46 in the mandibular part (14). Reviewing the available literature, we did not find any similar studies reporting results on the number of Ms in the TGs. The findings of the present research indicated the homogenous presence of Tryp+ Ms in the proximal and distal segments of the SSCGs, and the MsD had a value of 4.35 and 4.45 respectively. Comparing our findings with the previous results, 4.5 and 4.7 of Tryp+ Ms per field, from an extended examination of micromorphological characteristics of sympathetic neurons in the SSCGs, we absolutely confirmed the values of MD found in the two examined parts of SSCGs (15). This original contribution to the scientific literature, with no similar results is the first description of the MsD in the SSCGs.

Our final comparison of higher average value of MsD in two parts of the SSCGs with the lower number of mastocytes identified in three parts of the TGs showed a statistically significant difference in MsD of Tryp+ Ms between TG and SSCG. There are rare attempts to explain the role of Ms in the two studied ganglions, as well as their influence on neurons function. The assumption is that the above results on the presence of Ms indicate that the release of stress hormones occurs in response to physiological stress, activating Ms, which leads to the excitation of ganglionic nerve cells in the SSCG (16). Sympathetic vasoconstriction of the brain arteries and arterioles is a consequence of stimulation of SSCG ganglionic neurons. Restriction of cerebral circulation and reduction of pressure in microvessels is a mechanism by which the brain is protected from increased intra-arterial pressure (17). Cerebral vasospasm as a consequence of

subarachnoid hemorrhage following aneurysm wall rupture is causally related to neuroinflammation, which may also have activation of Ms from the TG and SSCG as a potential factor in its occurrence (18, 19). The question is whether the number of Ms can have a specific effect on the stimulation and function of ganglion nerve cells in the SSCG. Larger number of Ms in the vicinity to the nerve fibers and nerve bodies, possibly change the function of neurons, and augment nerve damage, inflammation and nociception to induce hyperalgesia (6). Mastocytes have role both in peripheral and central sensitization (2). Neurogenic inflammation starts with peripheral nerve sensitization. Central sensitization is a state of continuous excitation of nociceptive neurons of the central nervous system (2). Mastocytes present in the dura mater, brain and ganglions may have direct interactions with these nociceptive neurons (2, 10). These interactions depend not only on the proximity of mast cells to nociceptive neurons and their pathways, but also on their complex interplay with surrounding glial cells—including satellite glial cells within ganglia (2). All of these factors trigger the release of substances from mastocytes and glial cells, thereby amplifying neuroinflammation (2). Some studies suggest that quite a small number of mastocytes in the brain is sufficient to cause neuroinflammation and for this reason mastocytes are thought of as pivotal participants of central sensitization process (2, 10).

Degranulation of mastocytes happens within minutes after they are stimulated (2, 4). Secretory granules loaded with proteases, cytokines, biogenic amines and many other mediators degranulate through the activation of FceR1 (2, 4). Activation of this high-affinity IgE receptor causes actin and microtubule cytoskeletal rearrangements. Microtubule polymerization facilitates the translocation of secretory granules to the plasma membrane (4). Activated FceR1 leads to the activation of Lyn and Syk, which help fusion of granules and membranes and lead to the degranulation of mastocytes (4). Degranulation of mastocytes is the first very important step in inflammation process. When mastocytes release their contents into the blood stream, clinical manifestations of these actions are visible. This investigation suggests that Ms are of great interest for further understanding of the patophysiology of headaches and pain conditions, together with microvessels, sensory nerve fibers and sympathetic neurons. Inhibition of Ms degranulation should be possible therapeutic treatment for these conditions (13, 20). Future research should aim to validate our findings on MD and compare them with the data on microvessel and ganglionic neuron density in the TGs and SSCGs in order to establish correlations between the obtained data.

CONCLUSION

This is one of the first analyses of micromorphological characteristics of human tryptase-positive mastocytes and mastocytes density (MsD) in three segments of the human trigeminal ganglions (TGs): ophthalmic, maxillary and mandibular, as well as in two parts: proximal and distal of the sympathetic superior cervical ganglions (SSCGs), showing very high statistically significant differences in MsD compared to the TG and SSCG. This micromorphological description of the human tryptase-positive mastocytes in the trigeminal ganglions and in the sympathetic superior cervical ganglions showed that these cells need particular attention in the therapeutic treatment.

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Author Contributions: Conception and study design: MĆ, AMil, JB, DL. Histological analysis, data collection and data analysis: MĆ, AMil, JB, AMir. Manuscript preparation and interpretation of the data: MS, NB, MM. Writing the manuscript: MĆ, JB, AMil, DL. Critical revision of the manuscript: MS, NB, AMir. Final approval of the manuscript before submission: MĆ, AMil, JB, MS, DL, NB, AMir, MM.

Ethical approval: This research protocol and examination have been approved by the authorities of the Institute of Histology and Embryology, the Institute of Anatomy, the Institute of Pathology and the Ethics Committee of the Faculty of Medicine (No. 1322/VII-23, Date 07.07.2022).

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MIKROMORFOLOŠKE KARAKTERISIKE MASTOCITA U TRIGEMINALNIM I HUMANIM SIMPATIČKIM GORNJIM VRATNIM GANGLIONIMA

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Sažetak

Uvod: Mastociti (Mt) se obično nalaze u blizini mikrosudova i završetaka nerava koji inervišu moždanice i unutrašnje organe. Granule Mt sadrže brojne medijatore koji utiču na nervne ćelije i modifikuju njihove reakcije.

Cilj rada: Naše proučavanje imalo je za cilj da se analizira prisustvo i distribucija mastocita (Mt), kao i gustina mastocita (GMt) u tri dela humanih trigeminalnih gangliona (TG): oftalmički, maksilarni i mandibularni, kao i u dva dela: proksimalni i distalni, humanih simpatičkih gornjih vratnih gangliona (SGVG), kao i da se postavi korelacija između proučavanih parametara.

Materijal i metode: Pet TG i pet SGVG odraslih osoba obrađeno je za ovu histohemijsku i imunohistohemijsku studiju. Preparati su serijski sečeni i obojeni hematoksilinom i eozinom i Masson trihrom metodom, a sledeći isečci su pripremljeni za imunohisthemijsko bojenje triptaze mastocita.

Rezultati: Prosečan broj triptaza-pozitivnih (Trip+) Mt u mikroskopskim poljima, GMt, bio je 1,4 u oftalmičkim delovima, 1,45 u maksilarnim delovima i 1.5 u mandibularnim delovima TG. Broj Trip+ Mt u mikroskopskim poljima bio je od 2 do 6 (Med = 4,5) u proksimalnim delovima i od 3 do 6 (Med = 5) u distalnim delovima SGVG. Distribucije Mt u tri dela humanih TG i dva dela SGVG bile su uniformne, bez specifičnih mikromorfoloških varijacija. Ovo je prva mikromorfološka analiza Trip+ Mt i GMt u humanimTG i SGVG.

Zaključak: Nismo pokazali statistički značajnu razliku između aritmetičkih sredina GMt u tri segmenta TG (p = 0.883). Takođe, nismo pokazali statistički značajnu razliku između grupa GMt u dva segmenta SGVG (p = 0.899). Postojala je statistički značajna razlika u GMt Trip+ Mt između TG i SGVG (p<0.001). GMt u dva dela SGVG bila je značajno veća nego GMt u tri dela TG.

Ključne reči: mikromorfološka analiza, mastociti, gustina mastocita, simpatički gornji vratni ganglion, trigeminalni ganglion.

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ORIGINAL ARTICLE

Reproductive challenges of endometrial polyps: the influence of women's age and associated risk factors

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Summary

Introduction / Aim: Endometrial polyps (EPs) are abnormal benign growths that originate from the endometrium. Risk factors associated with EPs are hormonal changes related to ageing, Tamoxifen use, hormone replacement therapy, overweight or obesity. The exact causal relationship between EPs and infertility remains unclear, but multiple mechanisms including mechanical obstruction and biochemical changes are proposed. The "gold standard" for diagnosing and treating EPs is hysteroscopy. This study aims to provide comprehensive understanding of the interplay between EP, age and infertility**.**

Material and methods: Our study included 301 women undergoing hysteroscopic polypectomy due to infertility treatment, which were divided into two groups based on their age (age ≤ 35 and age > 35). Data were collected from patients' medical histories and pathology reports. Study variables included infertility type, type 2 diabetes mellitus, insulin resistance, polycystic ovary syndrome, endometriosis, hypothyreosis, previous unsuccessful intrauterine insemination and *in vitro fertilization (IVF)*, menstrual cycle irregularities and abnormal uterine bleeding, polyp size and histopathology type.

Results: At least one out of five participants had insulin resistance, polycystic ovary syndrome and abnormal uterine bleeding. Endometriosis, insulin resistance and primary infertility were more common in the younger group then in the older group. Previous history of unsuccessful IVF cycles, hypothyreosis and secondary infertility were associated with older age.

Conclusion: The prevalence of EPs tends to increase with advanced women's age, period marked by natural decline in ovarian reserve, hormonal changes and changes in endometrial health. Addressing EPs through appropriate diagnostic and therapeutic measure and understanding the hormonal influence on EPs and EM is crucial for improving fertility outcomes.

Keywords: endometrial polyps, infertility, hysteroscopy

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INTRODUCTION

Endometrial polyps (EPs), also known as uterine polyps, are abnormal growths that originate from the endometrium. They consist of glands, stroma, and blood vessels. The size of EPs can range significantly, they can be as small as 5 mm, but they can also fulfill the entire uterine cavity. Based on their attachment to the uterine surface, there are two main types of EPs. If an EP is attached by a narrow-elongated pedicle, then it is known as pedunculated. However, if they have a large flat base, they are known as sessile. The gross morphological appearance of these polyps can vary, but they often present as smooth structures with a tan to yellow coloration. EPs are quite common among women, with estimates suggesting they affect about 25% of women. While EPs can occur at any age, they are statistically more prevalent in women aged 40 to 49 years, primarily due to hormonal changes and fluctuations that this age group tends to experience (1,2). Estrogen's role in stimulating endometrial growth and thickening each month makes it a significant factor in the development of EPs. Understanding and managing conditions that result in elevated estrogen levels are essential in minimizing the risk of polyp formation and ensuring reproductive health (3). Aside from hormonal changes related to aging, other risk factors associated with EPs have also been identified, such as Tamoxifen use and hormone replacement therapy (HRT). There is an increased risk of EPs in women who are overweight or obese as well. Higher levels of adipose tissue can lead to increased estrogen production, which may contribute to the development of polyps (3,4).

EPs are typically asymptomatic but they can manifest as abnormal uterine bleeding (AUB). The AUB include irregular menstrual periods (unpredictable timing and flow), unusually heavy flow during menstrual period, bleeding or spotting between periods, vaginal spotting or bleeding after menopause. AUB may be the primary symptom of EPs, occurring in more than a half of all women with the condition, and its incidence appears to increase with age (5,6).

Correlation between EPs and infertility is a special concern. While the exact causal relationship between EPs and infertility remains unclear, multiple mechanisms including mechanical obstruction and biochemical changes are proposed. EPs may induce an inflammatory response in the endometrium, which could disturb embryo implantation, similar to the effects seen with an intrauterine device. Also, EPs have been found to exhibit altered levels of matrix metalloproteinases (MMPs), cytokines like interferon gamma, and proteins such as glycodelin and placenta protein 14. These biochemical changes can interfere with sperm function, inhibit embryonic development, and affect the endometrial environment needed for successful implantation (1,7).

Hysteroscopy with guided biopsy not only serves as the definitive method for diagnosing EPs but also facilitates precise localization and allows for immediate therapeutic intervention through simultaneous resection. These advantages underscore its status as the "gold standard" in clinical practice for managing EPs. While there is still disagreement in the literature regarding the routine removal of EPs before attempting pregnancy (both natural and assisted), there is a tendency towards removing polyps before initiating assisted reproductive technologies (ART) such as intrauterine insemination (IUI) and in vitro fertilization (IVF). Therefore, the decision-making process should involve a thorough assessment of each patient's unique circumstances and preferences, guided by current evidence-based practices and clinical judgment (8).

This study aims to provide comprehensive understanding of the interplay between EP, age and infertility, ultimately guiding more effective diagnostic and therapeutic strategies to enhance reproductive success for women experiencing such challenges.

MATERIAL AND METHODS

Study design and participants

The study was performed in the Clinical Hospital Centre (CHC) "Dr Dragisa Misovic - Dedinje" in Belgrade, Serbia. It included 301 women undergoing hysteroscopic polypectomy due to infertility treatment. The age ranged from 24 to 44 (mean 35.91 ± 4.63). Patients were treated at the Hospital of Gynecology and Obstetrics and recruited from 2019 to 2024. The study was approved by the CHC's Institutional Review Board (No 18328/4-2024, Date: 29.08.2024).

Study participants were divided into two groups based on their age.

The first, younger group (age ≤ 35) consisted of 131 participants where age ranged from 24 to 35 (mean 31.50 ± 3.00). Second, older group (age > 35) included 170 participants, with age ranging from 36 to 44 (mean 39.31 ± 2.12).

Study inclusion and exclusion criteria

Study included women aged between 18 and 45 who were treated during a five year long time frame at the CHC "Dr Dragisa Misovic - Dedinje". Women older than 45 or younger than 18 were excluded from the study. All women included in the study were normal weight and had body mass index (BMI) of between 18.5 and 24.9 kg/m2. Obese women were excluded.

Study variables

Data on infertility type (primary or secondary), type 2 diabetes mellitus, insulin resistance, polycystic ovarian

syndrome, endometriosis, hypothyreosis, previous unsuccessful intrauterine insemination and in vitro fertilization, previous hysteroscopic polypectomy, menstrual cycle irregularities and abnormal uterine bleeding, were collected from patients' medical histories. Data on polyp size and histopathology type were collected from pathology reports.

Statistical analysis

Descriptive statistics are presented as frequencies and percentages for categorical data, and as mean, median, SD and range for continuous data. The descriptives were presented for the total sample, and for the younger and older group of participants. To test the relationship between age groups and other relevant variables in our study, we used Fisher's Exact Test for categorical data and Mann-Whitney U test for continuous data. A significance level of $p < 0.05$ was set. Statistical analysis was conducted using IBM SPSS version 23.0.

RESULTS

The characteristics and prevalence of comorbidities, previous ART and hysteroscopy procedures and menstrual cycle disorders in the sample

In order to describe the characteristics of the age groups regarding significant variables related to infertility, we calculated the number of conditions within two groups and in the total sample. In order to examine the relationship between age groups and significant variables, we performed Fisher's Exact test. The results including frequencies, percentages and p values (two-tailed) are displayed in **Table 1**.

Looking at the results for the whole sample presented in **Table 1**, we can observe that at least one out of five participants have insulin resistance, PCOS and abnormal uterine bleeding – and those were the most common conditions. The majority of the sample (85.4%) had a regular menstrual cycle.

Inspecting the differences between the age group, we can see that in the younger group (age ≤ 35), the more common conditions are endometriosis ($p = 0.011$), insulin resistance ($p = 0.021$) and primary infertility ($p = 0.017$). The prevalence for endometriosis in the younger group was 8.4% compared to the 1.4% in the older group. Insulin resistance was present in 30.5% of cases in the younger group, compared to the 18% in the older group. Regarding primary infertility, there were 90.1% diagnosed participants in the younger group, compared to the 79.4% in the older group.

In the older group there was a significantly higher number of cases with hypothyroidism ($p = 0.014$), previous unsuccessful IVF ($p < 0.001$) and secondary infertility ($p = 0.017$). Every fourth patient had hypothyroidism (25.3%) in the older group compared to every sixth patient (13.7%) in the younger group. The prevalence of previous unsuccessful IVF was 18.8% in the older group compared to the 4.6% in the younger group. Regarding secondary infertility there were 20.6 % diagnosed women in the older group compared to the 9.9% in the younger group.

The characteristic polyp size and histopathology type in the sample

The size of EPs can vary significantly; they can be as small as 5mm, but they can also fulfill the entire uterine cavity. EPs are usually benign, but in some cases, they can be associated with a risk of malignancy, especially in postmenopausal women or in those with abnormal bleeding

Table 1. Frequencies, percentages of participants with endometriosis, insulin resistance, PCOS, hypothyroidism, unsuccessful IUI and IVF, previous hysteroscopic polypectomy, regular menstrual cycle, abnormal uterine bleeding withing total sample and subsamples and statistical significance (p value, two tailed) of Fisher's Exact test.

	total sample (n = 301)	**age ≤ 35 (n = 131)**	**age > 35 (n = 170)**	**p**
	N. yes (%)	N. yes (%)	N. yes (%)	
endometriosis	14 (4.7)	11 (8.4)	3 (1.8)	0.011
insulin resistance	72 (23.9)	40 (30.5)	32 (18.8)	0.021
PCOS	73 (24.3)	38 (29.0)	35 (20.6)	0.104
hypothyroidism	61 (20.3)	18 (13.7)	43 (25.3)	0.014
IUI	3 (1.0)	3 (2.3)	0 (0)	0.081
IVF	38 (12.6)	6 (4.6)	32 (18.8)	<0.001
previous hysteroscopic polypectomy	48 (15.9)	16 (12.2)	32 (18.8)	0.153
regular menstrual cycle	257 (85.4)	115 (87.8)	142 (83.5)	0.327
abnormal uterine bleeding	79 (26.2)	33 (25.2)	46 (27.1)	0.792
primary infertility	252 (84.1)	118 (90.1)	135 (79.4)	0.017
secondary infertility	48 (15.9)	13 (9.9)	35 (20.6)	0.017

Note: PCOS – polycystic ovarian syndrome; IUI – unsuccessful intrauterine insemination; IVF – unsuccessful in vitro fertilization

patterns. In the total sample the size of the polyp ranged from 2 mm to 50 mm (M = 12.88; SD = 6.57; Median = 10). We used Mann-Whitney U test to discover whether there was a significant difference in average size between the age groups. There were no significant differences (p = .813) between the Mean Rank in the younger group (149.67) compared to the older group (152.03).

We also checked the histopathology type and compared the groups. In the total sample in 96.7% of cases it was confirmed polyp, and in 3.3% cases other diagnosis. In the younger group the prevalence of other diagnosis was 3.1% whereas in the older group was 3.5%. The results of Fisher's Exact test showed that these differences were not statistically significant (p = 1.000).

Association Between Endometriosis and Type of Infertility

To investigate the potential association between endometriosis prevalence and the type of infertility (primary vs. secondary), we performed Fisher's Exact Test. The prevalence of endometriosis was 5.1% among patients with primary infertility and 2.1% among those with secondary infertility. However, this difference was not statistically significant (p = 0.706).

DISCUSSION

Our study included 301 women dealing with infertility, who were diagnosed with EPs. Subjects were divided in two subgroups, based on age, which was defined for the purpose of this study as older or younger than 35. A total of 12 variables related to EP and infertility treatment were assessed, but statistical relevance was found for only two parameters, concomitant presence of endometriosis and the history of previous IVF failure.

The age for the diagnosis of type 2 diabetes mellitus (T2DM) has decreased over the last few decades (9). Guidelines specifically created for this group of patients are still lacking, and recommendations are primarily extrapolated from the evidence in older people (10). However, only three cases of T2DM were observed in the group of women who were older than 35, and there were no participants with T2DM among participants in the first group (35 or younger) so therefore statistical comparison between the two groups was not performed.

Women's fertility starts to decline after the age of 30, and after the age of 35 the fertility decline rate accelerates. The underlying etiology is associated with the oocyte number and quality depletion, and this is the primary reason why women over 35 are more commonly seeking fertility counselling and are more commonly exposed to the methods of assisted reproduction (11). The highest number of ART procedures in the United States (US) are actually carried out among women aged between 35 and 40. Centers for Disease Control and Prevention (CDC) reported the average age of women undergoing ART procedures to be 36.2 years. Women aged between 35 and 40 represent 43.3% of all women undergoing ART procedures in the US (12).

Advanced women's age is a well-known risk factor for prolonged time to conception. Without involvement of ART techniques before the age of 30 women have approximately 85% chance to conceive within one year, while after the age of 35 these chances drop to 66%, and go further down reaching a probability of 44% by the age of 40. Unfortunately, reproductive challenges at this age are not limited only to the conception difficulties. At this age women deal with other burdens of unfavorable pregnancy outcomes as well, such as a miscarriage. More than a quarter of all clinical pregnancies terminate as a miscarriage at the age of 40, compared to 16% at the age of 30 (11,13).

Our results are in unison with the fact that advanced women's age is associated with higher number of unsuccessful IVF cycles (14), and this is the most probable explanation why women over 35 in our study did undergo IVF procedures more often compared to the group of women who were 35 years or younger.

Our study showed higher prevalence of endometriosis in younger patients with EP. Both EP and endometriosis are conditions related to the uterine lining, but they are distinct in their nature and presentation. While EP are growths that occur in the endometrium, endometriosis is a condition where endometrial tissue grows outside the uterus, causing pain, irregular bleeding and fertility issues. Even though EP and endometriosis are two different conditions, there's often an overlap in clinical presentation and possibly in the pathophysiological mechanism leading to it. Although the exact pathophysiological mechanism is not completely understood yet, several research groups have reported increased prevalence of EP in women with endometriosis dealing with infertility simultaneously (15,16).

It is known that adhesions associated with anatomical disturbances are the leading mechanism resulting in infertility in endometriosis patients, but the importance of endocrine and immunological disturbances should not be neglected either, especially since the exact pathophysiological mechanism explaining endometriosis is so far not fully understood (17).

Endometriosis and EP are influenced by hormonal changes, particularly those related to estrogen, but they do so in different manners. Estrogen stimulates the growth of the endometrial lining resulting in the development of EP, and estrogen fluctuations affect the growth of EP in a more complex manner. Estrogen receptor pattern and aromatase activity expression are altered in both EP and endometriosis patients, and estrogen fluctuation levels are the probable intrinsic factor linking EP, endometriosis and infertility (18-20). Furthermore, vascular changes associated with Endometriosis could be

in relationship with the evolution of the EP vessel's axis as well. Machado et al. have found that levels of inflammatory markers in endometrium, such as vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMP) and angiogenesis factors 1 and 2, are higher in patients with EP who suffer from endometriosis at the same time (21). Additionally, Wang et al. have reported significant association between endometriosis and EP in their research and emphasized that all patients dealing with endometriosis should be carefully evaluated for the concomitant presence of EP, regardless of the ultrasonography findings (22).

Unlike EP which have a peak incidence between the age of 40 and 49 (23), endometriosis typically affects women during their 30s. Even though between 10 and 15% of all women of reproductive age are affected by endometriosis, and despite all the improvements and progress in technology and diagnostics, there is still a shocking average delay of nearly seven years in establishing endometriosis diagnosis (24).

Estrogen levels reach their peak during women's late 20s. By the age of 50 there is a 50% decline in estrogen levels compared to their peak levels, with further dramatical decrease during and after perimenopause (25). Endometriosis is known to be highly dependent on estrogen, but it is known that even if estrogen level would decline, endometriosis symptoms would not necessarily decrease (26). In this context, younger age, 35 or less, is beyond doubt related with higher estrogen levels and consequently with higher endometriosis incidence, and our result supports this interdependence.

Endometriosis affects women's quality of life in a similar manner as Crohn's disease, T2DM and rheumatoid arthritis, and despite a significant percentage of women of reproductive age living with it, therapeutic approaches for endometriosis are still limited exclusively to the management of symptoms caused by endometriosis. In fact, treatment options are oriented only to infertility or pelvic pain treatment, without any targeted therapy designated for the treatment of endometriosis (27). Endometriosis is defined as an inherited, autoimmune, long-life diseases, which has to be treated from the time of diagnosis, continuously with the individually tailored treatment according to patients' overall status, age and complaints, in order to control the pain and further dissemination (28). Therefore, associations between endometriosis and EP, as well as the role of estrogen and its receptors, must be demystified, and more comprehensive and causal treatment offered to patients affected by it.

CONCLUSION

The prevalence of EPs tends to increase with advanced women's age, a period marked by natural decline in ovarian reserve, hormonal changes and changes in endometrial health. Addressing EPs through appropriate diagnostic and therapeutic measures and understanding the hormonal influence on EPs and EM is crucial for improving fertility outcomes. Ongoing research and clinical practice must continue to focus on optimizing treatment strategies to address EP pathology, ultimately supporting better reproductive outcomes for women across various age groups. The interplay between EPs, age and infertility underscores the importance of comprehensive reproductive evaluation and tailored interventions to enhance fertility perspectives and support women towards their journey to better reproductive outcomes.

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REPRODUKTIVNI IZAZOVI KOD ŽENA SA ENDOMETRIJALNIM POLIPIMA: UTICAJ STAROSTI I POVEZANIH FAKTORA RIZIKA

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Sažetak

Uvod / Cilj: Endometrijalni polipi (EP) predstavljaju benigne tumore poreklom iz endometrijuma. Najčešći faktori rizika koji dovode do pojave EP su hormonske promene povezane sa starenjem, upotreba tamoksifena, supstituciona hormonska terapija, prekomerna težina i gojaznost. Tačna uzročna veza između EP-a i infertiliteta ostaje nejasna, ali najčešće predlagani patofiziološki mehanizmi jesu mehaničke prepreke i biohemijske promene na nivou endometrijalnog tkiva. „Zlatni standard" dijagnostike i lečenja EP je histeroskopija. Cilj ove studije je da se ispita povezanost godina starosti, endometrijalnih polipa i infertiliteta. **Materijal i metode:** Istraživanje je obuhvatilo 301 pacijentkinju podvrgnutu histeroskopskoj polipektomiji radi lečenja infertiliteta, koje su podeljene u dve grupe na osnovu starosti (starost ≤ 35 i starost > 35 godina). Podaci su prikupljeni iz istorija bolesti i histopatoloških izveštaja, i analizirane su sledeće varijable: tip infertiliteta, prisustvo insulinske rezistencije, sindroma policističnih jajnika, endometrioze, hipotireoze, prethodni neuspešni pokušaji intrauterine inseminacije i vantelesne oplodnje (*IVF*), nepravilnosti menstrualnog ciklusa i abnormalno krvarenje iz materice, veličina polipa i histopatološki tip. **Rezultati**: Najmanje jedna od pet ispitanica imala je insulinsku rezistenciju, sindrom policističnih jajnika i abnormalno krvarenje iz materice. Endometrioza, insulinska rezistencija i primarni infertilitet bili su češći u mlađoj grupi nego u starijoj. Prethodna istorija neuspešnih *IVF* ciklusa, hipotireoza i sekundarni infertilitet bili su povezani sa uznapredovalim godinama.

Zaključak: Prevalencija EP ima tendenciju rasta sa godinama starosti žene, periodom koji je obeležen prirodnim padom rezerve jajnika, hormonskim promenama, kao i promenama u strukturi endometrijuma. Primena odgovarajućih dijagnostičkih i terapijskih mera, kao i razumevanje uticaja godina, hormonskog statusa i povezanosti EP i endometrioze, ključni su u lečenju EP, kao i u sveukupnom poboljšanju ishoda lečenja infertiliteta.

Ključne reči: endometrijalni polip, infertilitet, histeroskopija

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REVIEW ARTICLE

Emotion dysregulation and trauma in youth: a perpetuum mobile

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Summary

Both emotion dysregulation (ED) and traumatic experiences, especially interpersonalnal trauma in childhood, are being increasingly recognized as transdiagnostic risk factors for mental disorders during developmental age as well as later in life. The aim of this review is to explore the existing data regarding the potentially bidirectional relationship between ED and trauma, focusing on the way they could maintain each other to create a "perpetuum mobile" that shapes the mental health outcomes of youth. There is relatively consistent evidence that developmental traumatic experiences, especially child maltreatment, have adverse effects on ED, through various psychological mechanisms (such as social learning, interpretation of social cues, attachment, active invalidation of their emotional experiences) as well as neurobiological mechanisms (such as structural and functional abnormalities of the emotion-regulation-related neural areas caused by aberrant stimulation, stress-response system hyperactivity, and involvement of other physiological systems). In turn, certain evidence indicate that ED may maintain and worsen trauma by impairing trauma processing and eliciting trauma-related symptoms and conditions, as well as by inducing new interpersonal traumatic exposures via factors such as shame, mental disorders and proneness to aggressive behaviors. This potential "perpetuum mobile" could be active not only during a person's lifetime, but possibly spread through generations via different mechanisms. More research is needed to explore the nature of this complex relationship, and especially the ways ED in youth sets the ground for further maintenance of trauma. It is challenging to empirically conclude what the true direction of this relationship in different studies is, implying the need for methodological rigor in future investigations. Practical implications refer to all levels of prevention when it comes to both youth exposure to trauma and ED, but also to stopping the potential "perpetuum mobile", by preventive actions in both directions.

Keywords: developmental trauma, emotion dysregulation, interplay, adolescents, youth

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INTRODUCTION

The developmental age is a turbulent period comprising dramatic physical, cognitive, emotional, and social transformations (1). The interplay between biological aspects of maturation and the dynamics of environmental experiences results in vulnerability to the mental health difficulties (Mastorci, 2024). It is approximated that among all lifetime mental disorders, 50% begin by the age of 14, and 75% by the age of 24 (2,3), with the highest prevalence for depression, anxiety, and externalizing disorders (4). Mental disorders in youth may be associated with significant adverse consequences. They affect the current functioning as well as the development itself (for review see in (5)), increasing the risk for mental disorders in adult life (6). Mental health in youth shows dynamic course with often unclear or overlapping presentations of mental problems. Mental disorders at this age may be difficult to distinguish from common variations in developmental changes (7), while various psychiatric disorders can have varying developmental trajectories such as remission, diagnostic shift to another disorder, or comorbidity (8). This dynamic nature and low stability refers especially to disorders other than neurodevelopmental (8). Therefore, the transdiagnostic perspective on mental health disorders in warranted, in order to identify which pathological processes cut across different disorders (9). In terms of practical implications, such transdiagnostic factor could be a target of interventions aimed to address multiple diagnostic representations.

Emotion regulation (ER) has been proposed as one of the promising transdiagnostic factors, implied in the development and maintenance of multiple psychopathological outcomes in youth (10). It is defined as the process by which individuals influence their emotions, as well as their own experience of their emotions (11). It involves modifying various components of emotions, such as their intensity, duration and expression, in order to adapt to situational demands (11). *Emotion dysregulation (ED)* occurs when individuals experience difficulties in restoring their emotional balance and therefore are involved in prolonged, intense emotional states (12). ED is associated with a wide array of internalizing and externalizing mental health problems and psychiatric conditions in developmental period and in adulthood (13, 14). It has been indicated that mental health outcomes may be dependent on the type of ER strategies a person predominantly uses. The adaptive strategies such as cognitive reappraisal have been linked to lower levels of psychological distress and better emotional resilience (11), whereas the maladaptive strategies, such as rumination or suppression, are associated with increased risks of anxiety, depression, and other psychological disorders. It is suggested that an important role is played by the experiential avoidance, a response pattern that refers to the general unwillingness to remain in contact with particular private experiences that acts as a functional dimension through the use of maladaptive avoidance strategies (15). The effectiveness of the ER can also reflect on the long-term well-being, through affecting social relationships, career success, and overall quality of life, with those who excel in ER tending to form healthier interpersonal relationships and manage work-related stress more effectively (11), demonstrating better classroom behaviors and achieving stronger academic outcomes, while early disruptions in ER may contribute to academic challenges, particularly among children exposed to early adversities (14,16). Emotion dysregulation has also been associated with physical health outcomes. Inability to efficiently regulate emotions may result in chronic stress, negatively affecting the immune system, cardiovascular health, and overall physical well-being. In reverse, effective ER may have a buffering role in stress-related physiological responses, promoting superior health outcomes (11).

Traumatic experiences are increasingly being recognized as a transdiagnostic risk factor for mental disorders (17,18). They refer to experiences that have a high potential for harm (19), and resulting in toxic stress. Trauma exposure has a particularly detrimental effect when it happens early in life due to heightened brain plasticity which occurs in childhood and adolescence (20). It has been shown that the individuals who have experienced psychological trauma are at a nearly threefold higher risk of developing mental health disorders compared to those without such experiences, such as anxiety disorders, mood disorders, psychotic disorders and personality disorders (18). Child maltreatment represents a specifically detrimental traumatic risk factors associated with a variety of different mental health dysfunctions and disorders (21). The individuals who were maltreated in childhood show higher odds of hospital admissions for psychiatris disorders by the age of 30, including schizophrenia, bipolar disorder, depression, anxiety and PTSD (22). Developmental trauma such as child maltreatment has been associated with various mechanisms through which it adversely affects mental development and health outcomes. Among psychological mechanisms, child maltreatment has been associated with impaired development of secure attachment, mentalization, social and cognitive learning, etc (for review see in (21)). Important neurobiological mechanisms refer to the neural structures important for socio-emotional processes being affected by the abberant stimulation resulting from child maltreatment experiences, by chronic sensitization of stress-response system, of immunological system (23), and other systems such as oxytocin (for review see in (21)). The dysfunctional activity of some of the aforementioned physiological systems has also been associated with various physical health consequences of child maltreatment (for review see in (21)). Distressing and conflictuous relationships with parents have shown to predict mental disorders such as depression and anxiety even in emerging adulthood (24).

In addition to exploring some of the key transdiagnostic factors, it is of importance to take *their interplay* into account. Indeed, it is suggested that integrating the role of context into ER research is crucial, since empirical work has not fully embraced this perspective, leaving significant gaps in our understanding—particularly regarding its connection to trauma and subsequent psychopathology (25). On one hand, as mentioned earlier, child maltreatment has repeatedly shown adverse effects on the mental health and development, which could lead to ED, suggesting ED as an adverse consequence of traumatic experiences. On the other hand, ED is a concept with dynamic course through development, and could possibly, vice versa, give context to the experience and further processing of trauma, as well as increase risk of further exposure to traumatic experiences. While the effects of developmental trauma on ED are considerably supported by research, the data is scarce on the reverse direction of this relationship.

This narrative review aims to address this question by exploring various findings on the current understanding of the relationship between emotion dysregulation and traumatic experiences, in the way they could maintain each other over time to jointly create a "perpetuum mobile" that shapes the mental health trajectory of children and adolescents.

EMOTION DYSREGULATION AS A CONSEQUENCE OF TRAUMATIC EXPERIENCE

Emotions can be regulated through ER mechanisms corresponding to each phase of the emotion generation process. A person can decide which situations to approach or avoid to influence potential emotional outcomes (*situation selection),* change the aspects of a situation to alter its emotional impact (*situation modification),* direct attention toward or away from emotional triggers (*attentional deployment),* reappraise or reinterpret a situation to change its emotional meaning (*cognitive change),* and finally, one can influence behavioral, experiential, or physiological responses to an emotion after it has been fully generated (*response modulation*) (11). The development of adaptive ER abilities is facilitated by the interaction of multiple skills such as the awareness of emotions, the ability to identify and label emotions, to interpret emotion-related body sensations correctly, to understand the prompts of emotions, to support oneself in distress, furthermore the ability to actively modify negative emotions to feel better, to accept emotions, to be resilient and to tolerate negative emotions, and the ability to confront emotionally distressing situations in order to attain important goals (for review see in (26)).

How does ER develop?

The ER develops across the lifespan, being affected by biological maturation, social experiences and environmental factors. Multiple subsystems interact and influence the emotional development, including caregiver–child, co-parental, and sibling relationships. The development of ER includes several milestones. In infancy and early childhood, a child relies heavily on caregivers for external regulation of emotions, such as soothing when distressed. As children grow, they start developing internal regulation skills, such as distraction or simple cognitive strategies, often modeled and supported by caregivers and social interactions. In middle childhood and adolescence, more sophisticated ER strategies develop under the influence of cognitive and social development, such as cognitive reappraisal. In adolescence, ER is faced with the increased emotional challenges resulting from hormonal changes, experiences with peers, and identity formation. When an individual reaches full adulthood, with various life experiences and having an opportunity for refining cognitive strategies, the ER shows greater stability and adaptability. Older adults may use selective attentional deployment, focusing more on positive aspects of situations to maintain emotional well-being (11). The ER strategies can be seen as extrinsic, when one's emotion regulation is helped by external factors (other individuals), such as a caregiver soothing a distressed child; and intrinsic, when individuals regulate their own emotions to meet personal or external demands (11). From infancy to adulthood, therefore, the ER develops from more extrinsic, to more intrinsic (27).

Early trauma - when ER development goes wrong

Throughout childhood, adolescence, and transitioning to adulthood, the development of patterns for regulating emotions may be disrupted, by both innate vulnerabilities and adverse experiences, resulting in emotional imbalance and increased risk for mental health problems (11,14). Early exposure to traumatic events, and especially the interpersonal trauma in childhood such as child maltreatment, has been related to various conditions with emotional dysregulation as a core feature that could underlie this risk (28). Existing literature gives considerable support to the association between child maltreatment and ED throughout lifetime (29-37), including using fewer ER strategies, less acceptance of one's emotions and less emotion clarity, as well as a greater use of maladaptive strategies such as suppression and rumination, mediating general psychopathology risk (38). There are different hypotheses on the underlying mechanisms of the relationship between early trauma and EDR, both psychological and biological. Some of them will be presented in the following text.

Psychological mechanisms

A meta-analytic review on effects of maltreatment on coping and ER in childhood and adolescence (32) confirmed association between child maltreatment and poor ER, as well as increased avoidance, emotional suppression, and expression of negative emotions in response to stress. Defects in acquiring ER strategies and coping against the psychosocial problem development were associated with the decreased exposure to healthy models of ER and coping, and increased exposure to the examples of maladaptive stress response such as aggressive behavior (39). This implies the importance of the *social learning mechanism* (for review see in (21)). In case of child abuse, parents model maladaptive strategies in emotionally challenging situations, resulting in children possibly using similar problematic strategies, while in case of neglect, parents fail to provide the ER modeling at all, leaving neglected children with poorer understanding of emotions and with poor repertoire of ER strategies (40). It is noted that early-life maltreatment may actually create a double effect in terms that children experience the stressors that are far beyond their developmental ability to cope, while at the same time lacking the input of adequate stimuli of modelling appropriate responses to stress (41). This could result in children reaching out to the maladaptive strategies that may bring short-term relief but may increase the psychosocial risk in the long run, when the children apply them in normative situations of lower threat (41). Eventually, the situations of such developmentally challenging stressor, result in youth not only failing to acquire adaptive ER strategies, but learning the maladaptive strategies as well, imposing the increased risk of further psychosocial problems. This is in line with the finding showing that coping and ER may act as important mediators in the relationship between child maltreatment and the psychopathology (42). Studies show that deficits in caregiver responsiveness may inflict the development in interpersonal communication and *interpretation of social cues*. The data point out that maltreated children, independently of the type of maltreatment, have delays or deficits understanding and regulating emotions, and that they anticipate negative reactions if they show sadness and anger (28). This may be in close relationship with the development of *secure attachment*, which is regarded as essential for developing adaptive ER. Attachment (43) refers to an internal representation of the self and others in significant interpersonal relationships, starting with the attachment patterns with the caregivers and representing a "blueprint" for interpersonal relationships throughout life. Studies have found that maltreated children have disorganized attachment and negative internal representations of the caregiver, while also having ED and decreased social competence. In response to angry adult interactions simulation, maltreated children significantly more manifested problems with ER, associated with anxious and depressed symptoms as well as behavioral problems (44). Although all types of childhood maltreatment experiences fail to provide benevolent interpersonal environment, some data show that emotional maltreatment may be specific due to the experience of chronic active *invalidation of their emotional experiences*, which can lead to poor ER strategies later in life, especially emotional inhibition or avoidance (for review see (45)).

Biological mechanisms

It is suggested that, especially after repeated childhood trauma, the disruption of ER skill acquisition may result from the neurobiological effects of maltreatment (46). These mechanisms include molecular modifications of the stress response systems, further affecting neurogenesis, myelination, neuronal morphology, and synaptogenesis in brain regions important for ER. This results in attenuated structural development of the hippocampus, amygdala, left neocortex and corpus callosum as well as functional changes in the development of the left hemisphere, decreased integration between the hemispheres, increased limbic irritability, and decreased functional activity of the cerebellar vermis (for review see in (21,28)). The aforementioned neural structures, important for socio-emotional processing, emotion regulation and executive functions (the future "social brain") are the structures with extensive postnatal development and at the same time, the structures that are most sensitive to the effects of stress due to the high density of glucocorticoid receptors. Therefore, these areas are adversely affected by both aberrant neurostimulation resulting from child maltreatment, and by the toxic effects of cortisol, mostly mediated by glutamate (for review see in (21)). Other mechanisms that could affect these structures may be related to the possible sensitization of the immune system by child maltreatment, building a chronic low-grade inflammation and resulting in inflammatory cytokines crossing the blood– brain barrier; additionally, the development of the key structures could be affected by lower levels of oxytocin as a consequence of child maltreatment (for review see in (21)).

EMOTION DYSREGULATION AS A CONTRIBUTOR TO FURTHER TRAUMA

Processing trauma and contributing to trauma-related symptoms

While there is a number of findings confirming and exploring EDR as a consequence of developmental trauma, the data on the opposite direction of this relationship is insufficient. Most of the findings relate to the EDR effects on the processing of trauma and further development of

trauma-related conditions such as posttraumatic stress disorder (PTSD) and other symptoms and conditions (45,47-50). It is shown that posttraumatic EDR acts as a predictor of PTSD after trauma exposure (50). Posttraumatic symptoms were associated with the *lack of acceptance of emotional experiences, lack of emotional clarity, limited access to strategies and difficulties in goal-directed behavior and abstaining from impulsive behaviors while being upset* (51). Other findings further indicate that different facets of EDR predict different trauma-related conditions. Lack of emotional clarity predicted PTSD symptoms, speaking in favor of a potential mechanism inducing worsening symptoms. On the other hand, lack of regulation strategies, has been suggested as a marker of helplessness in post-traumatic depression (47). ED after traumatic experience has been shown to associate with the *ability to recover from posttraumatic symptoms*, even after controlling for the effects of symptoms (48), and it acted as a mediator between PTSD symptomatology and *dissociation symptoms* (52). The manifestations of EDR may, in fact, be seen as k*ey substrate of the complex trauma* since they represent the core features of complex PTSD in ICD-11 (53), and can also be closely related to the manifestations of borderline personality disorder (BPD) (54) and of developmental trauma disorder that was proposed but not included in DSM-V (55).

Interpersonal dysfunctions as a source of new trauma exposures

ER is a precondition of adequately navigating emotional responses in interpersonal communication. Therefore, ED may be associated to interpersonal dysfunctions and consequent exposure to stimuli that induce toxic stress. In addition to the aforementioned association of *insecure attachment* and interpersonal distress, it has been shown that ER is predictive of *shame and guilt proneness*, with higher use of maladaptive strategies and lower use of adaptive strategies being associated with shame-proneness, while the opposite pattern being associated with guilt-proneness. Shame and guilt are both negatively valenced emotions induced in similar situations, but shame has shown to be more detrimental since it includes in inferior and worthless views of self, and a tendency to hide or escape, and is predictive of various mental health conditions (for review see in (56)) potentially affecting interpersonal relationships. It is shown that EDR may act as a mediator between traumatic experiences in childhood and various *mental health problems*. This goes for early traumatic exposures, but even later in development, during emerging adulthood (18-24 years of age) it has been found that, for example, distressing relationships among youth and parents have predicted the occurrence and the severity of depression though maladaptive ER strategy as a mediator (57). Mental disorders may be associated with daily dysfunctionality as well as stigma, which could expose youth to distressing interpersonal situations. *Proneness to aggression* associated with ED, as found in certain studies (58,59), could be another mechanism contributing to interpersonal dysfunctiong leading to possible trauma exposure to both the person with ED and the person aggression is aimed at. As a neurobiological correlate, the altered thickness in superior frontal gyrus mediated the association between ED and physical aggression (60). Furthermore, as already mentioned EDR has been one of the core features in *BPD*, marked by both aggressive behaviors and interpersonal stress in the affected person. One of the consequences of ED being associated to aggression proneness, could be related to *intergenerational child maltreatment*, where ED has been hypothesized as one of the mechanisms between the child maltreatment history, and parental aggressive behavior towards child in the next generation (for review see in (21,61)). The intergenerational effects could also be mediated by the epigenetic markers of stress-response system dysregulation, found even in fetuses of parents who were child abuse victims (for review see in (21)).

CONCLUSION: MAINTAINING (AND STOPPING) A PERPETUUM MOBILE

"Perpetuum mobile" is a machine that moves endlessly, so that it produces more energy than was invested in it (62). Despite attempts, such a machine does not exist in the world of physics yet, as it defies the first and second law of thermodynamics. However, in the realm of youth mental health problems, could ED and trauma make each other survive through time, as a true perpetuum mobile?

Studies consistently indicate the adverse effects of developmental traumatic experiences, especially child maltreatment, on emotion dysregulation in adolescence and adulthood, through various psychological mechanisms (such as social learning, interpretation of social cues, attachment, active invalidation of their emotional experiences) as well as neurobiological mechanisms (such as structural and functional abnormalities of the ER-related neural areas caused by aberrant stimulation, stress-response system hyperactivity, and involvement of other physiological systems). In turn, some evidence indicate that emotion dysregulation may maintain and worsen trauma through the adverse contribution to trauma processing and eliciting trauma-related symptoms and disorders, as well as to opening doors to new interpersonal traumatic exposures via factors such as shame, mental disorders and proneness to aggressive behaviors. This potential "perpetuum mobile" could be active not only during a person's lifetime, but could possibly spread through generations via various mechanisms.

In terms of scientific implications, more research is needed to explore the nature of this complex relationship, and especially the ways emotion dysregulation in youth sets the ground for further maintenance of trauma

throughout one's lifespan and through generations. Furthermore, since emotion dysregulation can be both a consequence and a risk factor when it comes to trauma, the "ex post" assessment makes it difficult to clarify the true direction of this relationship (63); hence, rigorous methodological criteria are needed in future investigations. Practical implications refer to all levels of prevention when it comes to both youth exposure to trauma and emotion dysregulation, but also to stopping the potential "perpetuum mobile", by preventive actions in both directions. This could include the screening for emotion regulation patterns and difficulties among youth who have suffered trauma, as well as screening for previous but also current victimization among youth with emotion dysregulation. Finally, timely treating both trauma outcomes (including intergenerational effects) and emotion regulation difficulties is warranted, because both are modifiable by contemporary approaches (trauma-informed care, trauma-focused CBT (64), emotion-regulation-based transdiagnostic therapies (65)), built upon a good therapeutic relationship – an important platform for healing interpersonal wounds.

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EMOCIONALNA DISREGULACIJA I TRAUMA MLADIH: PERPETUUM MOBILE

Marija Mitković-Vončina[1,2], Marija Lero[2], Sanja Leštarević[2], Milica Pejović-Milovančević[1,2]

Sažetak

Emocionalna disregulacija (ED) i traumatska iskustva, posebno interpersonalna trauma u detinjstvu, sve se više prepoznaju kao transdijagnostički faktori rizika za mentalne poremećaje kako u razvojnom dobu, tako i kasnije tokom života. Cilj ovog preglednog članka je da istraži postojeće podatke u vezi sa potencijalno dvosmernim odnosom između ED i traume, fokusirajući se na način na koji bi ova dva faktora mogla da održavaju jedan drugog, čineći „perpetuum mobile" koji oblikuje ishode mentalnog zdravlja mladih. Postoje relativno dosledni dokazi da razvojna traumatska iskustva, posebno maltretiranje dece, imaju štetne efekte na ED, kroz različite psihološke mehanizme (kao što su socijalno učenje, interpretacija socijalnih signala, afektivno vezivanje („atačment"), invalidacija emocionalnih iskustava) kao i neurobiološke mehanizme (strukturne i funkcionalne abnormalnosti za emocionalnu regulaciju relevantnih neuralnih zona usled neadekvatne stimulacije, hiperaktivacije sistema odgovora na stres, i uticaja drugih fizioloških sistema). S druge strane, određeni dokazi ukazuju na to da ED može održati i pogoršati traumu ometanjem procesiranja traume i izazivanjem simptoma i stanja povezanih sa traumom, kao i stvaranja okolnosti za nova interpersonalna traumatska iskustava putem faktora kao što su stid, mentalni poremećaji i sklonost ka agresivnom ponašanju. Ovaj potencijalni „perpetuum mobile" mogao bi biti aktivan ne samo tokom života jedne osobe, već bi se mogao širiti i kroz generacije putem različitih mehanizama. Potrebno je više istraživanja da bi se istražila priroda ovog složenog odnosa, a posebno načini na koje ED mladih postavlja osnovu za dalje održavanje traume. Izazovno je empirijski zaključiti koji je smer ovog odnosa u različitim studijama, što implicira potrebu za metodološkom strogošću u budućim istraživanjima. Praktične implikacije odnose se na sve nivoe prevencije kada je u pitanju izloženost mladih traumi i ED, ali i zaustavljanje potencijalnog „perpetuum mobile-a", preventivnim delovanjem u oba smera.

Ključne reči: razvojna trauma, emocionalna disregulacija, međuodnos, adolescenti, mladi

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CASE SERIES

Sporadic inclusion body myositis – single center case series of 8 patients from a fifteen-year period

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Summary

Objective: Sporadic inclusion body myositis (IBM) is a slowly progressive inflammatory myopathy. Clinical presentation comprises asymmetric distal and proximal limb weakness and dysphagia. Muscle biopsy showing rimmed vacuoles are the main diagnostic indicator.

Method: We showed 8 patients with an IBM at the Neurology Clinic of the University Clinical Center Serbia in the period 2009-2024. We analyzed medical records, focusing on the following characteristics: sociodemographic data, age and presenting symptoms at disease onset, comorbidities, findings from neurological examinations, IBM functional rating scores, and results from laboratory and diagnostic procedures.

Results: The average age of years at the onset of the disease was 57.7±0.4 years. The first signs of disease were difficulty walking, dysphagia, hand weakness and eyelid ptosis. The average IBMFRS was 28.7±7.8. Three patients had clinical and ENG signs for polyneuropathy. In four patients, MRI revealed muscle degenerative changes consistent with grade 2b. Muscle biopsy was performed in seven patients and they fulfilled the criteria for clinically defined IBM. Five patients were treated with IVIG with minimal and short-term improvement.

Conclusion: Clinical examination and muscle biopsy are essential for establishing a diagnosis of IBM. Early diagnosis and early administration of the right therapy would make the greatest contribution to slowing the progression of the disease.

Key words: sporadic inclusion body myositis, case series, muscle biopsy, muscle MRI

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INTRODUCTION

Sporadic inclusion body myositis (IBM) is the most common inflammatory myopathy which begins over 45 years of age. The 272nd ENMC International Workshop, held in June 2023, marked a significant milestone in the understanding and management of IBM. Building upon the 2013 ENMC diagnostic criteria, the workshop introduced several key updates reflecting advancements over the past decade. The updated criteria aim to enhance diagnostic accuracy by incorporating recent insights into IBM's pathogenesis and clinical presentation. Emphasis is placed on early recognition, especially in atypical cases and younger patients, acknowledging the broader spectrum of disease manifestations. The criteria now integrate novel diagnostic tools, including muscle imaging techniques like MRI and ultrasound, and serological testing for cytosolic 5'-nucleotidase-1A (cN1A) antibodies (1). The prevalence of IBM is 24.8/1 000 000 (2). Studies have shown an association of IBM with the HLA-DRB1*03, DRB*03:01, DRB1*01, DRB1*01:01, DRB1*15:02, B*08, and the DQB1*02 allele (3). The IBM pathogenesis is still undetermined, and probably multifactorial, but there are two possible hypotheses: the autoimmune hypothesis and the degenerative hypothesis (2,4).

The diagnostic criteria for IBM are: common presentation (age ≥ 45 years at symptom onset, ≥12-month history of progressive weakness, CK ≤ 15x ULN) with common muscle IBM involvement pattern at presentation (often asymmetric and accompanied by dysphagia): deep finger flexor (FF) weakness and/or knee extensor (KE), and muscle biopsy findings (mandatory: inflammation consisting of endomysial lymphocytes surrounding non-necrotic muscle fibers (with or without invasion)), or supportive (1. rimmed vacuoles *and/or* cytoplasmic protein aggregates; 2. mitochondrial abnormalities (COX-SDH+ fibers > age-related); 3. anti-cN1a autoantibody positive; 4. typical muscle MRI appearance *and/or* typical muscle ultrasound pattern). Diagnosis of IBM is confirmed when there is: common presentation with FF and KE weakness, and mandatory investigation finding (1).

Antibodies targeting cytosolic 5'-nucleotidase 1A (cN-1A) are currently the only serum-based diagnostic marker for IBM. Although enzyme-linked immunosorbent assay (ELISA) is the most widely used method for their detection, the test's sensitivity remains relatively low (30% to 50%). In contrast, the specificity of cN-1A antibodies is generally high—over 90%. However, this specificity decreases significantly in patients with other connective tissue disorders, including systemic lupus erythematosus, Sjögren's syndrome, and dermatomyositis, where up to one-third may test positive for cN-1A antibodies despite not having IBM. Therefore, cN-1A antibody results should be interpreted with caution and always considered in the context of the patient's clinical presentation (5).

To-date literature underlies that most of the investigated therapeutic strategies were insufficiently successful and that further randomized controlled trials are needed. However, there are conflicting results for some drugs (intravenous immunoglobulin (IVIG), arimoclomol, follistatin, canakinumab, bimagrumab,…). As a symptomatic approach, botulinum toxin injections in upper esophageal sphincter can improve dysphagia (6,7). Therefore, IBM surely represents a great economic burden of the health system (8).

Our study provides a clinical and pathological workup of a single center group of 8 patients with IBM from a fifteen-year period.

MATERIAL AND METHOD

We performed a retrospective analysis of all hospitalized patients at the Department of Neuromuscular Diseases of the Neurology Clinic of the University Clinical Center Serbia (UCCS), Belgrade in the period 2009-2024. with a diagnosis of myositis. We identified 8 patients with an IBM diagnosis. This retrospective study has been conducted in accordance with all ethical principles of the Declaration of Helsinki and in accordance and approval with all national and institutional ethical standards. All patients had negative family history.

We analyzed medical records and following characteristics: sociodemographic (age and gender), age and symptoms/signs at onset of disease, co-morbidities, neurological examination, fulfillment of the 2024. diagnostic criteria for IBM (1), analysis of all other laboratory and other supplementary available diagnostic procedures performed during hospitalization (muscle CT, muscle MR, ENG, EMG, muscle biopsy). After the biopsy of the muscles, the sample was analyzed by microscopy. We calculated an IBM functional rating score (IBMFRS) and Barthel Index based on functional disability. IBMFRS has a maximum value of 40 indicating that the patient is without functional disability in the 10 domains examined by this scale (9).

Depending on the type of variables and the normality of the distribution, data will be presented as n (%), mean ± standard deviation, or median (range).

RESULTS

The average age of years at the onset of the disease was 57.7±10.4 years, and the average age at the time of first hospitalization was 63.2±8.17 years. The average duration of disease until the first hospitalization and definite diagnosis at the Neurology Clinic UCCS was 6.75 (min: 0, max: 18 years). All patients had a characteristic distribution of hypotrophy and muscle weakness of varying degrees (weakness of the flexors of the hands, hypotrophy and weakness of the m. quadriceps, weakness of the muscles in the anterior compartment of the lower legs) **(table 1)**.

Table 1. Characteristics of patients with sporadic inclusion body myositis (sIBM)

	Number of patients							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Sociodemographic characteristics								
Gender	M[1]	M	M	F[2]	F	M	F	F
Age	59	66	70	70	51	64	73	71
Characteristics of diseases								
Comorbidities	HTA[3] Hemorrhoids Hepatomegaly Hiatus hernia HLD[4]	HTA	HTA AF[5] HLD Lichen chronicus	Right eye cataract HLD	No	HTA AAA[11]	Tachy-cardia Discus hernia	AF HTA
Symptoms on IBM onset	Walking	Walking	Dysphagia	Walking	Ptosis (right)	Hands	Walking	Walking
Duration of disease (y)	18	4	3	2	6	2	13	15
Dysphagia	Yes	No	Yes	Yes	Yes	No	No	Yes
Diagnostic criteria for clinically defined IBM								
I	41	62	67	68	45	62	52	65
II (years)	18	4	3	2	6	2	13	15
III	641	747	599	1145	161	831	500	800
IV	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
V	Yes	NA[6]	Yes	Yes	Yes	Yes	Yes	Yes
Other clinical and diagnostic characteristics								
LDH[7] (220-460 IU/L)	583	716	703	580	374	439	482	NA
Asymmetry	Right	Right	Left	Right	No	No	No	No
Myopathic EMG[8]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Neuropathy on ENG[9]	No	Yes	Yes	No	No	No	Yes	No
Radiculopathy on ENG	No	No	Yes	No	No	No	Yes	No
IBMFRS	28	37	21	32	14	29	34	35
Barthel index	60	80	60	65	25	88	90	90
Mobility	Amb[10]	Amb	Amb	Amb	Wheelchair	Amb	Amb	Amb
Therapy								
Therapy during disease	No	No	IVIG[12]	IVIG	IVIG	IVIG	No	IVIG

I- Age of onset > 45 years; **II**- Duration > 12 month; **III**- Creatine kinase < 15x normal (0-200 U/L); **IV**- With common IBM muscle involvement pattern at presentation: Deep finger flexor (FF) weakness AND/OR Knee extensor (KE) weakness; **V**- Inflammation consisting of endomysial lymphocytes surrounding non-necrotic muscle fibers (with or without invasion)
[1]Male [2]Female [3]Hypertension [4]Hyperlipidaemia [5]Atrial fibrilation [6]Not available [7]Lactate dechydrogenase [8]Electromyography [9]Electroneurography [10]Ambulatory[11] Abdominal aortic aneurysm [12] Intravenous immunoglobulin

Muscle biopsy was performed in 7/8 (87.5%) patients and the diagnosis of IBM was confirmed. The eighth patient had not undergone a muscle biopsy and therefore met the criteria for a probable diagnosis of IBM. The creatine kinase (CK) level was 694 U/L (min 161 U/L, max 1145 U/L) and LDH level was 553.9±129.6 U/L. Anti-cN1a autoantibodies were not performed on any patient. At the time of hospitalization, the average IBMFRS was 28.7±7.8 and Barthel index was 69.7±22.3.

Five patients were treated with IVIG at a dose of 0.4 g/kg daily for five days, followed by a booster dose of 0.4 g/kg every 6-8 weeks for at least 6 months. Minimal

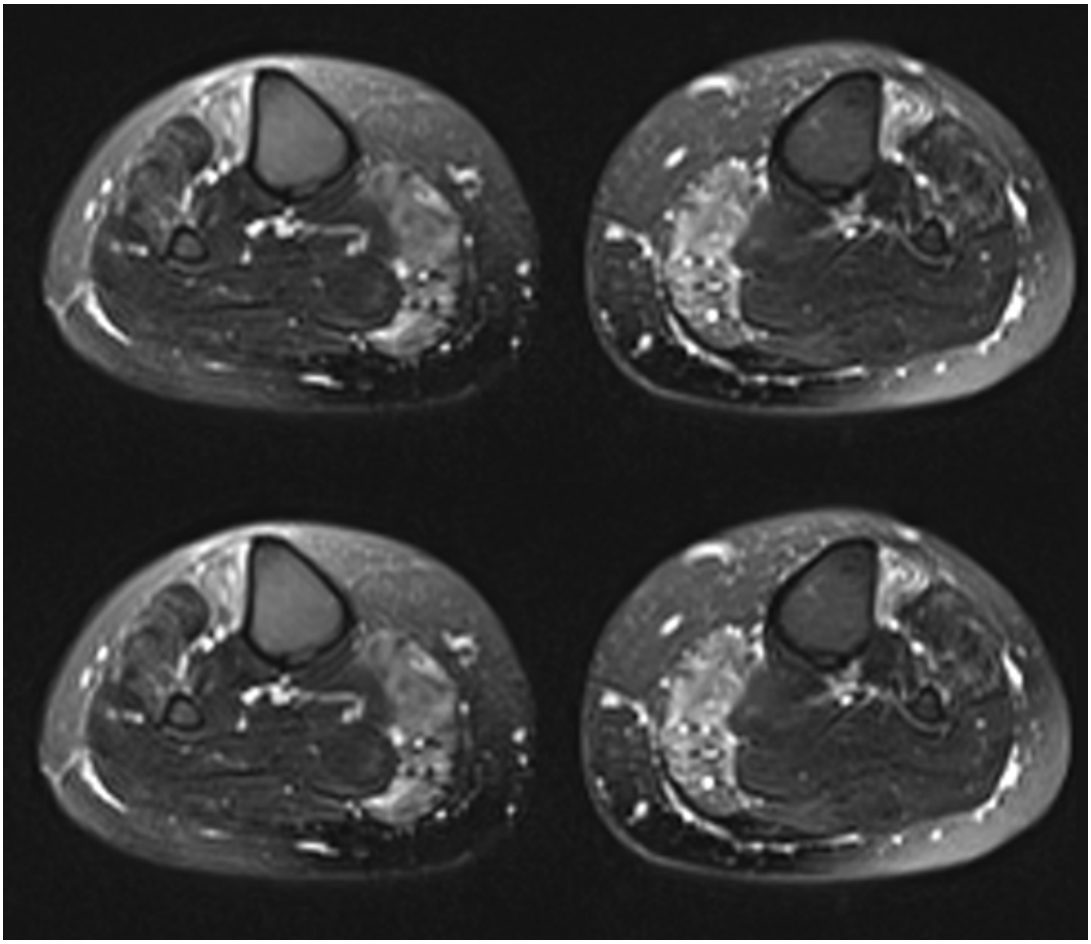


Figure 1. Symmetrical diffuse muscle hypotrophy of all compartments of both upper legs and lower legs, with diffuse edema and fatty degeneration in the distal third of the vastus medialis and lateralis m. gastrocnemius medialis, m. tibialis anterior.

and short-term improvement was noted. These and other clinical and laboratory characteristics were shown in **Table 1**.

Serum protein immunoelectrophoresis in patient 1 revealed the presence of polyclonal IgG antibodies, while urine protein immunoelectrophoresis results were normal. On the abdomen ultrasound, the diameter of the liver was larger (190 mm) than normal. CT of the proximal third of the thighs showed atrophy and fat infiltration and CT of the lower legs were normal. Patient number 2 had dysesthesias on his legs in the form of short socks and hands in the form of short gloves, while electroneurography (ENG) showed sensory and motor axonal and demyelinating polyneuropathy on upper extremities (UE) and lower extremities (LE), which was more expressed on LE. The etiology of motor-axonal polyneuropathy remained idiopathic. For patients 4,6,7, and 8 MRI of the proximal third of the thighs and the lower legs showed muscle degenerative changes and thighs muscle edema **(Figure 1)** which was consistent with IBM diagnosis.

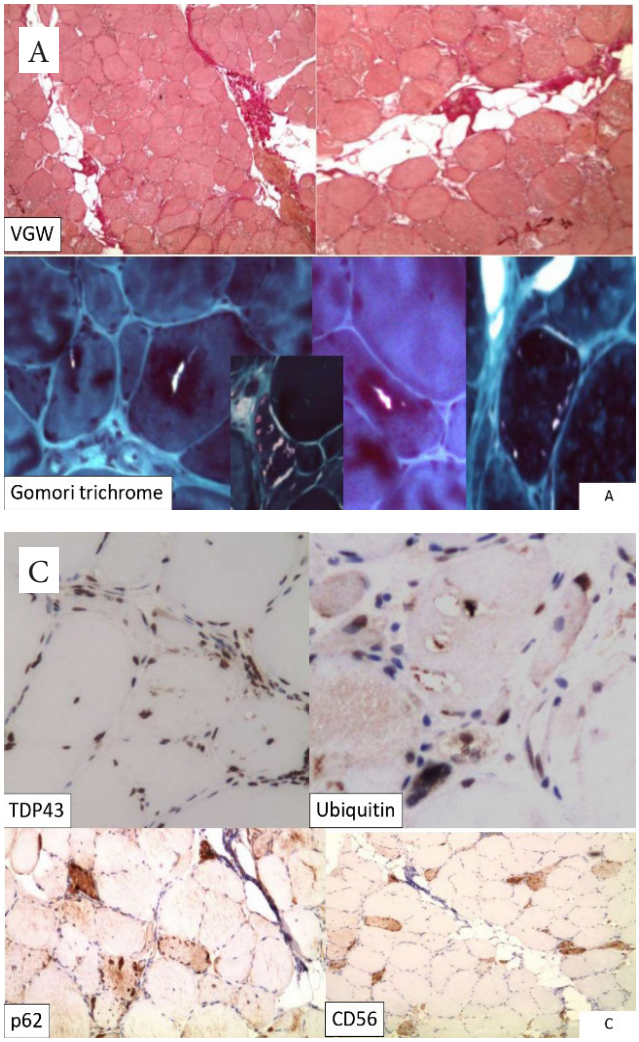
In the majority of patients (7/8), pathohistological examination showed findings characteristic of IBM and in all of them the pathohistological criteria for IBM were fulfilled **(Figure 2A, 2B, 2C)**.

DISCUSSION

We showed 8 patients with IBM and 7 patients had typical muscle biopsy findings for IBM. The course of the disease was atypically very slow but still progressive in three patients. The onset of disease in one of our patients in the form of ptosis was a very rare clinical manifestation.

In the largest percentage of patients, the IBM begins with difficulty walking, mostly due to the weakness of m. quadriceps. In five patients the first sign was difficulty walking, and in two patients the first problem was dysphagia. Dysphagia was a common presenting symptom being more frequent in women than men and was during the disease course reported in 74% of men and 84% of women (10). In our patient number 5, the first sign was right eyelid ptosis that lasted a month. In this patient, IBM started 6 years before hospitalization, but she has the highest functional disability (IBMFRS 14). Extraocular muscle weakness and ptosis is a very rare initial manifestation of IBM described only as a case report (11).

The average duration of the disease until definitive diagnosis is 4.6-5.8 years (1). In 5/8 (62.5) of our patients the diagnosis was made within 6 years, while in patient 1, a total of 18 years has passed from the onset of the disease. The very slow progressive course of the disease contributed significantly to this delay. Change in percentage of



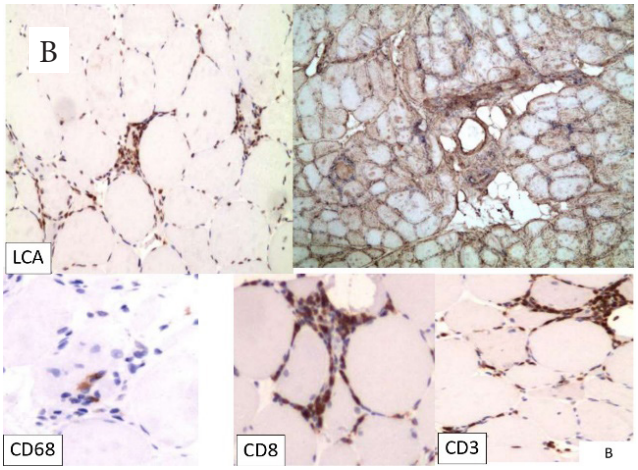


Figure 2. A. VGW: Proliferation of connective and fatty tissue is moderate, more pronounced interfascicularly. PAS: Glycogen content is preserved. Gomori trichrome: The distribution and density of mitochondria is grossly disturbed. Irregular to oval vacuoles are clearly visible and most of them contain material that is colored red (according to the "rimmed" vacuole type). Significant cytoplasmic bodies can also be observed in certain fibers.
B. A focal inflammatory infiltrate of the mononuclear type (LCA+) was observed with the presence of macrophages (CD68+) and a clearly expressed phenomenon of myophagocytosis. The expression of the host compatibility complex (MHC I) is disturbed with increased intensity both in the inflammation zone and with expression on the membrane and in the cytoplasm. The immune profile of lymphocytes is T type (dominantly CD8+ and CD3+).
C. The presence of positive fibers, sarcoplasmically granulated in the form of protein aggregates of the autophagic marker p62 and the positivity of the protein aggregating marker TDP-43, as well as Ubiquitin was observed. Angular fibers and fibers in active denervation express NCAM (CD56+) on the membrane and in the cytoplasm.

IBMFRS score over time yielded an average decline of 6.3% per year, with steeper decline in the initial years. Older age of onset was associated with a more rapid IBMFRS decline (12).

Up to 25% of IBM patients may have normal serum CK level (13). CK level is not associated with muscle strength, age, age at onset, and duration of disease (14). Our patient number 5 with the most severe deficit had a CK at the reference values. LDH was elevated in 5/8 (62.5%) of our patients. The recent case series showed that LDH levels were slightly elevated, periodically very elevated and that LDH levels did not correlate with CK levels (15). Also, serum markers did not have a statistically significant correlation with any of the clinical measures (16).

Some studies have shown an increased incidence of hypertension (HTA), hyperlipidemia (HLD) and myocardial infarction in the patients with IBM (17). In our sample, 6/8 (75%) patients had some of these diagnoses **(Table 1)**. On the other hand, more recent research has not confirmed this, but has found a higher incidence of peripheral neuropathy, Sjogren's syndrome and hematologic malignancies. (18) Three (37.5%) patients had clinical and ENG signs for sensorimotor axonal and demyelinating peripheral neuropathy. Even after all the diagnostic tests were done, the polyneuropathy remained idiopathic.

Although IBM is a slowly progressive disease that leads to wheelchairs for a mean time of 12 years (10), some patients had minimal progress after 12 years of disease (18). Despite having lived with IBM for 18, 13, and 15 years respectively, patients 1, 7, and 8 remain ambulatory, though with marked difficulty. IBM was associated with increased mortality risk compared with population controls, with hazard ratio per 1-year increase of 2.69 [1.74, 4.15] ($p < 0.0001$) (18).

Three placebo-controlled studies were performed with IVIG in patients with IBM. Although there was an improvement in the study groups in comparison with the control group, the differences were not statistically significant. Owing to its limited quality, this study was excluded from meta-analyses. Better-designed studies are needed (6,7,20). Our four patients received the IVIG recommended dose regimen, but the effects were short-lived (about three months) and therapy had the greatest positive effect on dysphagia. However, based on the most recent guidelines, IVIG is no longer considered an appropriate treatment for IBM, and it is no longer used for this purpose in Serbia.

Limitations

This study is limited by the small sample size which restricts the generalizability. The retrospective design may also introduce bias due to incomplete or inconsistent medical records. Not all patients underwent the same diagnostic procedures leading to potential variability in diagnostic certainty. Additionally, treatment outcomes, particularly with IVIG, were not assessed using standardized follow-up protocols, limiting conclusions regarding therapeutic efficacy. Finally, the absence of a control group prevents comparison with other myopathies or treatment modalities.

CONCLUSION

The IBM is a slow progressive disease of unknown etiology and unclear pathogenesis, with no effective therapy. Clinical examination and muscle biopsy are paramount in making the diagnosis. Early diagnosis and the detection of disease mechanisms in these patients are very significant. Early administration of the right therapy would make the greatest contribution to slowing the progression of the disease.

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Authors' Contributions:

Conception and design: DA, IB, VRS; Data collection: DA, IB, VRS; Statistical analysis: DA, IB, VRS; Writing the article: DA, IB, VRS; Critical revision of the article: DA, IB, IK, SG, IB, VRS; Final approval of the article: DA, IB, IK, SG, IB, VRS

Ethical Approval: This retrospective study has been conducted in accordance with all ethical principles of the Declaration of Helsinki and in accordance and approval with all national and institutional ethical standards.

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SPORADIČNI MIOZITIS SA INKLUZIVNIM TELAŠCIMA – SERIJA SLUČAJEVA 8 PACIJENATA IZ JEDNOG CENTRA IZ PERIODA OD PETNAEST GODINA

Dejan Aleksić[1], Ivo Božović[2], Ivana Kezić[3], Sanja Gluščević[4], Ivana Basta[5], Vidosava Rakočević-Stojanović[5]

Sažetak

Uvod: Sporadični miozitis sa inkluzionim telima (IBM) je sporo progresivna inflamatorna miopatija. Klinička slika se sastoji od asimetrične slabosti distalnih i proksimalnih mišića ekstremiteta i disfagije. Biopsija mišića koja pokazuje "rimmed" vakuole je glavni dijagnostički indikator.

Metod: Prikazali smo 8 pacijenata sa IBM na Klinici za neurologiju Univerzitetskog kliničkog centra Srbije u periodu 2009-2024. Analizirali smo medicinsku dokumentaciju i sledeće karakteristike: sociodemografske podatke, uzrast i znakove bolesti, komorbiditete, neurološki nalaz, IBMFRS, rezultate laboratorijskih i dijagnostičkih procedura.

Rezultati: Prosečna starost godina na početku bolesti bila je 57,7±0,4 godine. Prvi znaci bolesti bili su otežano hodanje, disfagija, slabost ruku i ptoza očnih kapaka. Prosečan IBMFRS bio je 28,7±7,8. Tri pacijenta su imala kliničke i ENG znakove polineuropatije. Kod četiri pacijenta MR je pokazala degenerativne promene mišića stepena 2b. Biopsija mišića urađena je kod sedam pacijenata i oni su ispunjavali kriterijume za klinički definitivan IBM. Pet pacijenata je lečeno IVIG sa minimalnim i kratkotrajnim poboljšanjem.

Zaključak: Klinički pregled i biopsija mišića su najvažniji u postavljanju dijagnoze IBM. Rana dijagnoza i rana primena prave terapije dali bi najveći doprinos usporavanju progresije bolesti.

Ključne reči: sporadični miozitis sa inkluzionim telima, serija slučajeva, biosija mišića, MR mišića

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CASE REPORT

Severe autonomic dysfunction associated with autoimmune thyroiditis in post-COVID-19 patient

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Summary

Introduction: We present a case of Hashimoto's thyroiditis and severe autonomic dysfunction three months after the resolution of the acute phase of COVID-19 infection in a patient with no prior thyroid disease and autonomic dysfunction.

Patient Review: We discuss our patient's clinical presentation, diagnostic evaluation, and subsequent management and follow-up. The patient had previously tested positive for COVID-19. A 53-year-old female developed Hashimoto's thyroiditis with normal thyroid function and severe autonomic dysfunction. Her heart rate variability (HRV) is depressed, and her High-Frequency power (HF) is high.

Conclusion: Our findings suggest that COVID-19 could have long-term negative effects on antithyroid antibodies. Therefore, testing antithyroid antibodies should be included in the follow-up algorithm for COVID-19 survivors. Autonomic nervous system dysfunction related to COVID-19 may be reversible and early aggressive therapy is necessary. The new technique is important for the early detection of post-acute sequelae of SARS-CoV-2 infection (PASC) in the autonomic nervous system.

Keywords: COVID-19, Hashimoto's thyroiditis, autonomic dysfunction

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INTRODUCTION

World Health Organization (WHO) defines post-acute sequelae of SARS-CoV-2 infection (PASC) as the development of new, continuing, or recurring symptoms 3 months after the initial SARS-CoV-2 infection, lasting at least 2 months (1,2). One of the most common long COVID entities is referred to dysautonomia which is manifested as fatigue, dizziness, syncope, dyspnea, orthostatic intolerance, nausea, vomiting, and heart palpitations (3). It has been observed that within the complex pathogenesis of SARS-CoV-2 infection, especially if there is an aberrant immune response, some autoimmune diseases could be triggered during an active infection, but also during recovery. As for the endocrine system, it has been shown that one of the most prevalent COVID-related autoimmune diseases is Hashimoto's thyroiditis (4). We present a case of severe dysautonomia and autoimmune thyroid disease following SARS-Cov-2 infection.

CASE REPORT

A 53-year-old female patient was hospitalized in February 2021 for bilateral COVID-19 pneumonia. Among the symptoms on admission, the patient reported high fever, severe weakness, headache, and shortness of breath. At the emergency room, a chest X-ray revealed bilateral pneumonia, a nasopharyngeal swab was positive for SARS-CoV-2 and her blood test revealed an inflammatory state with a marked increase in CRP (C-reactive protein) - 21 mg/L and a D-dimer within the range. She was on oxygen therapy, corticosteroids, and other symptomatic therapy. In her personal history, she reported asthma in childhood which was treated until 2016 with a Symbicort inhaler. Family history is positive for diabetes mellitus type 2, cardiovascular diseases, and colorectal carcinoma.

Three months later, due to severe fatigue accompanied by palpitations, dizziness or lightheadedness (especially when standing up), fatigue, weakness, chest pain, shortness of breath, lack of concentration, frequent headaches, trouble swallowing, and constipation, she visited a cardiologist. The cardiologist's objective finding was that the patient was conscious, oriented, compensated, and normally nourished (BMI (body mass index) 25 kg/m2). The cardiac function was described as rhythmic, with clear tones, and without murmurs. Over the lungs, there was a normal respiratory sound without accompanying findings. The liver and spleen were not palpable as enlarged, kidneys were insensitive to percussion and the lower extremities were without edema and varicose veins. The ECG (electrocardiogram) finding was normal with sinus rhythm, frequency 78/min, without changes in the T wave and ST segment.

As part of additional diagnostics, the function of the thyroid gland was evaluated because the patient had fatigue, trouble swallowing, and constipation. The level of thyroid hormones was within the physiological range (FT4 14 pmol/l FT3 5.14 pmol TSH 1.2 uIU/ml). Thyroid antibodies were elevated (Anti-TPO 114.48 IU/ml and Anti-Tg antibodies 4.14 IU/ml) which indicated autoimmune thyroid diseases. Ultrasonographically, the thyroid gland was described as hypoechogenic, with regular position and size, and with a nonhomogeneous pseudo-nodulated structure. The patient did not respond adequately to conventional hormone replacement therapy with levothyroxine. She was treated with Prednisone therapy one month after COVID-19 pneumonia. Throughout this period, she consistently took supplements including selenium, zink, magnesium, and vitamin D to support thyroid hormone metabolism. The patient had no other viral infections such as Herpes (HSV), Epstein-Barr (EBV), or influenza after COVID-19 pneumonia.

Furthermore, the patient was tested according to the protocols in the Neurocardiology Laboratory for

Table 1. Short-term heart rate variability analyses

Parameter	First value*	Second value**	Normal range
IBI (INTERBEAT INTERVAL)	791	855	785 -1160
SDNN	55	75	102 - 180
PNN 50%	6-29	15-32	>3%
RMSSD	46	39	19 - 107
TOTAL POWER	2514.1	2198.5	750 - 12000
VLF	600	1358.1	400 - 1750
LF	1344	664.1	300 - 1750
HF	557.6	163	50 - 120
LF/HF ratio	2.4	4.1	1.5 -2

**First value on the beginning **Value after 3 years IBI interbeat interval SDNN Standard deviation of NN intervals*
PNN% Percentage of successive RR intervals that differ by more than 50 ms RMSSD Root mean square of successive RR interval differences VLF -very low- frequency power LF- low- frequency power HF -high- frequency power
LF/HF ratio <1,5 Parasympathetic dominance
LH/HF ratio>2 Sympathetic dominance

Table 2. Ewing battery test scoring system

	Normal Result (0)	Borderline Result (1)	Pathological Result (2)
Handgrip Test	> 16 mmHg	11-15 mmHg	< 30 mmHg
Orthostatic Hypotension	< 10 mmHg	11-29 mmHg	> 30 mmHg
Valsalva Maneuver Test	> 1.21 mmHg	1.11-1.20 mmHg	< 1.10 mmHg
Deep Breathing Test	> 15 mmHg	11-14 mmHg	< 10 mmHg
Stand Up Test	> 1.04 mmHg	1.01-1.03 mmHg	< 1.0 mmHg

accessing ANS function. The protocol included complete testing of the ANS Ewing battery tests (Ewing DJ, Clarke BF 1982.), 24-hour Holter ECG, and ambulatory blood pressure monitoring. Complete testing first included cardiovascular reflex tests, followed by short-term heart rate variability (HRV) mathematical analysis in frequency-domain and time domain **(Table 1)**.

Ewing battery of autonomic tests consists of various analyses of heart rate variations during deep breathing (DB), lying to standing (LS), standardized Valsalva maneuver (VM), analysis of blood pressure variations on standing (PH) and sustained handgrip (SHG). The existence of autonomic dysfunction is determined by the scoring system for the group of tests that the Ewing battery consists of and 24-hour Holter ECG **(Tables 2,3,4)**.

Table 3. Results of Ewing`s cardiovascular reflex tests

Tests for accessing autonomic function	
Hand grip test	2
Median (min-max)	(0-2)
Orthostatic hypotension	0
Median (min-max)	(0-2)
Sympathetic dysfunction	1
Median (min-max)	(0-1)
Valsalva maneuver	2
Median (min-max)	(0-2)
Deep breathing test	1
Median (min-max)	(0-2)
Stand up test	2
Median (min-max)	(0-2)
Parasypathetic dysfunction	2
Median (min-max)	(0-2)
Total	**7 (severe autonomic dysfunction)**

A 24-hour blood pressure Holter showed that the systolic blood pressure was within the reference values in 67% of the measurements, while the diastolic pressure was in the reference values in 74%, the maximum blood pressure measured was 145/104 mmHg - detailed report in the attachment. The results of ANS testing showed that the patient had severe complete autonomic dysfunction **(Table 4)**. Short-term frequency-domain spectral analysis showed higher HF, associated with modification in parasympathetic activity. Tilt-Table Test (5,6) was positive (orthostatic hypotension). The patient had reduced HRV during a deep breathing maneuver. The LF/HF (Low Frequency/High Frequency power) ratio was high **(Table 1)**.

Follow-up after three years indicated that the treatment provided by endocrinologists and cardiologists was effective. The function of the thyroid gland is good, function of the autonomic nervous system has improved. The level of thyroid hormones is within the physiological range (FT4 15.4 pmol/l, FT3 4.88 pmol/l, TSH 2.35 mIU/l). Thyroid antibodies are a little higher than 3 years ago (Anti-TPO antibodies 148.0 IU/ml and Anti-Tg antibodies 77.0 IU/ml) **(Table 5)**.

Table 5. Hormonal and immunological analyses

Parameter	First value*	Second value**	Normal range
FT4	14	15.4	9.0-19.1 pmol/l
FT3	5.14	4.88	2.63-5.70 pmol/l
TSH	1.2	2.35	0.35-44.94 mIU/l
Anti-TPO	114.48	148	0.00-5.61 IU/ml
Anti-Tg	4.14	77	0.00-4.11 IU/ml

**First value on the beginning **Value after 3 years*
FT4-free thyroxine
FT3-free triodothyronine
TSH-thyroid-stimulating hormone
Anti-TPO-Thyroid peroxidase antibodies
Anti-Tg-Thyroglobulin antibodies

DISCUSSION

Globally, 6,957,216 people have died from COVID-19, with an estimated mortality rate of approximately 1% in Serbia (7, 8). However, PASC is a much greater burden for the health system. One study found that PASC led to more than 80 disability-adjusted life years, or DALYs, for every 1,000 people who weren't hospitalized for their initial infection (9). Most patients tested positive for

Table 4. Level of ANS dysfunction scoring system

Score	**0-1**	**2-3**	**4-6**	**7-10**
Level of ANS dysfunction	Normal Result	Mild dysfunction	Moderate dysfunction	Severe dysfunction

COVID-19 and recovered, but the latest data indicate that every fifth patient experiences a variety of mid- and long-term effects after convalescence (10). For the new treatment for PASC, it is important to consider viral persistence or reactivation of viruses. The persistence of SARS-CoV-2 RNA or antigens has been reported in some organs, but the pathogenic immune response remains unclear (11). The most common symptoms of PASC are chronic fatigue, cognitive symptoms, orthostatic hypotension, chronic pain, etc. (12). Many of these symptoms could be found in patients with autonomic dysfunction and hypothyroidism, such as our reported case.

Minnotti et al. (2024) retrieved 35 studies with data on 42,934 children, adolescents, and adults under 20, who had post-viral infection symptoms (13). These studies reported both physical and physiological symptoms, with similar symptom duration for PASC and post-EBV (gastrointestinal virus syndromes, ranging between 10 days to 18 months.

As expected, fatigue was a dominant persistent symptom of the post-EBV and PASC conditions in older children and adolescents, which likely hindered their everyday tasks and activities. However, neurocognitive symptoms such as the lack of concentration and loss of taste and smell were specific to post-COVID conditions, and even their underlying mechanics were unestablished (13). Some of the previous studies described similar cases to the one we are presenting. Some people with Hashimoto's thyroiditis have normal thyroid-stimulating hormone (TSH). The treatment for Hashimoto's disease with normal TSH usually does not involve medication. Instead, there are lifestyle changes a person can make, like getting optimal nutrition that can help them manage the diseases. The patient in our reported case consumed selenium, zinc, magnesium, and vitamin D supplements to improve thyroid hormone metabolism. Three years later thyroid function is normal but thyroid antibody is high. The patient in our reported case had severe autonomic dysfunction. She was treated with diet without gluten, lactose, and refined sugar, beta-blockers (Concor) and natural antioxidants (glutathione, coenzyme Q10), antioxidant vitamins (Vitamin C, Vit.B12, Vit D). She used NADH which plays a role in generating energy in organisms and peptan collagen which is helpful for joint, bone, and muscle health.

Agnihotri et al. presented a case of autonomic neuropathy as a PASC in a 47-year-old woman. The patient reported palpitations, hyperhidrosis, and tremulousness but in comparison to our patient, without syncopal events. Autonomic testing results indicated sympathetic vasomotor impairment but without a significant drop in blood pressure in a 10-minute head-up tilt test. During autonomic testing, tachycardia response in the second half of the tilt period could explain symptoms such as palpitations, agitation, and vertigo (14). Test results of our patient did not indicate a drop in pressure in the Tilt-up test, but other tests showed that there is a pathological response of ANS.

In a Mayo Clinic study of patients with symptoms related to postinfectious autonomic dysfunction after COVID-19, 63% were found to have abnormal findings on standardized tests of autonomic function, such as cardiovagal function, which analyzes heart rate responses to deep breathing and the Valsalva maneuver (15).

Our findings suggest that our patient had depressed HRV during the deep breathing test, indicating parasympathetic dysfunction.

Jammoul et al. investigated the possible pathophysiological mechanism of autonomic dysfunction as PASC (16). They suggested persistent inflammation, hypoxia, sympathetic overactivation due to higher release of pro-inflammatory cytokine, and tissue damage as one of the key factors involved in the pathogenesis of the disease. Imbalance of the renin-angiotensin aldosterone system, due to the binding of the SARS-Cov-2 virus to ACE-2 receptors with consequent effects on blood pressure and volume, and viral invasion of autonomic centers could also be the possible mechanisms (17).

It has been shown that virus infection in predisposed individuals could lead to the activation of some autoimmune diseases (18). Since the SARS-Cov-2 infection outbreak, many cases of endocrine autoimmune diseases have been reported during the active infection or recovery period. Feghali et al. noted several cases of autoimmune thyroid gland disease related to COVID-19. One of the patients was a 38-year-old health worker who tested positive for COVID-19 and a month later noted thyroid enlargement along with fatigue, dry skin, and depression. Laboratory tests pointed to elevated TSH, anti-thyroid peroxidase antibodies, anti-thyroglobulin antibodies, and decreased levels of thyroid gland hormones. Results from fine needle aspiration biopsy verified the diagnosis of chronic lymphocytic thyroiditis – Hashimoto's disease (4). The results of our patient are the same with the exception that the thyroid hormones are in the physiological range. However, it is not excluded that later autoimmune thyroiditis will become manifest and lead to hypothyroidism. Some people with Hashimoto's disease have normal TSH levels. In such cases, treatment usually does not involve medication. Instead, lifestyle changes, such as obtaining optimal nutrition, can help manage the disease. The patient in our case report consumed selenium, zinc, magnesium, and vitamin D supplements to improve thyroid hormone metabolism. Three years later, thyroid function is normal, but thyroid antibody levels remain high. The patient also had severe autonomic dysfunction. The patient was treated with a diet free of gluten, lactose, refined sugar, beta-blockers (Concor), natural antioxidants (glutathione, coenzyme Q10), and antioxidant vitamins (vitamin C, vitamin B12, vitamin D). She also used NADH, which plays a role in generating energy in the body, and peptan collagen, which is beneficial for joint, bone, and muscle health.

Hypothyroidism is a commonly seen endocrine disorder with a well-known pathophysiological background and a thoroughly established association with other medical disorders (19). The underlying mechanism of most of the cardiovascular and neurological manifestations of hypothyroidism could be due to autonomic dysfunction. Antony et al. investigated the connection between the hypofunction of the thyroid gland and dysautonomia. Results showed that severe autonomic dysfunction is far more common in hypothyroid individuals in comparison to euthyroid patients. About ¾ of the patients were diagnosed with sympathetic dysfunction and of ⅔ individuals had parasympathetic dysfunction. No significant association between autonomic dysfunction and the duration of hypothyroidism was found (20). Since sympathovagal imbalance is associated with an increased risk of development of arrhythmias and sudden cardiac death one of the required tests is heart rate variability (HRV). Brusseau et al. (2022) investigated in their meta-analysis how hyperthyroidism influences HRV parameters (21). Their study concluded that heart rate variability is decreased in hyperthyroid patients, with increased sympathetic and decreased parasympathetic activity (21). These results could be explained by the various effects thyroxine has on the cardiovascular and autonomic systems (22). Although short-term spectral analysis showed higher, it cannot be claimed with certainty that the cause of heart rate variability is autoimmune thyroiditis, because the patient was euthyroid all the while. A worsening of symptoms can certainly be expected if thyroiditis progresses to hypothyroidism.

Ashrafi et al. (2024) found in meta-analysis a 26% prevalence of NTIS (non-thyroidal illness syndrome) and a 10% prevalence of thyrotoxicosis (23). Therefore, it can be inferred that SARS-CoV-2 can affect the thyroid either directly (via direct viral effects) or indirectly (through immune system dysregulation). It is noteworthy that some patients experiencing thyroiditis after COVID-19 experience a subclinical hypothyroidism phase about three months later (24). Furthermore, Graves' disease and Hashimoto's thyroiditis can occur several months after subacute thyroiditis, raising the possibility that viral infection may cause autoimmune thyroid disease (24).

The connection between Hashimoto's thyroiditis and dysfunction of the autonomic nervous system remains unclear.

The cerebellum plays a vital role in the regulation of the hypothalamus which is most important in the regulation of ANS. Initial brain MRI of Hashimoto's thyroiditis was normal but follow-up MRI showed diffuse cerebellar atrophy. Similar outcomes have been reported in Anti-TPO antibodies that have been shown to bind to cerebellar glial cells (25).

CONCLUSION

Our findings suggest that COVID-19 could have long-term negative effects on antithyroid antibodies. Therefore, testing antithyroid antibodies should be included in the follow-up algorithm of COVID-19 survivors. The COVID-19 dysfunction of the autonomic nervous system may be reversible, and early aggressive therapy is necessary. The new technique is important for the early detection of PASC in the autonomic nervous system.

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TEŠKA AUTONOMNA DISFUNKCIJA POVEZANA SA AUTOIMUNIM TIREOIDITISOM KOD POST-KOVID 19 PACIJENTA

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Sažetak

Uvod: Cilj našeg rada je prikazati slučaj Hašimoto tiroiditisa i teške autonomne disfunkcije koji su se razvili tri meseca nakon oporavka od akutne faze infekcije izazvane kovidom 19 kod 53-godišnje pacijentkinje bez prethodne istorije bolesti štitaste žlezde i autonomne disfunkcije.

Prikaz slučaja: Klinička evaluacija pokazala je očuvanu funkciju štitaste žlezde uprkos dijagnozi Hašimoto tiroiditisa, uz izraženu autonomnu disfunkciju — smanjenu varijabilnost srčane frekvencije (HRV) i povišenu komponentu visoke frekvencije (HF) u spektru snage. Diskutovani su klinički tok, dijagnostički pristup i strategije lečenja, kao i praćenje pacijentkinje.

Zaključak: Naša istraživanja ukazuju na to da infekcija virusom kovid 19 može imati dugoročne negativne efekte na antitireoidna antitela. Stoga, testiranje antitireoidnih antitela treba da bude uključeno u algoritam praćenja kod osoba koje su preležale kovid 19. Disfunkcija autonomnog nervnog sistema uzrokovana kovidom 19 može biti reverzibilna, te je neophodna rana i agresivna terapija. Stoga je važno rano otkrivanje disfunkcije autonomnog nervnog sistema u PKS (post-kovid sindromu).

Ključne reči: kovid 19, Hašimoto tiroiditis, disfunkcija autonomnog nervnog sistema

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