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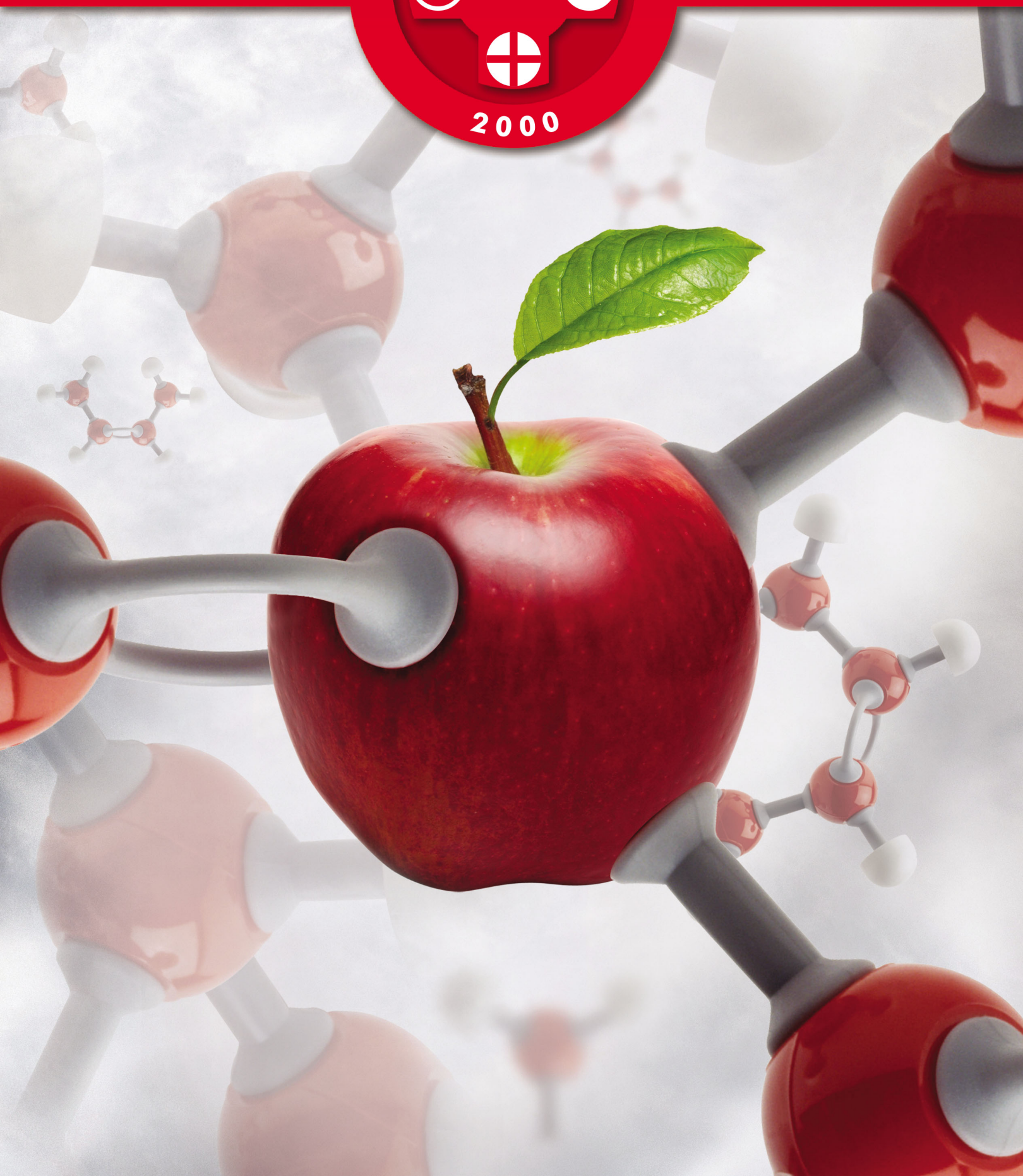

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Table Of Contents

REGULATORY ROLE OF PERITONEAL B CELLS IN EAE

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REGULATORNA ULOGA PERITONEALNIH B LIMFOCITA U EAE

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ABSTRACT

B cells play a dual role in the pathogenesis of autoimmune diseases. In experimental autoimmune encephalomyelitis (EAE), an experimental model for multiple sclerosis, B cells contribute to disease progression, while their regulatory role predominates in the initial phases of disease development. Several studies have identified different subsets of regulatory B cells, mostly in the spleen, which are all sources of IL-10. However, peritoneal regulatory B cells are also important producers of IL-10, can migrate towards inflammatory stimuli, and could have an immunoregulatory function. As we have observed expansion of regulatory B cells in the peritoneum of resistant mice after EAE induction, herein we discuss the regulatory roles of B cells in EAE pathogenesis and the possible role of peritoneal regulatory B cells in resistance to EAE induction.

Keywords: *multiple sclerosis, EAE, B cells, peritoneal regulatory B cells*

SAŽETAK

B limfociti imaju dvojnu ulogu u patogenezi autoimunskih bolesti. B limfociti doprinose progresiji eksperimentalnog autoimunskog encefalomijelitisa(EAE), eksperimentalnog modela za multiplu sklerozu, a u inicijalnim fazama razvoja bolesti dominira njihova regulatrona uloga. U nekoliko studija je identifikovano nekoliko subpopulacija regulatornih B limfocita, uglavnom u slezini, a sve produkuju IL-10. Međutim i peritonealni B limfociti produkuju IL-10, migriraju ka inflamatornim stimulusima i mogu da imaju imunoregulatornu funkciju. Pošto smo uočili ekspanziju regulatornih B limfocita u peritoneumu miševa rezistentnih na indukciju EAE, ovde razmatramo regulatorne uloge B limfocita u patogenezi EAE i moguću ulogu peritonealnih regulatornih B limfocita u rezistenciji na indukciju EAE.

Ključne reči: *multipla skleroza, EAE, B limfociti, peritonealni regulatorni B limfociti*

B cells are effector cells of the adaptive humoral immune system that act by producing specific antibodies. However, B cells express numerous innate immune receptors, including Toll-like receptor (TLR)-3, TLR4, TLR7, TLR8 and TLR9 (1,2); stimulation of these receptors also induces antibody production. It is well established that two populations of B cells exist—B1 and B2 cells—that can be distinguished according to their phenotype, ontogeny, anatomical location and function (3-5). B2 cells, which include follicular and marginal zone B cells, originate from bone marrow precursors and circulate throughout the blood and secondary lymphoid tissues. These cells respond to a broad range of T-dependent and T-independent antigens. In contrast to B2 cells, B1 cells develop during foetal and neonatal development, have the capacity to self-renew, and predominantly localize to peritoneal and pleural cavities (3-5). B1 cells are innate immune cells that produce the majority of "natural" immunoglobulins, which are encoded by germline immunoglobulin genes. These natural immunoglobulins act as a first line of defence against pathogens, such as encapsulated polysaccharide-expressing bacteria (4, 5). There are two functionally distinct subsets of B1 cells that can be delineated by differential expression of CD5 (4). B1 cells that express CD5 are known as B1a cells, and those that lack CD5 expression, but have other hallmarks of B1 cells, are known as B1b cells. $CD5^+$ peritoneal B1a cells produce an abundance of interleukin-10 (IL-10) following stimulation with TLR agonists, such as lipopolysaccharide (LPS) (6).

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B cells in multiple sclerosis

Multiple sclerosis (MS) is classically viewed as a predominantly T cell-mediated autoimmune disease, based on the finding that the disease can be induced in healthy experimental animals by the adoptive transfer of T cells from diseased animals (7,8). T cells can also have a protective role in MS. A specialized population of T cells, $CD4^+$ regulatory T cells, are critically important for disease attenuation by limiting the activation of T cells during MS and other autoimmune diseases (9,10), in part through the production of IL-10 (11). However, B cells also regulate immune responses and can contribute to MS pathogenesis (12,13) by functioning as antigen-presenting cells for $CD4^+$ T cell activation (14) and by producing pro-inflammatory cytokines that affect T cell function (15). Recent clinical trials in MS patients that used depleting CD20 monoclonal antibody (Rituximab) suggest that pan-mature B cell depletion has clinical efficacy for the treatment of MS (16,17) in addition to demonstrated efficacy for other autoimmune disorders (13). Additionally, B cells, as with T cells, can have a regulatory role in autoimmune diseases. B cells from patients with multiple sclerosis produce decreased amounts of IL-10 (18). MS patients have significantly higher frequency of $CD20^+$ B cells, but among B cell subsets they have a reduced frequency of B1 cells, which are known to have a regulatory role in immune responses (19).

Opposite roles of B cells have also been identified during the initiation and progression of an experimental model for MS, experimental autoimmune encephalomyelitis (20). Depletion of mature B cell in mice before EAE induction significantly exacerbates disease symptoms, whereas B cell depletion during EAE progression dramatically inhibits these symptoms. Thereby, the balance between opposing positive and negative regulatory B cell functions shape the normal course of EAE immunopathogenesis. Mice lacking B cells develop an extremely severe and chronic form of EAE, which confirms the regulatory role of B cells in autoimmune diseases (21). The regulatory role of B cells in EAE results from the production of IL-10 (22). A suppressive function for IL-10 produced by B cells has also been demonstrated in a models of inflammatory bowel disease and collagen-induced arthritis (23,24), suggesting a general role for IL-10-producing B cells in immune homeostasis.

Regulatory B cells

B cell subsets that can down-regulate immune responses by secreting interleukin IL-10 are known as regulatory B cells. These regulatory B cells, which are functionally defined by their immune-suppressive action either *in vitro* or *in vivo* (25), include splenic $CD21^{hi}CD23^{hi}CD1d^{hi}$ transitional 2 marginal zone precursor B cells described by the group of Mauri (26,27) and IL-10-producing B cells, termed B10 cells, characterized by the group of Tedder. The latter are mainly found within the $CD1d^{hi}CD5^+$ splenic B cell subset (28,29). Regulatory B cells must be activated to exert suppressive functions, and the activation of regulatory B cells presumably occurs *in vivo* in the context of inflammation. Activated regulatory B cells are more potent suppressors of autoimmunity than their non-activated counterparts (27,30). $CD1d^{high}CD5^+$ B cells can be induced to express cytoplasmic IL-10 following 5 hours of *in vitro* stimulation with LPS, phorbol 12-myristate 13-acetate, and ionomycin, in the presence of monensin to block IL-10 secretion. Splenic B10 pro-B cells have also been functionally identified in mice and are found within the $CD1d^{high}CD5^+$ B cell subpopulation (31). These B10 pro-B cells require 48 hours of *in vitro* stimulation with LPS or via CD40 before they acquire the ability to express cytoplasmic IL-10 (31). Although B10 cells normally represent only 1–2% of splenic B cells, they significantly inhibit the induction of Ag-specific inflammatory reactions and autoimmunity (20,29). Depletion of B10 cells in mice before disease initiation accounts for exacerbated disease, which can be ameliorated by the adoptive transfer of splenic $CD1d^{high}CD5^+$ B cells (32). There is evidence that B regulatory cell-mediated protection in chronic inflammatory diseases is antigen-specific, as B regulatory cells that are *in vivo* activated by one antigen do not protect in inflammatory models induced by a second antigen (27,29). *In vitro*, B regulatory cells can be activated in an antigen-nonspecific manner to secrete IL-10 and suppress immune activation triggered by stimuli, including activation by TLR ligands (31,33-35), CD40 ligation (36,37), a combination of these two pathways (38), or the cytokine IL-21 (39).

Regulatory B cells in MS

B regulatory cell-mediated amelioration in EAE is dependent upon IL-10. Many reports indicate that B regulatory cells can influence T cell activation. B cell-deficient mice (40) and mice with IL-10-deficient B cells (22) exhibit heightened lymph node T helper 1 (Th1)-cell responses to immunization. B-cell-mediated regulation of EAE is also associated with the suppression of Th17 cells, suggesting that IL-10 from B cells influences disease progression by instructing T cell differentiation (33). The adoptive transfer of B regulatory cells often correlates with a reduction in the frequency of interferon (IFN)-γ, IL-17 and/or tumour necrosis factor-α positive T cells (27,39,41,42), and sometimes increased amounts of $Foxp3^+$ regulatory T cells (43) or IL-10-producing T cells (44). IL-10-producing B cells may contribute to modulation of T-cell responses also indirectly by limiting dendritic cells function and by inhibition of the innate immune responses (45,46). IL-10 produced

by B cells can repress IL-6 and IL-12 production by DCs, thereby inhibiting the differentiation of Th17 and Th1 cells, respectively (33). Additionally, after immunization, DCs from B cell-deficient mice produce higher amounts of IL-12 compared with DCs from wild-type mice (40). Changes in the balance of IL-10 and IL-12 levels have important effects on the pathogenesis of EAE (47). Accordingly, B cell-mediated regulation of EAE begins in the draining lymph nodes within days of immunization. B cells can therefore orchestrate the regulation of autoimmune diseases from within secondary lymphoid organs both directly by inhibiting pathogenic cells (autoreactive T cells and innate immune cells) and indirectly by inducing regulatory activity in different T cell populations.

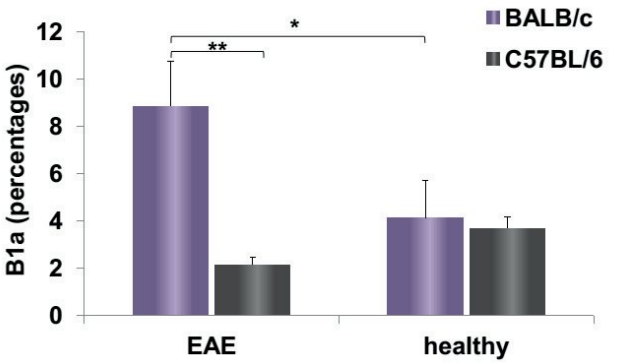

Figure 1. Immunization with $MOG_{35–55}$ induces a significant increase in peritoneal regulatory B1a cells. BALB/c and C57BL/6 mice were immunized with $MOG_{35–55}$/CFA. Then, 7 days later, mononuclear cells from the peritoneum were isolated and analysed for the cell surface expression of CD19, CD5, and CD11b by flow cytometry. The frequencies of B1a cells ($CD19^+CD11b^+CD5^+$ cells) are presented as the means from three independent experiments; 15 mice per group ± SEM; *P<0.05; ** P<0.005. Statistical significance was assessed using Student's *t*-test.

B1 cells in EAE

Although splenic regulatory B cells share some functional and phenotypical characteristics with B1a cells, as both cell populations produce IL-10 and express CD5, limited data are available about the regulatory role of B1 cells in EAE. One study showed a reduced severity of demyelination and overall pathology in the brain after the depletion of peritoneal B1 cells during the effector phase of EAE (48). Depletion during the induction phase of disease resulted in an increased incidence of progressive EAE. The experiments in this study were carried out with A.SW mice, which produce anti-MOG antibodies that contribute to EAE pathogenesis, rather than the C57BL/6 mice that are typically used. Attenuation of EAE induced by the depletion of B1 cells could be accounted for by changes in the production of natural antibodies. However, no data are yet available about the IL-10-mediated regulatory role of B1 cells in MS or EAE. It is known that components from *Mycobacterium tuberculosis* that are present in complete Freund's adjuvant, which is used to induce EAE, provide TLR agonists that can stimulate TLRs (22). Furthermore, the *in vitro* stimulation of B cells with TLR agonists produces a cytokine milieu that can inhibit T cell activation in an IL-10-dependent manner, whereas activation of DCs in the same manner induces very low amounts of IL-10 production that is not sufficient to inhibit T cell proliferation (33). Thus, stimulation with TLR agonists that are present in the adjuvants that are used for EAE induction can also trigger regulatory function in B1 cells. Our preliminary findings revealed a significantly higher percentage of $CD5^+$ B1a lymphocytes in the peritoneum of BALB/c mice, which are resistant to EAE induction with $MOG_{35–55}$ peptide (49), compared to susceptible C57BL/6 mice. By contrast, there was no significant difference in the frequencies of B1a or B1b cells in the peritoneum of healthy C57BL/6 versus BALB/c mice (Fig. 1). This finding is in accord with previous findings that *in vivo* stimulation with different microbes (TLR agonists) could induce the expansion of peritoneal B1 cells. It has been shown that among peritoneal cells, B1a cells are the main source of IL-10 after stimulation with TLR agonists (50). Immunization of susceptible strains, such as C57BL/6, with myelin antigens in adjuvants induces the expansion of inflammatory $CD4^+$ T cells that gain the capacity to induce inflammation in the CNS. Modulation of immune responses has been suggested to exacerbate or attenuate EAE in susceptible strains of mice (51). Alternatively, our previous study recently showed that alteration of an immunoregulatory pathway by deleting components of the IL-33/ST2 axis may enhance susceptibility to EAE in the resistant BALB/c strain by inducing an inflammatory phenotype in antigen presenting cells (52, 53). In accord with the significantly increased expansion of IL-10-producing peritoneal B1a cells in BALB/c mice compared with susceptible C57BL/6 mice and the fact that peritoneal B cells migrate to lymph nodes (54), it could be assumed that the increased number of peritoneal B1 cells could contribute to the regulatory phenotype of dendritic cells in draining lymph nodes and the resistance of BALB/c mice to the development of EAE.

Further studies will be needed to explore in more detail the regulatory role of B1a cells in EAE and to assess the relative contribution of splenic B regulatory cells and peritoneal regulatory B cells in the pathogenesis of EAE.

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CORRELATION BETWEEN TIMI RISK SCORE AND CLINICAL OUTCOME IN PATIENTS WITH UNSTABLE ANGINA PECTORIS

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Clinic of Cardiology, Clinical Center Kragujevac, Kragujevac, Serbia

KORELACIJA TIMI RISK SKORA I KLINIČKOG ISHODA KOD PACIJENATA SA NESTABILNOM ANGINOM PEKTORIS

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ABSTRACT

Given Taking that the TIMI score is a major predictor of MACE, this study aimed to determine the value of the TIMI risk score in predicting poor outcomes (death, myocardial infarction, recurrent pain) in patients presenting with unstable angina pectoris in short-term observation. A total of 107 patients with APns were examined at the Clinical Centre Kragujevac and were included in the investigation. The TIMI score was determined on the first day of hospitalization. During hospitalization, the following factors were also observed: troponin, ECG evolution, further therapy (pharmacologic therapy and/or emergency PCI or CABG), age, hypertension and hyperlipidaemia. The low-risk group (TIMI 0 – 2) included 30.8% of patients, whereas 47.6% of patients were in the intermediate-risk group (TIMI 3 – 4), and 21.5% of patients were in the high-risk group (TIMI 5 – 7). Good outcomes (without adverse event) and poor outcomes (death, myocardial infarction, and recurring chest pain) were dependent on the TIMI risk score. The increase in TIMI risk score per one unit increased the risk of a poor outcome by 54%. Troponin and TIMI risk score were positively correlated. Our results suggest that the TIMI risk score may be a reliable predictor of a poor outcome (MACE) during the short-term observation of patients with APns. Moreover, patients identified as high-risk benefit from early invasive PCI, enoxaparin and Gp IIb/IIIa inhibitors. Thus, routine use of the TIMI risk score at admission may reduce the number of patients not recognized as high-risk.

Keywords: *TIMI risk score, unstable angina pectoris, poor outcome*

SAŽETAK

Ako uzmemo u obzir da je TIMI risk skor glavni prediktor MACE, istraživanje je imalo za cilj da identifikuje vrednosti TIMI risk skora koji mogu da predvide loš ishod (smrt, infarkt, rekurentni bol) kod pacijenata sa nestabilnom anginom pectoris u kratkoročnom praćenju. U ispitivanje je uključeno 107 pacijenata primljenih u Klinički centar Kragujevac kao APns. TIMI risk skor je utvrđivan prvog dana hospitalizacije. Tokom hospitalizacije praćeni su i sledeći faktori: troponin, ECG evolutivnost, dalja terapija (medikamentna terapija i/ili urgentni PCI, PCI, CABG), godine, hipertenzija i hiperlipidemija. U grupi malog rizika (TIMI 0-2) je 30,8 % pacijenata, 47.6% pripadalo je umereno rizičnoj grupi (TIMI 3-4) a 21.5% je u visoko rizičnoj grupi (TIMI 5-7). Dobar ishod (bez neželjenih događaja) i loš ishod (smrt, infarkt i rekurentni bol) su u zavisnosti od TIMI risk skora. Povećanje TIMI risk skora za jedan povećavao je rizik za loš ishod za 54%. Troponin i TIMI risk skor su u pozitivnoj korelaciji. Naši rezultati ukazuju da TIMI risk skor moze biti dobar prediktor lošeg ishoda (MACE) za vreme kratkoročnog praćenja pacijenata sa APns. Pacijenti identifikovani kao visoko rizični imaju benefit od rane invazivne PCI, enoksaparina, GpIIb/IIIa. Rutinska upotreba TIMI risk skora u prijemnim ambulantama mogla bi redukovati broj pacijenata koji nisu prepoznati kao visokorizični.

Ključne reči: *TIMI risk skor, nestabilna angina pectoris, loš rezultat.*

ABBREVIATIONS

ACS - acute coronary syndrome
APns - unstable angina pectoris
CABG - coronary artery bypass grafting
MACE - major advanced cardiovascular events
NSTEMI - non-ST elevation/ST-segment depression
STEMI - ST-segment elevation
PCI - percutaneous coronary intervention
PVD - peripheral vascular disease

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INTRODUCTION

Patients with APns/NSTEMI are a heterogeneous group who may have a good prognosis with the administration of conventional therapy if they belong to the low-risk group; patients in the high-risk group require aggressive antithrombotic therapy and invasive procedures. The mortality in patients with APns is less than 1.7%, whereas the mortality in patients with NSTEMI/STEMI is less than 5.1%, which was determined by short-term observation within a period of 30 days. However, long-term observation showed a similar incidence of nonfatal events and increased mortality in patients with APns/NSTEMI. Risk stratification is of vital importance for isolating and monitoring patients in the high-risk group, as well as those whose condition shows the greatest improvement as a result of timely diagnostic and therapeutic measures (1).

HoweverTthe TIMI risk score highlights seven independent factors (based principally on risk factors): 1. older than 65 years, 2. more than three risk factors for coronary artery disease, 3. arteriographically documented coronary artery disease (50% diameter stenosis), 4. denivelation of the ST-segment ≥ 0.5 mm on ECG, 5. more than two episodes of angina during the last 24 hours, 6. consumption of aspirin in the previous week and 7. higher marker levels for myocardial necrosis. The risk of death, myocardial infarction or emergency revascularization within 14 days of observation in patients with a risk score of 2 is 8.3%, whereas it is 19.9% in patients with a risk score of 4 and 40.9% in patients with a risk score of 6-7. Thus, we conclude that 75% of patients (those with a TIMI risk score of three or more) benefit from invasive compared with conservative strategies, and from enoxaparin compared with heparin and from GP IIb/IIIa inhibitors compared with the use of placebo healing strategies (2, 3).

Given taking the above-mentioned findings, this study aimed to determine the value of the TIMI risk score with respect to MACE (death, myocardial infarction, recurrent pain) in the short-term observation of patients.

MATERIALS AND METHODS

Study population

This retrospective study included 107 patients recruited at the Clinic of Cardiology, Clinical Centre in Kragujevac, from August 2006 until the end of 2007.

Protocol

At admission and after hospitalization, all patients were diagnosed with unstable angina pectoris. All patients had their TIMI risk score determined at admission. During hospitalizsation, the following factors were also assessed: age, sex, risk factors (diabetes, hypertension, hyperlipidaemia, smoking, obesity and heredity), previous cardiovascular disease (myocardial infarction, PCI, CABG, PVD), ECG, CK, CK-MB, troponin I, CRP, cholesterol and triglycerides. Troponin I, numerically presented and analysed on VIDAS instruments, was considered negative if it was less than 0.01, borderline if it ranged from 0.01 - 0.1, and high if it was more than 0.1. Other parameters were considered qualitatively normal or high. During their stay at the hospital, low-risk patients were given pharmacologic therapy, whereas emergent or invasive PCI was administered to patients in the high-risk group. Healing outcomes were registered for all patients fourteen days after hospitalization, where poor outcomes included death, myocardial infarction and recurrent chest pain.

Patients with secondary or post-infarction angina pectoris, as well as those with a troponin I ≥ 0.1, were not included in the study.

Heterogeneous groups and the unavailability of an interventional cardiologist and medical ward for daily 24-hour catheterization during the period of examination were obstacles during the study.

Table 1. Demographic characteristics of the patients

Age (X±SD (Med; min-max))		63.94±10.39 (66; 37-83)
Sex n (%)	male	65 (60.7%)
	female	42 (39.3%)

Statistical analyses

Statistical analysis was performed with SPSS 10.0 for Windows. The results are expressed as the mean ± standard deviation (median). Data were analysed by standard statistical tests (c2-test, ANOVA, univariate binary logistic regression and multivariate binary logistic regression).

RESULTS

Demographic characteristics

The demographic characteristics of the patients are presented in Table 1. Overall, 60.7% (65) of the investigated population was male, whereas 39.3% (42) was female. The average age of was approximately 64 (37-83 years of age).

Distribution of risk factors

The distribution of risk factors is shown in Table 2. Overall, 31.8% (34) of patients had DM, 72.9% (78) had HTA, 37.4% (40) had HLP, 37.4% (40) were smokers, 6.5% (7) were obese, and 49.5% (53) had positive family anamnesis.

Table 2. Risk factors

N° Risk factor	Name	Number of patients	%
1	Diabetes	34	31.8
2	Hypertension	78	72.9
3	Hyperlipidaemia	40	37.4
4	Smoking	40	37.4
5	Obesity	7	6.5
6	Heredity	53	49.5

Table 3. Previous cardiovascular disease

Nº Risk factor	Name	Number of patients	%
1	MI	38	35.5
2	underwent PCI	7	6.5
3	underwent CABG	14	13.1
4	PVD	8	7.5
5	CVD	11	10.3

Table 4. TIMI risk score

TIMI risk score	Number of patients	%
0	1	0.9
1	10	9.3
2	22	20.6
3	24	22.4
4	27	25.2
5	17	15.9
6	5	4.7
7	1	0.9

Table 4.1. TIMI risk score scale

TIMI risk score scale	Number of patients	%
Low (1-2)	33	30.8
Intermediate (3-4)	51	47.6
High (5-7)	23	21.5

Table 5. Outcomes after 14 days

Outcome	Number of patients	%
Death	3	2.8
Myocardial infarction	3	2.8
Recurrent pain	30	28
Without adverse event	70	65.4
No data	1	0.9

The distribution of previous cardiovascular disease is shown in Table 3. Overall, 72.9% (78) of patients suffered from previous cardiovascular disease, among whom 35.5% (38) had MI, 6.5% (7) underwent PCI, 13.1% (14) underwent CABG, 7.5% (8) had PVD and 10.3% (11) had CVD.

TIMI risk score

The number of patients with a different TIMI risk score was significantly different in the different groups (c2-test; p=0.000). The most common risk score was four – in 25.2% of patients. The low-risk group (TIMI 0 – 2) included 30.8% of patients, whereas 47.6% of patients were in the intermediate-risk group (TIMI 3 – 4), and 21.5% of patients were in the high-risk group (TIMI 5 – 7) (Table 4). Moreover, 36 out of 107 patients (34.6%) had poor outcomes (recurrent pain in the chest, myocardial infarction, death) (Table 5). Among them, 25% of patients were in the low-risk group (TIMI 1-2), 41.7% of patients were in the intermediate-risk group (TIMI 3-4), and 33.3% of patients were in the high-risk group (TIMI 5-7) (Table 4.1).

Clinical outcome

Based on multivariate binary logistic regression (Table 6), it was found that the outcomes werewas dependent on the TIMI risk score (p = 0.015). An increase in the TIMI risk score per 1 unit increased the risk of a poor outcome by 40.3%, with an odds ratio of 1.403 (1.026 - 1.918).

DISCUSSION

Patients with APns/NSTEMI can have a good prognosis with pharmacologic therapy if they are in the low-risk group, or they may require more aggressive antithrombotic and invasive (PCI) therapy if they are in the high-risk group. Clinical indications of an increased risk of a fatal event in patients with APns/NSTEMI include anamnesis, clinical presentation, ECG, laboratory analysis, and coronary angiogram results (4-7). There are many methods of calculating the risk of a new cardiovascular event. In the stratification of high-risk patient groups, several scores arewere used (GRACE, PURSUIT, TIMI, HEART). All of them have virtually the same predictive value in short-term observation, whereas in long-term observation, the most valid are the GRACE score (includes heart failure, which is very common in practice) and PURSUIT score (follows the creatinine value) (8). The GRACE risk score (based on clinical presentation) is a consequence of the PURSUIT study, which has proven increased mortality in the following patients: elderly people and patients with hypotension, tachycardia, ST-segment depression, signs of heart failure and higher levels of markers of myocardial necrosis (9-11).

We showed that the risk of a poor outcome (death, myocardial infarction, recurrent chest pain) was increased by 54.1% (odds ratio 1.541, table 6) when the TIMI risk score increased per one unit, which indicated the significance of the TIMI risk score in the stratification of patients with unstable angina pectoris. The TIMI risk score was chosen due to its simple usage at admission and during short-term observation to confirm that high-risk patients have poorer outcomes (14 days after hospitalization). Consistency in applying the recommended treatment measures is increased by the isolation of high-risk patients: required hospitalization, dual anti-platelet therapy, LMWH rather than UFH, Gp IIb/IIIa inhibitors, emergent/early invasive therapy as opposed to medical therapy, regular check-ups and stricter control of heart risk factors due to poorer long-

Table 6. Univariate and multivariate binary regression

	Univariate binary regression		Multivariate binary regression	
Variable	Odds ratio	p	Odds ratio	p
TIMI risk score	1.376 (1.020 – 1.856)	0.036	1.541 (1.089 – 2.180)	0.015

term prognosis (12). Moreover, 69.1% of our patients had a TIMI risk score ≥ 3, whereas 21.5% of patients were in the high-risk group (TIMI 5 – 7).

It should be emphasized tht comparedison with other previously reported scores, the TIMI risk score possesses an equal predictive value to that of GRACE or PURSUIT in short-term observation. However, TIMI can be constructed at admission (within the first hours of hospitalization) because it simple and does not require the clinical status of the patient. Otherwise, the GRACE score is primarily used in clinical practice and involves following the clinical status of the patient; thus, it is more reliable in long-term observation (2). Thus, it is obvious that the TIMI risk score is the most prompt stratification testand is and is particularly convenient for the emergency department.

The EXTRACT-TIMI 25 trial with enoxaparin in patients with APns/NSTEMI has shown a decrease in mortality, myocardial infarction and recurrent ischaemia by 20% compared with UFH, especially in patients with ST-segment depression, higher levels of troponin I (with normal CK-MB values) and TIMI risk scores ≥ 3 (13).

The present results indicate that (higher) TIMI scores affect the type of therapy. Namely, in our study, every tenth patient was treated with enoxaparin, although the TIMI risk score was ≥3, which was a consequence of the high price of enoxaparin.

In the TACTIS-TIMI 18 study, patients were treated with aspirin, heparin and the GP IIb/IIIa inhibitor tirofiban. Early invasive therapy (up to 48 hours) showed a benefit in patients with ST-segment changes (10% absolute risk reduction) and with positive troponin (39% risk reduction). The patients who benefited most from early invasive therapy were those with intermediate- (3-4) and high-risk (5-7) TIMI scores (14).

In the FRISC II study, 2457 patients with APns/NSTEMI were treated conservatively, or their arteriography was postponed because they had refractory angina (despite maximal anti-anginal therapy) or a positive cardiac stress test with ST-segment depression ≥0.3 mV within the first four days of symptom onset. Only 9% of patients fulfilling these criteria were treated with revascularization within a period of seven days. Six months later, the risk of death or myocardial infarction was significantly decreased in the group that received invasive therapy (9.4/12.1%) and was stable after one year (2.2/3.9%) (15).

The conditions of all patients with a TIMI risk score ≥3 (two-thirds of the patients in our study) improved after early invasive therapy; hence, medical wards for arteriography should be made more accessible, at least for high-risk patients.

In the short-term observation of patients with unstable angina (within 14 days of their hospitalization), 33.6% had poor outcomes, including death, myocardial infarction or recurrent pain. Most of these patients had recurrent chest pain; 28% showed signs of acute coronary disease instability (Table 5).

A positive correlation between TIMI risk score and troponin values indicated that patients with angina pectoris and borderline troponin values have an increased risk of a poor outcome, which may be an additional benefit of the utilization of the TIMI risk score.

CONCLUSION

The present study noteddemonstrated that the TIMI risk score was a reliable predictor of a poor outcome in the short-term observation of patients with unstable angina pectoris. The TIMI risk score is the most prompt stratification testand is and is particularly convenient for the emergency department. A high TIMI risk score, which can be determined as soon as the patient is hospitalized, determines the course of subsequent treatments.

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EFFECTS OF PROVINOLS ON CARDIODYNAMICS AND CORONARY FLOW IN ISOLATED RAT HEARTS

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EFEKTI PROVINOLA NA KARDIODINAMIKU I KORONARNI PROTOK IZOLOVANIH SRCA PACOVA

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ABSTRACT

Provinols are an alcohol-free extract of red wine that contains a wide range of polyphenols. Polyphenols are a group of chemical compounds found in diverse plants. Polyphenols are considered to protect against cardiovascular disease. Although some older epidemiological studies have indicated that the positive effects of red wine on heart disease can be attributed to the alcohol content alone, there is now powerful evidence that polyphenols present in red wine are responsible for these positive effects. The hearts of male Wistar albino rats (n = 36, 12 in each experimental group, 10 weeks old, body mass 250 ± 30g) were excised and retrogradely perfused according to the Langendorff technique at a gradually increasing perfusion pressure (40-120 cmH_2O). Parameters of cardiac function (dp/dt max, dp/dt min, SLVP, DLVP, HR, CF) were measured after perfusion with three different concentrations of provinols (5 μg/ml, 10 μg/ml and 50 μg/ml). Administration of the highest dose (50 μg/ml) induced a significant increase in dp/dt max, dp/dt min, HR and CF compared with control conditions at CPP = 40 cmH_2O, while an intermediate dose increased dp/dt max at the same CPP. Generally viewed, the results of the present study suggest that provinols may have a beneficial effect on the intact myocardium and coronary circulation. These findings could constitute an important step in further investigation of these polyphenols under different representative experimental conditions in the heart, as well as providing a good basis for potential clinical studies in this field.

Keywords: *Provinols, Isolated rat heart, Langendorff technique, Cardiodinamics, Coronary flow*

SAŽETAK

Provinoli predstavljaju bezalkoholni ekstrakt crvenog vina koji sadrži širok spektar polifenola. Polifenoli su grupa hemijskih jedinjenja koje se nalaze u različitim vrstama biljaka. Smatra se da mogu da umanje rizik od nastanka kardiovaskularnih bolesti. Iako su ranije epidemiloške studije ukazale da pozitivni efekti crvenog vina na razvoj oboljenja srca potiču samo od alkoholne komponente, sada postoje čvrsta saznanja da su zapravo polifenoli zaslužni za ove efekte. Srca muških Wistar albino pacova (n = 36, 12 u svakoj grupi, 10 nedelja starosti, telesne mase 250 ± 30g) su izolovana i retrogradno perfundovana prema modifikovanoj Langendorff-ovoj tehnici, uz postupno povećawe koronarnog perfuzionong pritiska (40-120 cmH_2O). Parametri srčane funkcije (dp/dt max, dp / dt min, SLVP, DLVP, HR, CF) su registrovani tokom perfuzije sa tri različite doze Provinola (5 μg/ml, 10 μg/ml i 50 μg/ml). Administracija najviše doze (50 μg/ml) je uzrokovala značajno povećanje vrednosti dp/dt max, dp/dt min, HR i CF u poređenju sa kontrolnim uslovima (pri CPP = 40 cmH_2O), dok je srednja doza povećala samo vrednosti dp/dt max na istom CPP. Generalno posmatrano, rezultati sadašnje studije sugerišu da Provinoli mogu da imaju pozitivne uticaje i na zdrav miokard i koronarnu cirkulaciju. Ova saznanja mogu da budu važan korak u daljem ispitivanju ovih polifenola u različitim eksperimentalnim uslovima na srcu, kao i dobra osnova za potencijalne kliničke studije iz ove oblasti.

Ključne reči: *Provinoli, Izolovano srce pacova, Langendorff-ova tehnika, Kardiodinamika, Koronarni protok*

ABBREVIATIONS

CF - Coronary flow
CPP - Coronary perfusion pressure
FDRW - Freeze-dried red wine
LDL - Low density lipoprotein
NO - Nitric oxide
VGSCs - Voltage-gated sodium channels

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INTRODUCTION

Provinols are an alcohol-free extract of red wine that contains wide range of polyphenols (1). These extracts have been studied in association with the discovery of the "French Paradox", describing a low incidence of cardiovascular disease in French people despite a diet rich in saturated fats. Researchers believe that this may be due to high consumption of wine in this country (2). Although some older epidemiological studies have indicated that the positive effects of red wine on heart disease can be attributed to the alcohol content alone (3, 4), there is powerful evidence that polyphenols present in red wine are responsible for these positive effects (5-7).

Polyphenols are a group of chemical compounds found in a vast variety of plants (8). Polyphenols are considered to protect against cardiovascular disease (9, 10) and some cancers (11). Additionally, a large number of studies have demonstrated strong antioxidant properties and inhibition of the peroxidation of polyunsaturated fatty acids (12). These properties of polyphenols can be explained by low density lipoprotein and platelet aggregation (13). Previous studies on red wine polyphenols have shown positive effects on the oxidation of LDL-cholesterol (14), arterial hypertension (13) and vasorelaxation (15). Based on these properties, diets supplemented with foods containing polyphenols might also protect various tissues against heart injury.

Reports describing the oral administration of red wine polyphenolic compounds, including provinols, indicate their ability to decrease blood pressure in normotensive rats (16). In addition, an accelerated decrease in blood pressure and improvement of structural and functional cardiovascular characteristics occur as a consequence of chronic inhibition of nitric oxide (NO) synthesis (17). All of these effects of provinols are associated with a greater increase in NO synthase (NOS) activity in the left ventricle and aorta (17, 18). Because of the above properties, polyphenols may interfere with the atherogenesis process and/or the thrombotic phenomena associated with atherosclerosis, which could at least partially explain the beneficial effects of these substances.

On the other hand, there is a lack of data on the direct effects of red wine polyphenols on myocardial function, especially on coronary circulation in the intact heart. Thus, this study aimed to assess the direct and acute influence of provinols on cardiac function and coronary flow, using an isolated rat heart model.

MATERIALS AND METHODS

Isolated heart preparation

Hearts of male Wistar albino rats (n = 36, 12 in each experimental group, 10 weeks old, body mass 250 ± 30 g) were isolated and perfused via retrograde perfusion using the Langendorff technique (Langendorff apparatus, Experimetria Ltd, 1062 Budapest, Hungary). After brief ketamine/xylazine narcosis, the animals were euthanized via cervical dislocation (Schedule 1 of the Animals/Scientific procedures, Act 1986, United Kingdom) and premedicated with heparin. This was followed by immediate thoracotomy and sudden cardiac arrest induced by superfusion with ice-cold isotonic saline. The hearts were rapidly excised, and the aortas were cannulated and retrogradely perfused at a pressure in the range of 40 to 120 cmH_2O. They were subsequently perfused in a reverse fashion via the aorta with Krebs–Henseleit solution (nutrient-rich, oxygenated solution). The composition of the nonrecirculating Krebs-Henseleit perfusate was as follows (mM/L): NaCl 118, KCI 4.7, $CaCl_2 \cdot 2H_2O$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.7, $NaHCO_3$ 25, KH_2PO_4 1.2, and glucose 11, equilibrated with 95% O_2 plus 5% CO_2 and warmed to 37 °C (pH 7.4).

All experimental procedures were performed in accordance with prescribed legislation (EU Directive for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes 86/609/EES) and the principles of ethics.

Physiological Assay and Experimental Protocol

After successful heart perfusion at a CPP of 60 cmH_2O, a 30 min period was allowed for stabilisation of the preparation. To test coronary vascular reactivity, all hearts were challenged via short-term occlusion (5–30 s), followed by a bolus injection of 5 mM/L adenosine (60 µL at a flow rate of 10 mL/min to elicit maximum coronary flow (CF)) during the stabilisation period. The hearts were discarded if the flow did not increase by 100% over the control value for both tests (approximately 25% of hearts). When the flow was considered stable (three measurements of the same values), coronary effluent samples were collected.

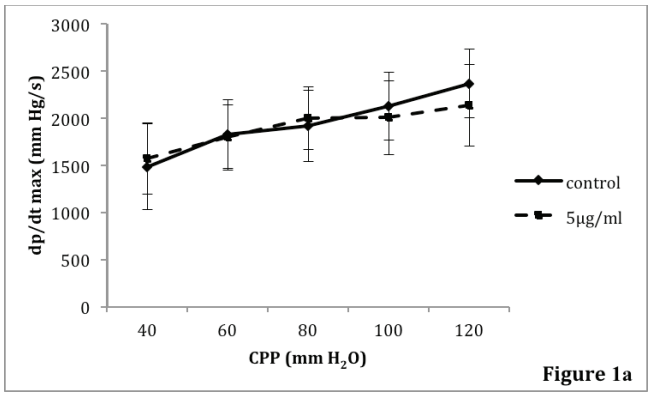
After control sets of experiments (control conditions), hearts were perfused with

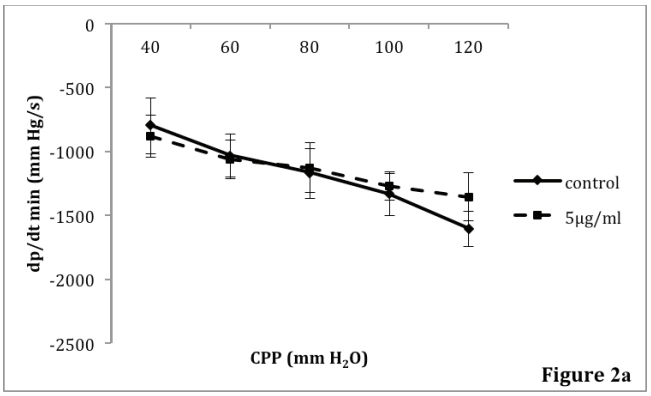
(1) provinols at a dose 5 µg/ml
(2) provinols at a dose 10 µg/ml
(3) provinols at a dose 50 µg/ml.

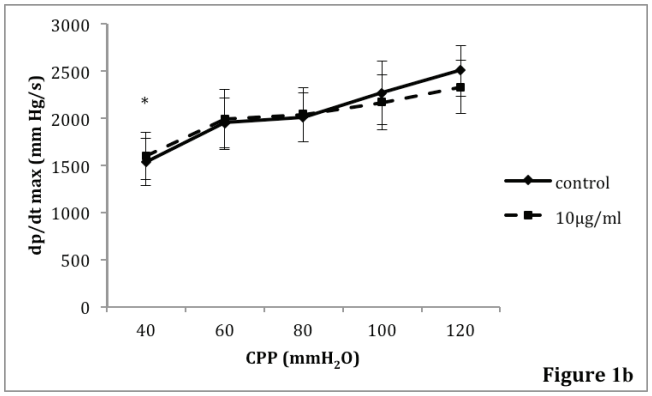
After establishing a stable heart rate, removal of the left atrium and rupture of the mitral valve allowed a sensor to be inserted (transducer BS4 73-0184, Experimetria Ltd., Budapest, Hungary) in the left ventricle for direct and continuous monitoring of the following parameters of left ventricular function:

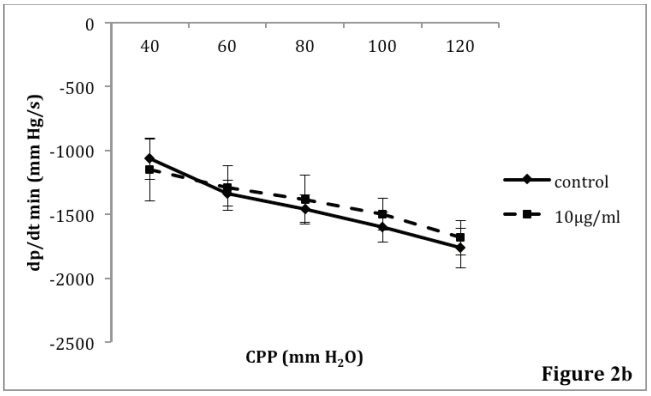
1. dp/dt max - maximum rate of pressure development in the left ventricle,
2. dp/dt min - minimum rate of pressure development in the left ventricle,
3. SLVP - systolic pressure of the left ventricle,
4. DLVP - diastolic pressure of the left ventricle,
5. HR - heart rate.

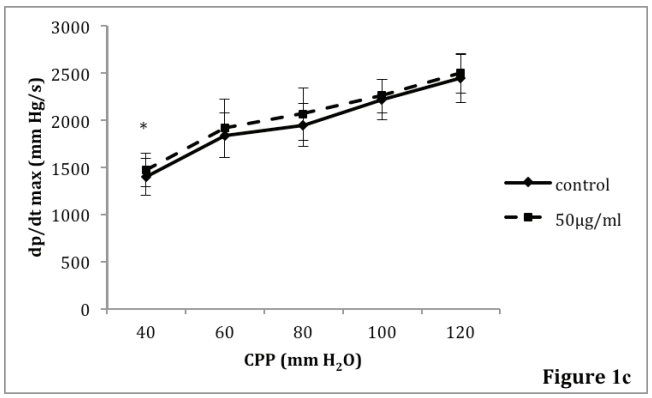
Coronary flow was measured using flowmetry. The substances tested within a series of acute experiments were administered via continuous perfusion under changing perfusion pressures, starting from a pressure of 60 cmH_2O, followed by 80 cmH_2O, 100 cmH_2O, and 120 cmH_2O, and finally, 40 cmH_2O at end of the experiment. For each perfusion pressure, functional parameters of the left ventricle were registered.











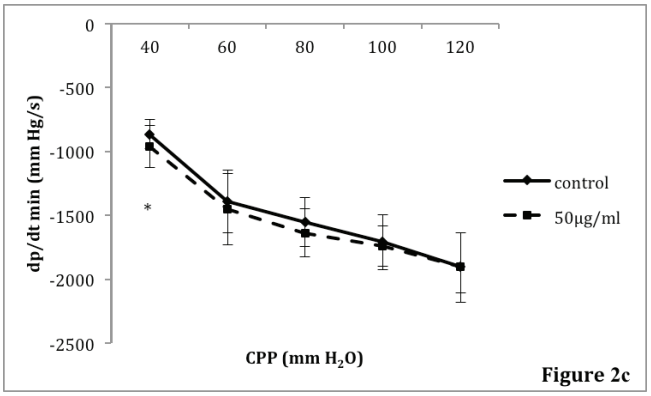


Figure 1a-c. The effects of 5 µg/ml provinol (1a), 10 µg/ml provinol (1b) and 50 µg/ml provinol (1c) on dp/dt max. The values represent X ± SE; *p<0.05, **p<0.01

Figure 2a-c. The effects of 5 µg/ml provinol (2a), 10 µg/ml provinol (2b) and 50 µg/ml provinol (2c) on dp/dt min. The values represent X ± SE; *p<0.05, **p<0.01

In the experimental work, the rules regarding the welfare of laboratory animals and the rules for the use of experimental animals of the Faculty of Medical Sciences, University of Kragujevac, were respected, which are compliant with the European Directive in this area.

Drug

Provinols were purchased from the French company VITIMED Groupe UDM Distillerie du Vivarais (Route de Ruoms BP 47, 07150 Vallon Pont d'Arc France).

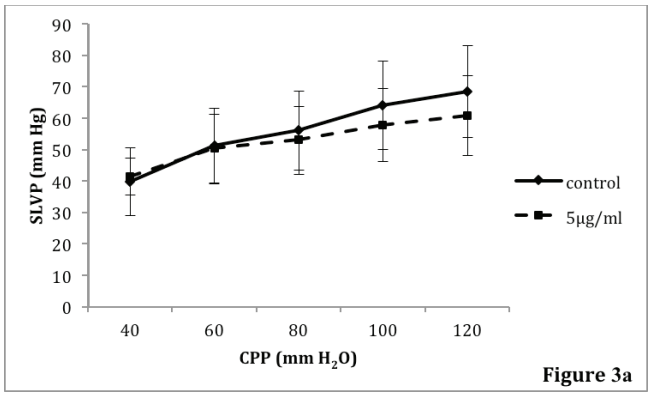
Statistics

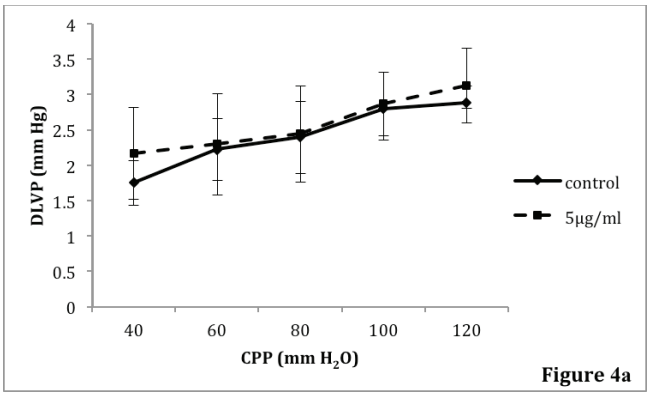
The statistical analysis of the experimental data included the following basic descriptive statistics: the mean value (X) ± the standard deviation (SD). The following statistical test was used to test the statistical significance of the results and to confirm the hypothesis: paired-samples T test. A database analysis of the results was performed using the software package SPSS 20th (SPSS Inc., Chicago, IL, USA). P values lower than 0.05 (p<0.05) were considered to be significant, while P values lower than 0.01 (p<0.01) were considered to be highly significant.

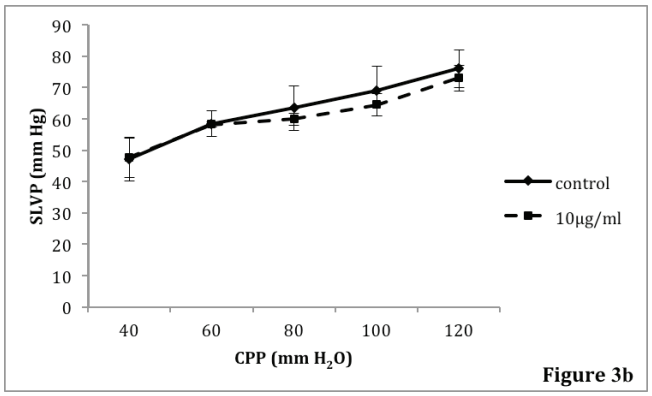
RESULTS

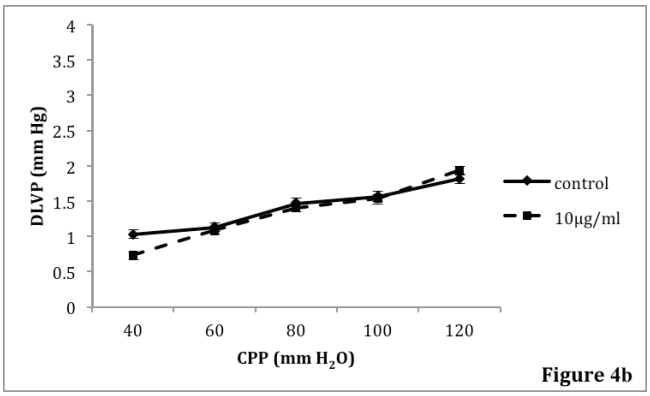
Maximum rate of pressure development in the left ventricle (dp/dt max)

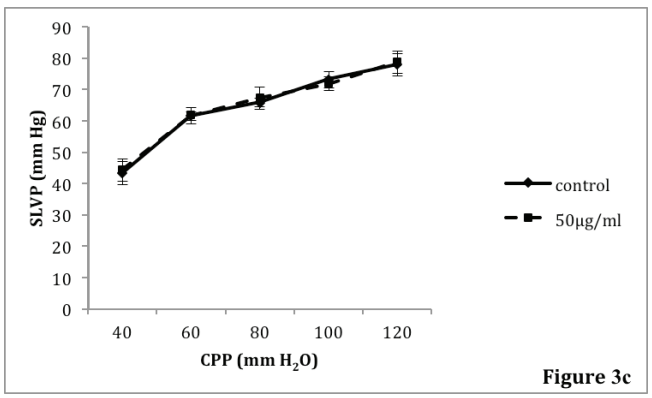
Parameters related to contractile force and systolic performance showed no significant changes between the control and











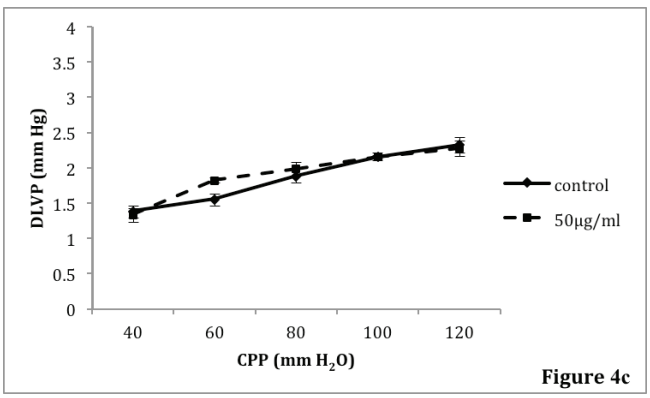


Figure 3a-c. The effects of 5 µg/ml provinol (3a), 10 µg/ml provinol (3b) and 50 µg/ml provinol (3c) on SLVP. The values represent X ± SE; *p<0.05, **p<0.01

Figure 4a-c. The effects of 5 µg/ml provinol (4a), 10 µg/ml provinol (4b) and 50 µg/ml provinol (4c) on DLVP. The values represent X ± SE; *p<0.05, **p<0.01

the lowest dose of provinols (5 µg/ml) when compared at the same CPP over the entire CPP range (p>0.05) (Fig 1a). Administration of the intermediate (10 µg/ml) and highest (50 µg/ml) doses induced a significant increase in dp/dt max compared with the control conditions at CPP = 40 cmH_2O (p<0.05) (Figs 1b, 1c).

Minimum rate of pressure development in the left ventricle (dp/dt min)

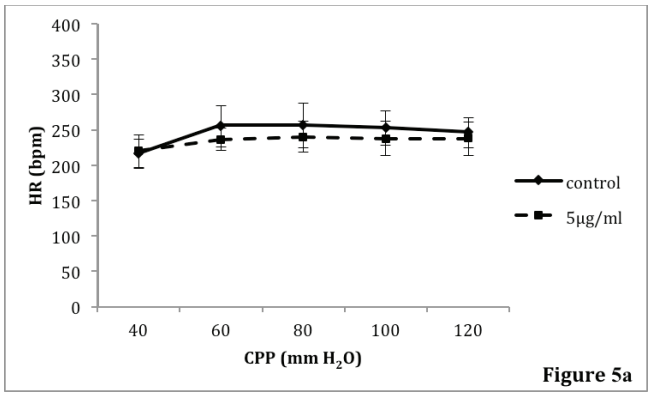
There were no statistically significant changes in the values of parameters describing the lusitropic effect (diastolic function) during the application of 5 µg/ml provinols or 10 µg/ml provinols over the entire CPP range (p>0.05) (Figs 2a, 2b). Perfusion with the highest dose of provinols (50 µg/ml) induced a significant increase in dt/dp min at CPP = 40 cmH_2O (p<0.05) (Figure 2c).

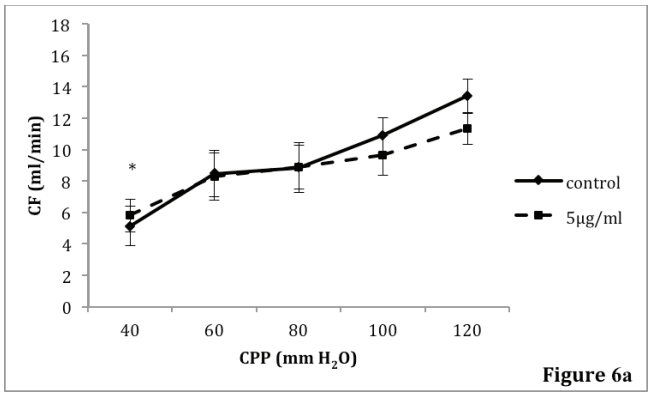
Systolic pressure of the left ventricle (SLVP)

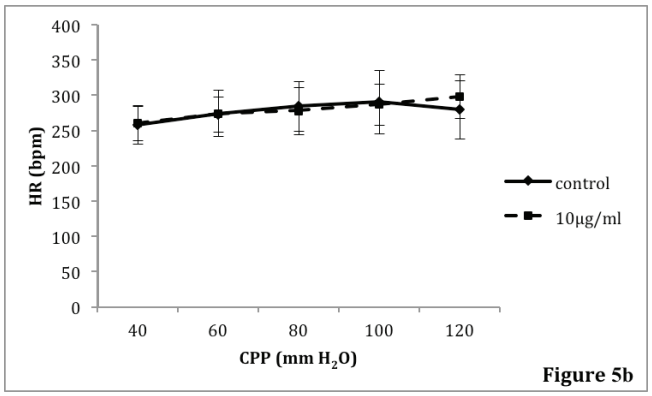
After the administration of provinols at doses of 5 µg/ml, 10 µg/ml and 50 µg/ml, we did not observe any statistically significant changes in systolic left ventricule pressure or parameters of myocardial function over the entire CPP range (p>0.05) (Figs 3a, 3b, 3c).

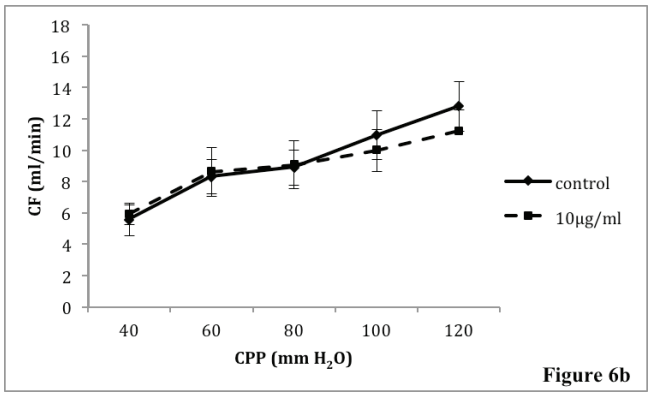
Diastolic pressure of the left ventricle (DLVP)

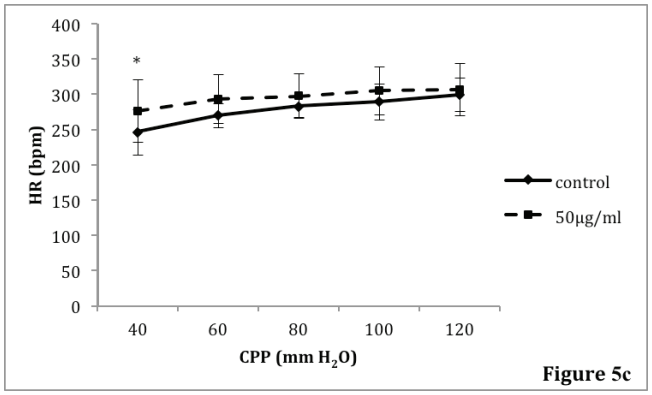
Diastolic left ventricular pressure did not change significantly with an increase in CPP in the control or in all other groups (p>0.05). There was no significant difference between the control and any of the groups at any of the set CPPs (p>0.05) after the administration of all three doses of provinols (5 µg/ml, 10 µg/ml and 50 µg/ml) (Figs 4a, 4b, 4c).











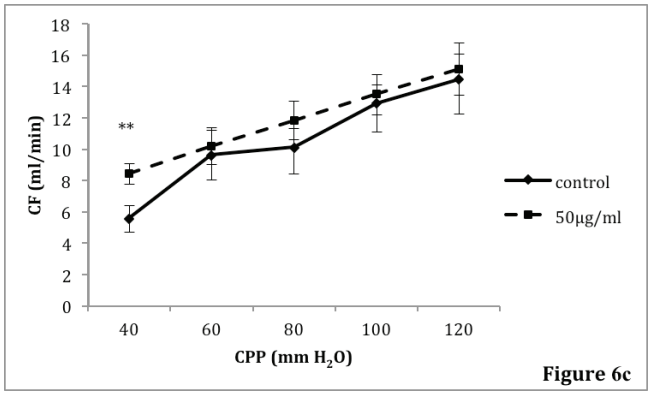


Figure 5a-c. The effects of 5 µg/ml provinol (5a), 10 µg/ml provinol (5b) and 50 µg/ml provinol (5c) on HR. The values represent X ± SE; *p<0.05, **p<0.01

Figure 6a-c. The effects of 5 µg/ml provinol (6a), 10 µg/ml provinol (6b) and 50 µg/ml provinol (6c) on CF. The values represent X ± SE; *p<0.05, **p<0.01.

Heart rate (HR)

The heart rate did not change significantly under the lowest (5 µg/ml) and intermediate (10 µg/ml) doses of provinols with an increasing CPP (p>0.05) (Figs 5a, 5b). The administration of the highest dose of provinols (50 µg/ml) induced a significant increase in HR (p>0.05) compared with control conditions (Figure 5c).

Coronary flow (CF)

This parameter was significantly increased after the application of provinols at dose of 5 µg/ml (p<0,05) at CPP = 40 cmH_2O (Figure 6a). After the administration of an intermediate dose of provinols (10 µg/ml), there was no significant difference detected over the entire CPP range (p>0.05) (Figure 6b). Compared with the controls, the coronary flow was increased in the 50 µg/ml group at CPP = 40 cmH_2O (p<0,01) (Figure 6c).

DISCUSSION

As previously noted, red wine polyphenols have been reported to possess beneficial properties for the prevention of cardiovascular diseases (13, 19), but the molecular mechanisms underlying their haemodynamic effects on the cardiovascular and renal systems are much more poorly understood (19). Polyphenolic compounds have been documented to relax precontracted smooth muscle of arteries with an intact endothelium. Moreover, some of these compounds were shown to relax endothelium-denuded ar-

teries (20, 21). Another therapeutically relevant effect of flavonoids may be their ability to interact with the generation of NO from the vascular endothelium, which leads not only to vasodilatation but also to the expression of genes that protect the cardiovascular system (22-24). Provinols have been shown to improve human endothelial vascular function (25) and reduce blood pressure in animal studies (16, 17, 19), but the results of human intervention studies investigating the effect of red wine polyphenols on blood pressure are inconsistent.

The aim of the present study was to assess the influence of acute administration of provinols on cardiac function and coronary flow in the isolated rat heart.

Cardiac contractility was estimated according to the maximum and minimum rate of left ventricle pressure development (dp/dt max and dp/dt min). The first parameter (dp/dt max) represents an indirect indicator of the contractile force of the myocardium (inotropic properties), while dp/dt min reflects the ability of the cardiac muscle to relax during diastole (lusitropic properties). The lowest dose of provinols did not induce any significant changes in the contractile force of the myocardium (dp/dt max), while the intermediate and the highest doses increased the values of this marker (at CPP=40 cmHg) (Figs 1b, 1c). Another parameter related to contractility (dp/dt min) showed the same trend of reactivity (Figs 2a-c). Furthermore, our results revealed a decrease in SLVP (Figs 3a-c) and DLVP (Figs 4a, 4b, 4c) following the acute administration of provinols at all tested doses. Using the similar study model, Ferrara and coauthors examined the effects of freeze-dried red wine (FDRW) on cardiac function and ECG in Langendorff-perfused rat hearts. These authors noted reduced left ventricular pressures, but at a 10 percent higher concentration in comparison with our highest dose (26). However, FDRW has a different content of polyphenols then red wine extracts produced without using a freeze-drying technique.

For HR, the results of present study showed that the highest dose of provinols induced an increase of HR, while the other doses did not significantly change this parameter (Figs 5a, 5b). Dillenburg and colleagues recently investigated the effects of the red wine polyphenol resveratrol on HR and other haemodynamic parameters in hypertensive rats. Their results indicated that resveratrol did not alter HR values in these rats (16). Differences between this previous study and the present study regarding the different experimental models used may be a likely explanation for the obtained results. On the contrary, human studies have documented that daily consumption of red wine (40 grams) for 4 weeks results in an increased 24-hour systolic HR in normotensive humans (27). Based on all of the above results, it seems that red wine may alter HR predominantly in the absence of hypertensive conditions.

The data describing the potential mechanism underlying the effects of polyphenols on the heart are still insufficient. Studies on the effects of the red grape polyphenols quercetin, catechin and resveratrol on cardiac voltage-gated sodium channels (VGSCs) suggest that some of their cardioprotective effects may involve inhibition of the Na^+ current (28). This protective mechanism involves improved myocyte calcium handling and contractility, downstream of the inhibition of the late Na^+ current.

On the other hand, the influence of red wine extracts on the coronary endothelium are less well understood. CF was increased after the administration of the lowest and highest doses of provinols tested in this work (Figs 6a, 6c), as shown by Shimada et al., who investigated the effects of red wine in healthy volunteers (29). The positive effects of polyphenols on the coronary endothelium could be related to a reduced oxidant status and increased production of NO, as indicated by the increase in NO synthase activity in both cardiac and aortic tissues (13, 17, 19). Such enhanced NO production could contribute to the relaxation of vascular smooth muscles and the increase in CF induced by provinols.

CONCLUSIONS

Generally viewed, the results of the present study suggest that provinols may have a beneficial effect on the intact myocardium and coronary circulation. These findings could be an important step in further investigation of these polyphenols under different representative experimental conditions in the heart (ischaemic/reperfusion injury), as well as providing a good basis for potential clinical studies in this field.

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Disclosures

The authors declare that they have no conflicts of interests relevant to the manuscript.

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LIVOLIN FORTE® AMELIORATES CADMIUM-INDUCED KIDNEY INJURY IN WISTAR RATS

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LIVOLIN FORTE* UMANJUJE KADMIJUMOM-INDUKOVANO OŠTEĆENJE BUBREGA KOD VISTAR PACOVA

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ABSTRACT

The kidney, which is an integral part of the drug excretion system, was reported as one of the targets of cadmium toxicity. Early events of cadmium toxicity in the cell include a decrease in cell membrane fluidity, breakdown of its integrity, and impairment of its repair mechanisms. Phosphatidylcholine and vitamin E have a marked fluidizing effect on cellular membranes. We hypothesized that Livolin forte® (LIV) could attenuate kidney damage induced by cadmium in rats. Twenty-five adult male Wistar rats were divided into five groups of five rats each: group I (control group) received 0.3 ml/kg/day of propylene glycol for six weeks; group II was given 5 mg/kg/day of cadmium (Cd) i.p for 5 consecutive days; group III rats were treated in a similar way as group II but were allowed a recovery period of 4 weeks; group IV was treated with LIV (5.2 mg/kg/day) for a period of 4 weeks after inducing renal injury with Cd similarly to group II; and group V was allowed a recovery period of 2 weeks after a 4-week LIV treatment (5.2 mg/kg/day) following Cd administration. A significant increase in plasma creatinine, urea, uric acid, and TBARS were observed in groups II and III compared to the control rats. Significant reductions in total protein, glucose, and GSH activity were also recorded. The urine concentrations of creatinine, urea, and uric acid in groups II and III were significantly lower than the control group. This finding was accompanied by a significant decrease in creatinine and urea clearance. Post-treatment with LIV caused significant decreases in plasma creatinine, urea, uric acid, and TBARS. Significant increases in total protein, glucose, and GSH activity of groups IV and V were observed compared to group II. A significant increase in urine concentrations of creatinine, urea, and uric acid and significant decreases in total protein, glucose, and GSH activity were observed in groups IV and V compared to group II. Photomicrographs of the rat kidneys in groups IV and V showed an improvement in the histology of their renal tissue when compared to group II, with features similar to the control rats. Additionally, group III showed an improvement in the histoarchitecture of the kidney compared with group II, although occasional atrophy of some glomeruli and shrinking of renal corpuscles was observed.

SAŽETAK

Bubreg, koji je sastavni deo ekskrecionog sistema lekova, je opisan kao jedan od ciljeva toksičnosti kadmijuma. Rani događaji toksičnosti kadmijuma u ćeliji uključuju smanjenje propustljivosti ćelijske membrane, narušavanje integriteta i oštećenje mehanizama reparacije. Fosfatidilholin i vitamin E imaju izrazit efekat na propustljivost ćelijske membrane. Naša pretpostavka je da Livolin FORTE® (LIV) može ublažiti oštećenje bubrega indukovanu kadmijumom u pacova. Dvadeset pet odraslih muških pacova Vistar soja, su podeljena u pet grupa sa po pet pacova u svakoj: grupa I (kontrolna grupa) primila je 0,3 mL/kg/dan propilen glikola šest nedelja; grupi II je administriran kadmijum (Kd) u dozi od 5 mg/kg/dan i.p. tokom 5 uzastopnih dana; grupa III- pacovi su tretirani na sličan način kao grupa II ali je dozvoljen period oporavka od 4 nedelje; grupa IV je tretirana LIV (5,2 mg / kg / dan) u periodu od 4 nedelje nakon izazivanja renalne povrede sa Kd slično grupi II; i grupa V je dozvoljen period oporavka od 2 nedelje posle 4 nedelje LIV tretmana (5,2 mg/kg/dan) nakon Kd administracije. Značajno povećanje nivoa kreatinina, uree, mokraćne kiseline i TBARS-a u plazmi zabeženo je u grupama II i III odnosu na vrednosti ovih parametara u kontrolnoj grupi. Primećeno je značajno smanjenje ukupnih proteina, glukoze i GSH aktivnosti u grupama IV i V u odnosu na grupu II. U urinu, značajno su povećane vrednosti kreatinina, uree i mokraćne kiseline i značajno smanjenji ukupni proteini, glukoza i aktivnost GSH-a u grupi IV i V u odnosu na grupu II. Fotomikrografije bubrega pacova u grupi IV i V su pokazale poboljšanje histološkog nalaza bubrežnog tkiva u odnosu na II grupu, sa karakteristikama sličnim u kontrolnoj grupi. Pored toga, u grupi III je primećeno poboljšanje u histoarhitekturi bubrega u poređenju sa grupom II, iako je zapažena mestimična atrofija nekih glomerula i skupljanje renalnih korpuskula. Rezultati ove studije ukazuju da LIV administracija ublažuje oštećenje bu-

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In conclusion, the results of this study indicated that LIV administration ameliorated Cd-induced kidney injury in rats. Thus, LIV represents a prospective therapeutic choice to prevent kidney injury inflicted by Cd exposure.

Keywords: *Cadmium, Livolin forte®, Phosphatidylcholine, Vitamin E, Rat*

brega izazvano kadmijumom (Kd). Dakle, LIV predstavlja potencijalno terapijsko sredstvo u prevenciji oštećenja bubrega uzrokovano izlaganjem kadmijumu.

Klučne reči: *Kadmijum, Livolin Forte*, fosfatidilholine, Vitamin E, pacov*

INTRODUCTION

Heavy metals exposure has become an increasingly recognized source of illness worldwide. Cadmium is a ubiquitous heavy metal in the environment and a known industrial pollutant. Exposure to cadmium through food, water, and occupational sources has been known p to cause a variety of adverse effects. Cadmium is known to cause reproductive disorders, renal and hepatic dysfunction, osteomalacia, neurological impairment, and pancreatic activity changes (1). Additionally, various structures and metabolic processes can be affected, such as nucleic acids, carbohydrates, energy metabolism, protein synthesis, and enzyme systems (2). Inhalation of cadmium causes respiratory stress and injury to the respiratory tract. Emphysema and chronic rhinitis have been linked to high cadmium concentrations in polluted air. Reduction in forced expiratory volume and a high incidence of respiratory distress syndrome were reported among people exposed to cadmium (3). Cadmium was also reported to be injurious to the heart (4). The kidney, which is an integral part of the drug excretion system, was reported as one of the targets of cadmium toxicity (5, 6). Long-term ingestion of cadmium causes kidney damage, the initial signs of which are proteinuria and ß$_2$ microglobulinuria (7).

Early events in cadmium toxicity in the cell include a decrease in cell membrane fluidity, breakdown of its integrity, as well as impairment of its repair mechanisms. All of these changes are associated with a number of disorders, including kidney and neurological diseases, various cancers, and cell death (8, 9). Phosphatidylcholine, particularly phosphatidylcholine rich in polyunsaturated fatty acids, has a marked fluidizing effect on cellular membranes.

Vitamin E, an antioxidant, is presumed to be incorporated into the lipid bilayer of biological membranes to an extent proportional to the amount of polyunsaturated fatty acids or phospholipids in the membrane (10). Because of its hydrophobic nature, vitamin E is readily held within the hydrophilic lipid region of the membrane and lipoprotein where its ability to quench free radicals becomes readily useful (11). As an important antioxidant, Vitamin E may interfere with cadmium toxicity by preventing auto-oxidation of cell membranes.

Livolin Forte® (Mega Lifesciences (Australia) Pty. Ltd.) is a drug that is used in the treatment and management of liver diseases. This agent basically contains phospholipids with vitamins including essential phospholipids-polyunsaturated phosphatidylcholine (300 mg), vitamin B_1 (thiamine mononitrate, 10 mg), vitamin B_2 (riboflavin, 6 mg), vitamin B_6 (pyridoxine HCl, 10 mg), vitamin B_{12} (cyanocobalamin, 10 mcg), nicotinamide (30 mg), and vitamin E acetate (alpha tocopheryl acetate, 10 mg). Livolin forte® (LIV) has been shown to promote a rapid arresting of clinical symptomatology and normalization of biochemical indices in patients with steatohepatitis (12). Additionally, LIV ameliorates the elevation in alanine transaminase in HIV-infected patients when commencing highly active antiretroviral therapy (13). LIV has also been reported to exhibit a significant hepato-protective effect in acute ethanol-induced fatty liver in Wistar rats (14).

Based on the constituents in LIV, we hypothesized that LIV could attenuate renal damage induced by cadmium in rats. Therefore, this study aimed at investigating the effect of LIV on cadmium-induced kidney injury in Wistar rats.

MATERIALS AND METHODS

Drug and Chemicals

Livolin forte® from Mega Lifesciences (Pakenham, VIC, Australia; batch number 107050), cadmium sulphate from Guangzhou Fischer Chemical Co., Ltd., Guangdong, and propylene glycol (Biovision, Milpitas, CA, USA) were the agents used in this study.

Drug Preparation

Each capsule containing 366 mg of LIV was dissolved in 20 ml of propylene glycol, after which 0.04 ml of the solution (equivalent to 0.78 mg of LIV) was administered orally to a 150 g rat. This dosage is equivalent to 5.2 mg/kg, which is the therapeutic dose of the drug in humans.

Animal Care and Management

Twenty (25) adult male Wistar rats weighing 140 g - 190 g were obtained from the Animal House of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife and allowed to acclimatize in the laboratory for two weeks before the commencement of the study. The

rats were kept under normal environmental conditions with a natural light/dark cycle and were fed a standard rodent pellet diet (Caps Feed PLC, Osogbo, Nigeria) and water *ad libitum*. They were housed individually in separate metabolic cages (Ohaus R Model; Ohaus, Pine Brook, New Jersey, USA) during the experiment to obtain a 24-hr urine sample. The experimental procedures adopted in this study were in strict compliance with Experimental Animal Care and Use of Laboratory Animals in Biomedical Research, College of Health Sciences, Obafemi Awolowo University, Ile-Ife.

Experimental Design

The rats were divided into five (5) groups of 5 rats each as follows: Group I (control group) received 0.3 ml/kg/day propylene glycol orally throughout the course of the study (6 weeks). Group II was administered cadmium (Cd) alone, 5 mg/kg/day (i.p), for 5 consecutive days to induce renal injury. The rats were sacrificed 24 hours after the last day of Cd administration. Group III (recovery group) was treated similarly to Group II, but the rats were allowed a recovery period of 4 weeks (after Cd intoxication) without treatment with LIV. Group IV was treated with LIV (5.2 mg/kg/day) for a period of 4 weeks after inducing renal injury with Cd similarly to Group II. Group V was allowed a recovery period of 2 weeks after a 4-week LIV treatment (5.2 mg/kg/day) following Cd-induced renal injury, after which the rats were sacrificed by cervical dislocation.

Blood samples from all rats were drawn via cardiac puncture, collected into separate EDTA bottles and centrifuged at 4000 rpm for 15 minutes at -4°C. A cold centrifuge (Centurium Scientific, Model 8881) was used in this study. Plasma was obtained and collected into separate plain bottles for the assessment of biochemical parameters. Thereafter, the kidney of each rat was carefully excised and fixed inside 10% formo-saline for histopathological studies.

Measurement of Body Weight

Weekly body weights of the rats were measured with the aid of a digital weighing balance (Hanson, China) to assess weekly weight gain or weight loss.

Measurement of Food Consumption, Water Intake, and Urine Volume

The food consumption, water intake, and urine output of the rat were measured. Water intake and urine volumes were measured with the aid of a measuring cylinder (Volac, Great Britain), while the food consumption was measured with the aid of a digital weighing balance (Hanson, China).

Biochemical Analysis

Levels of creatinine, urea, uric acid, and glucose in the plasma were determined by the use of appropriate biochemical kits purchased from Randox Laboratories (Crumlin, Co. Antrim, UK). The urine concentrations of urea, creatinine, uric acid, protein, and glucose were estimated in the last samples of urine collected from the rats before sacrifice, using the same methods that were used in the analysis of plasma. Creatinine clearance was subsequently calculated using a standard formula.

Reduced glutathione (GSH) was determined by the method of Beutler and Kelly (15). Total protein determination was carried out according to the method of Lowry et al. (16) as described by Holme and Peck (17). The level of lipid peroxidation in the renal tissue was determined by estimating the level of thiobarbituric acid reactive substances (TBARS) in the homogenate of the kidneys according to the method of Ohkawa et al. (18).

Histopathological Evaluation

The fixed kidney samples were dehydrated in graded alcohol and embedded in paraffin wax. The samples were then cut into 7-8 µm thick sections and stained with haematoxylin-eosin for photomicroscopic assessment using a Leica DM 750 Camera Microscope at x 400 magnification.

Statistical Analysis

The results obtained were expressed as the mean ± standard error of the mean (S.E.M.) and subjected to one-way analysis of variance (ANOVA). The data were further subjected to a post-hoc test using Student-Newman-Keuls method, and differences with probability values of $p < 0.05$ were considered statistically significant. The statistical analysis was performed with the aid of GraphPad Prism 5.03 (GraphPad Software Inc., CA, USA) and Microsoft Office Excel, 2007 package.

RESULTS

Food Consumption and Body Weight

The food consumption of the control group increased significantly throughout the study period. Similarly, a significant increase in body weight was observed in the control group throughout the course of the study (Figure 1). The food consumption of the experimental rats decreased significantly during the 5 days of treatment with cadmium when compared with their pre-treatment values. This reduction was accompanied by a significant decrease in body weight in groups II and IV. In the remaining weeks of the study, the food consumption of the experimental groups increased significantly compared to the food consumption during the 5-day treatment period but was significantly lower than the pre-treatment values in groups III and IV (Figure 1). The body weight of the experimental groups during the remaining weeks of treatment was not significantly different from their pre-treatment values, except for group IV, which showed a significant decrease in body weight in the 1st, 2nd, and 3rd weeks compared to the pre-treatment value (Figure 2). The body weight of rats in this group was significantly higher during the 4th week than the 1st and 2nd weeks.

Water Intake and Urine Volume

The water intake of the experimental groups dropped significantly during the 5 days of treatment with cadmium

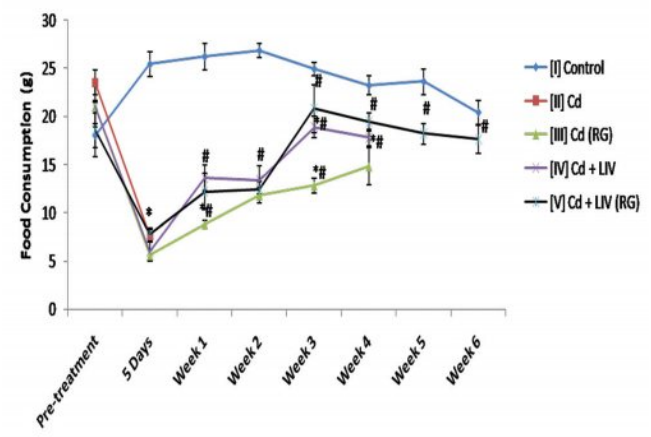

Figure 1: Effect of Livolin forte® on food consumption in cadmium- induced renal injury in rats. Values are given as mean ± SEM (n=5). * = Significantly different from pre-treatment value. # = significantly different from 5 days of treatment with cadmium.

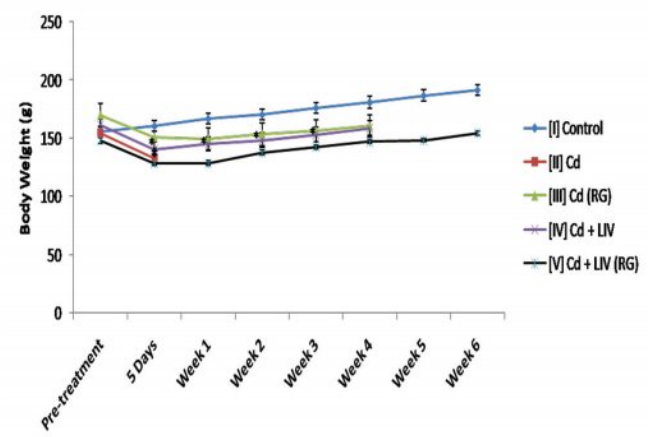

Figure 2. Effect of Livolin forte ® on body weight in cadmium- induced renal injury in rats. Values are given as mean ± SEM (n=5). * = Significantly different from pre-treatment value. δ = Significantly different from the 4th week of treatment.

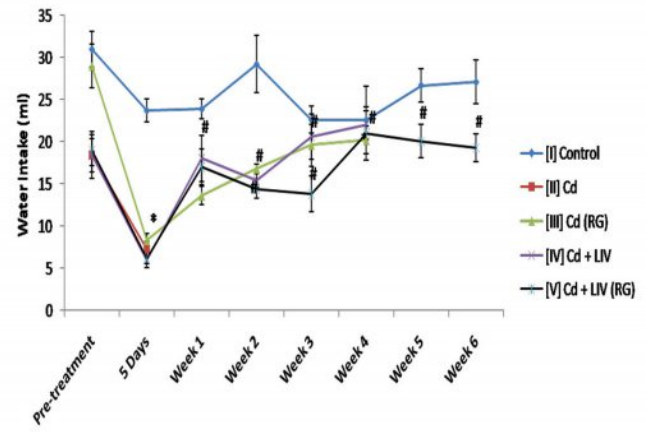

Figure 3. Effect of Livolin forte® on water intake in cadmium- induced renal injury in rats. Values are given as mean ± SEM (n=5). * = Significantly different from pre-treatment value. # = Significantly different from 5 days of treatment with cadmium

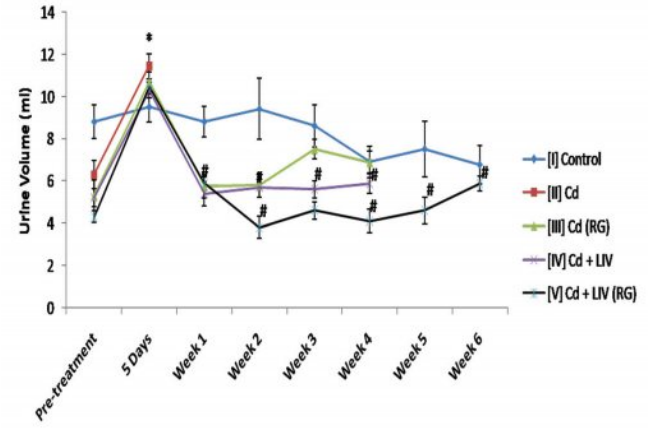

Figure 4. Effect of Livolin forte® on urine volume in cadmium- induced renal injury in rats. Values are given as mean ± SEM (n=5). * = Significantly different from pre-treatment value. # = Significantly different from 5 days of treatment with cadmium.

compared to their pre-treatment values (Figure 3). This reduction was accompanied by an increase in urine volume, which was significantly higher than the pre-treatment values (Figure 4). During the remaining weeks, a significant increase in water intake was observed in the experimental groups compared to the water intake during the 5-day treatment period with cadmium (Figure 3). However, the water intake in group III decreased significantly compared to the pre-treatment value.

Plasma Creatinine, Urine Creatinine, and Creatinine Clearance

A significant reduction in urine creatinine was observed in groups II and III compared to the control rats (Table 2). This finding was accompanied by an increase in the plasma concentration of creatinine (Table 1). The creatinine clearance in these groups dropped significantly compared to the control group and groups IV and V (Figure 5). However, the creatinine clearance and plasma and urine concentrations in groups IV and V were not significantly different from the control rats. Groups III, IV, and V showed a significant increase in the urine creatinine concentration compared to group II (Table 2). The plasma concentration of creatinine decreased significantly in these groups compared to group II. A significant increase in creatinine clearance was observed in these groups compared to group II (Figure 5).

Plasma Urea, Urine Urea, and Urea Clearance

The plasma urea concentration in groups II and III increased significantly compared to the control rats (Table 1). A significant decrease in the concentration of urea in the urine of groups II and III was observed compared to the control group (Table 2). A decrease in urea clearance in these groups was observed compared to the control group and groups IV and V (Figure 6). In contrast, urea clearance in groups IV and V was not significantly different from the

Table 1. Effect of Livolin forte® on plasma biochemical indices in cadmium-induced renal injury in rats

	I (Control)	**II** (Cd only)	**III**(Cd (RG))	**IV** (Cd + LIV)	**V** (Cd + LIV (RG))
Creatinine (mg/dl)	0.88 ± 0.02	1.90 ± 0.07*	1.16 ± 0.06*β	0.85 ± 0.06β§	0.85 ± 0.05β§
Urea (g/l)	42.25 ± 0.97	136.6 ± 10.16*	76.06 ± 4.95*β	43.69 ± 2.81β§	41.35 ± 2.69β§
Uric acid(mg/dl)	1.61 ± 0.38	5.51 ± 0.45*	3.04 ± 0.32*β	2.07 ± 0.34β	1.65 ± 0.21β§
Total protein (g/l)	48.14 ± 1.36	35.04 ± 1.86*	41.91 ± 1.85*β	46.29 ± 1.14β	44.18 ± 1.45β
Glucose (mg/dl)	5.31 ± 0.86	15.98 ± 0.58*	8.65 ± 0.56*β	5.84 ± 0.69β§	5.18 ± 0.91β§

Values are given as mean ± SEM (n=5). * = Significantly different from control. β = significantly different from Cd. § = significantly different from Cd (RG) (p<0.05). LIV, Livolin forte; Cd, cadmium; RG, recovery group.

Table 2. Effect of Livolin forte® on urine biochemical indices in cadmium-induced renal injury in rats

	I (Control)	**II**(Cd only)	**III**(Cd (RG))	**IV**(Cd + LIV)	**V**(Cd + LIV (RG))
Creatinine (mg/dl)	48.10 ± 1.11	14.63 ± 1.51*	30.94 ± 1.24*β	45.56 ± 2.40β§	47.22 ± 1.48β§
Urea (g/l)	203.7 ± 19.17	63.54 ± 4.46*	127.7 ± 6.79*β	173.3 ± 4.73β§	182.4 ± 15.08β§
Uric acid (mg/dl)	24.61 ± 3.68	5.86 ± 0.59*	19.66 ± 1.24β	22.15 ± 0.41β	26.51 ± 2.26β
Total protein (g/l)	26.07 ± 2.52	41.23 ± 0.70*	28.34 ± 0.83*β	24.83 ± 2.48β	26.18 ± 0.75β
Glucose (mg/ml)	1.10 ± 0.20	1.99 ± 0.04*	1.35 ± 0.16*β	1.08 ± 0.31β	1.15 ± 0.1β§

Values are given as mean ± SEM (n=5). * = Significantly different from control. β = significantly different from Cd. § = significantly different from Cd (RG) (p<0.05). LIV, Livolin forte; Cd, cadmium; RG, recovery group.

control group. The plasma and urine concentration of urea in groups IV and V was not significantly different from the control rats. The concentration of urea in the urine of groups III, IV, and V was significantly elevated compared to group II (Table 2). The plasma concentration of urea decreased significantly in these groups compared to group II. The urea clearance of these groups increased significantly compared to group II (Figure 6).

Uric Acid in Plasma and Urine

The concentration of uric acid in the urine of group II was significantly lower than the control rats (Table 2). This finding was accompanied by a significant increase in the plasma concentration of uric acid (Table 1). The urine concentration of uric acid in groups III, IV, and V was significantly higher than in group II but not significantly different from the control group. The plasma concentration of uric acid in groups IV and V decreased significantly compared to group II but was not significantly different from the control group (Table 1). The plasma concentration of uric acid in group III was significantly higher than the control rats but was significantly lower than group II.

Plasma and Urine Total Protein and Glucose

There was a significant decrease in the plasma concentration of total protein in groups II and III compared with

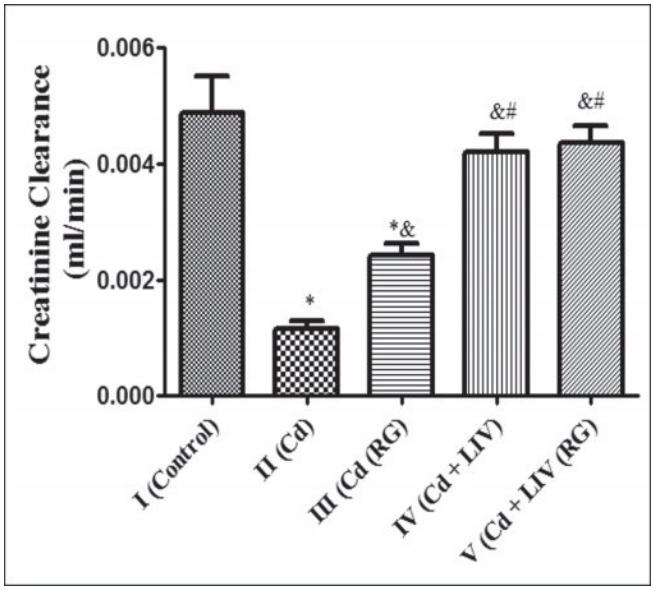

Figure 5. Effect of Livolin forte® on creatinine clearance in cadmium-induced renal injury in rat. Values are given as mean ± SEM (n=5). * = Significantly different from control. & = significantly different from Cd. # = significantly different from Cd (RG) (p<0.05)

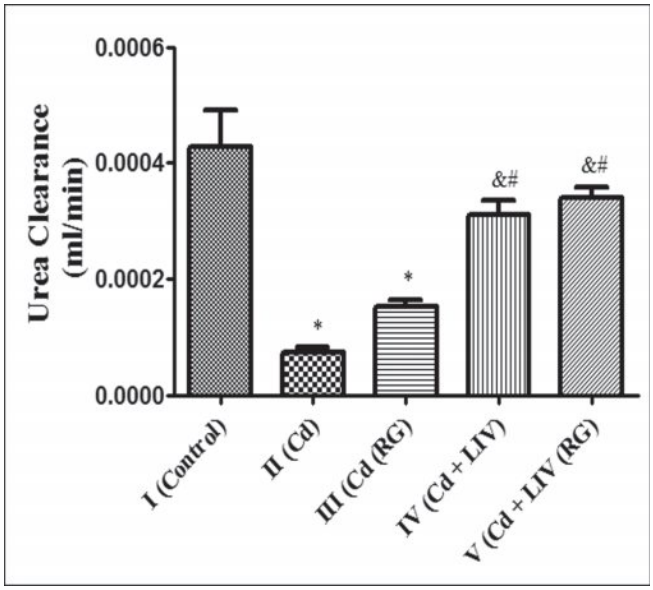

Figure 6. Effect of Livolin forte® on urea clearance in cadmium-induced renal injury in rats. Values are given as mean ± SEM (n=5). * = Significantly different from control. & = significantly different from Cd. # = significantly different from Cd (RG) (p<0.05)

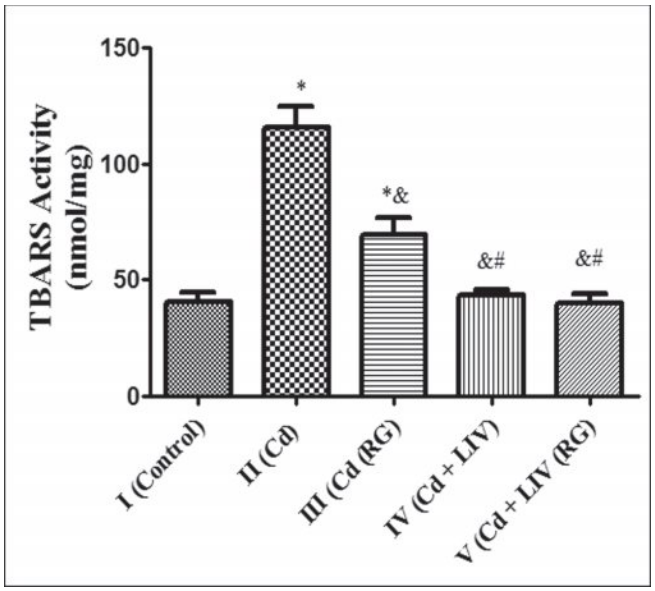

Figure 7. Effect of Livolin forte® on malondialdehyde level in cadmium-induced renal injury in rats. Values are given as mean ± SEM (n=5). * = Significantly different from control. & = significantly different from Cd. # = significantly different from Cd (RG) ($p<0.05$)

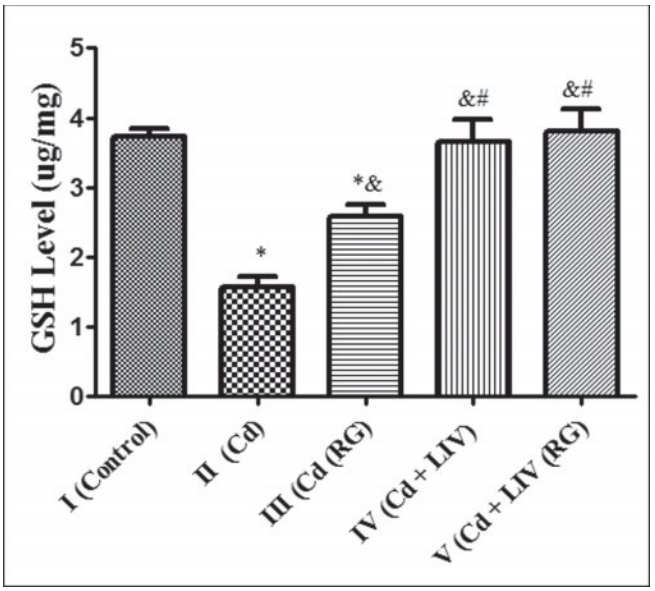

Figure 8. Effect of Livolin forte® on reduced glutathione level in cadmium –induced renal injury in rats. Values are given as mean ± SEM (n=5). * = Significantly different from control. & = significantly different from Cd. # = significantly different from Cd (RG) ($p<0.05$).

the control rats (Table 1). The urine concentration of total protein in group II was significantly higher than the control rats and the other experimental groups (Table 2). The plasma total protein concentration in groups III, IV, and V was significantly higher than group II.

The plasma glucose concentration in groups II and III was significantly elevated compared to the control rats (Table 1). Groups III, IV, and V showed a significant decrease in plasma concentration of glucose compared to group II. The plasma glucose concentration in groups IV and V was not significantly different from the control group. The urine glucose concentration in group II was significantly higher than the control rats and the other experimental groups (Table 2). Groups IV and V showed a significant decrease in urine glucose concentration compared to group III.

Reduced Glutathione and Thiobarbituric Acid Reactive Substances

A significant reduction in the reduced glutathione (GSH) level was observed in group II compared to the control rats and the other experimental groups (Figure 8). Additionally, the GSH level in group III dropped significantly compared to the control rats and groups IV and V. The thiobarbituric acid reactive substances (TBARS) level in group II increased significantly compared to the control rats (Figure 7).

Photomicrographs of the Kidneys

Photomicrographs of the kidneys from group II animals showed distorted renal corpuscles with atrophic glomeruli, severe cloudy swelling of the proximal convoluted tubules with renal tubular destruction, and severe loss of cellular constituents of the tubules compared to the control group, which showed intact renal corpuscles and normal-appearing glomeruli, tubules, and intact Bowman's spaces (Figure 9). Group III showed an improvement in the histoarchitecture of the kidneys compared to group II, although occasional atrophy of some glomeruli and shrinking of renal corpuscles was observed. Group IV exhibited histoarchitectural improvement of the renal cortices compared to Groups II and III. No evidence of atrophic or shrunken glomeruli was observed. The Bowman's spaces were intact with undamaged epithelial linings of the Bowman's capsules. The kidneys in Group V had features that were similar to the control rats (Figure 9).

DISCUSSION

Reduced growth rate is one of the symptoms indicative of toxicity in animals who were administered cadmium (19, 20). In this study, the food consumption of the experimental rats decreased significantly during the 5 days of treatment with cadmium compared to their pre-treatment values. This observed change is consistent with previously published reports (21, 22).

One of the hormones that plays a role in the regulation of appetite is ghrelin. Ghrelin is produced primarily by the stomach and proximal small intestine. In addition to the intestine, ghrelin has been identified in other peripheral tissues, such as the pancreas, ovaries, and adrenal cortex.

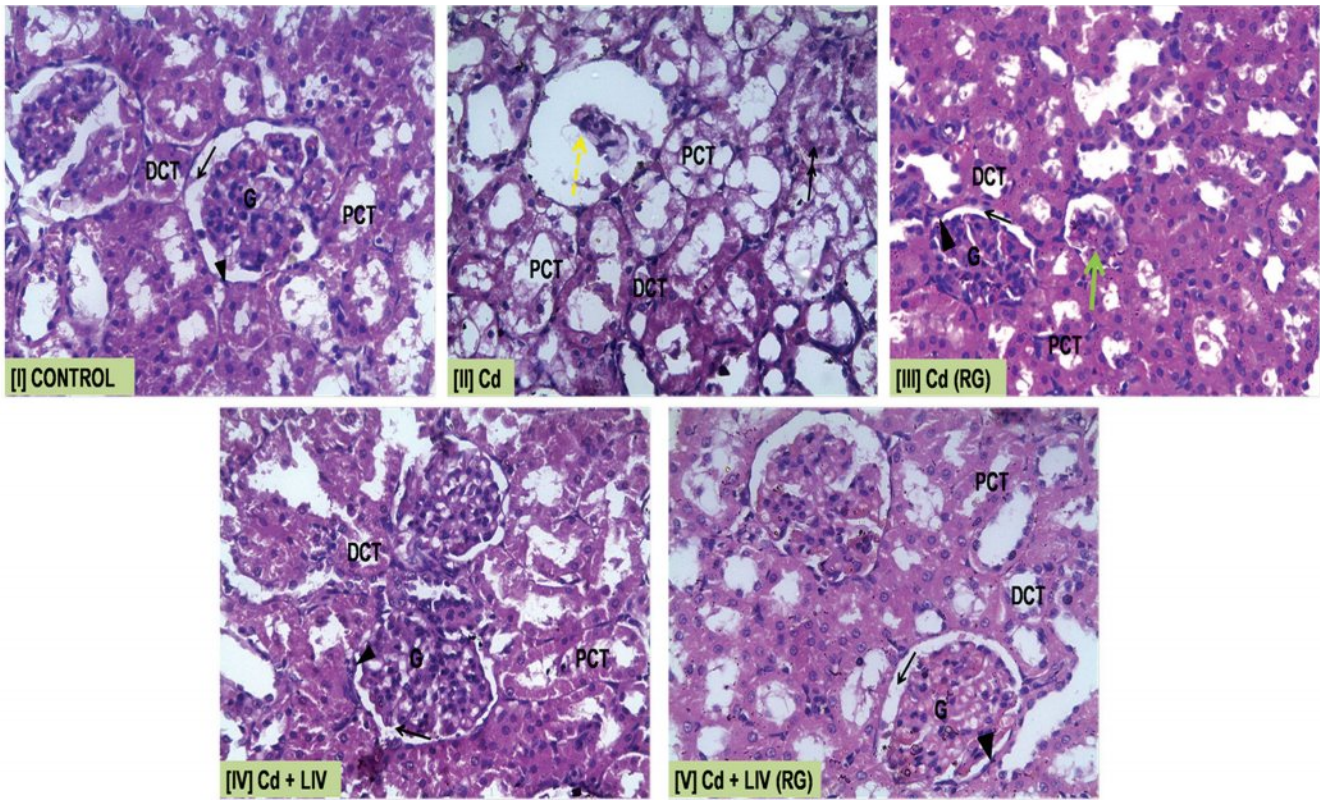

Figure 9. Photomicrographs of the kidneys. Control showed intact renal corpuscles with normal appearing glomeruli (G) and tubules (T); including the proximal convoluted tubules (PCT) and distal convoluted tubules (DCT), as well as intact Bowman's space (black arrow) and epithelial lining of Bowman's capsule (arrow head). Cadmium (Cd) showed distorted renal corpuscles with atrophic glomerulus (yellow arrow) and severe cloudy swelling of PCT (double arrow). Cd (RG) showed improvement in histoarchitecture, although with occasional atrophy of some glomeruli (yellow arrows) and shrinking of renal corpuscles (green arrows). There appear to be improvement in the histology of the tubules (T); including the PCT and DCT. Cd + LIV showed histoarchitectural improvement of the renal cortex. There appear to be no evidence of atrophic or shrunken glomerulus, Bowman's space is intact with intact epithelial linings of Bowman's capsule. Cd + LIV (RG) showed features that are similar to CN.

In the brain, neurons that produce ghrelin have been identified in the pituitary, hypothalamus, and the neuron group in the dorsal, ventral, paraventricular, and hypothalamic arcuate nucleus. Ghrelin strongly increases food intake and decreases GI motility and the secretion of insulin (23). If ghrelin production becomes inadequate as a result of the binding of cadmium to these organs, a decrease in appetite and malnutrition may occur, which shows that Cd has the capability of decreasing food intake by reducing appetite and caloric intake.

Weight gain depends on the availability and absorption of nutrients. Previous studies have shown that cadmium decreased nutrient digestion and absorption through its direct effect on the intestinal mucosal cells (24). Moreover, exposure to low levels of heavy metals has been reported to impair the glucocorticoid system (25). The glucocorticoid hormones play a vital role in glucose regulation as well as carbohydrate, lipid, and protein metabolism. Dysfunction in the glucocorticoid system has been linked to weight loss and weight gain. Therefore, the observed reduction in body weight of the experimental rats may have resulted from a decrease in food intake, an increase in the degeneration of lipids and proteins as a result of cadmium toxicity (26), or an impaired glucocorticoid system. A gradual increase in food consumption and body weight was observed in the treated groups. The gain in body weight of the rats could be attributed to the phosphatidylcholine that is present in LIV, which would correspond to the general effect of phospholipids as growth promoters (27).

Cadmium is actively transported into brain cells. By interfering with the normal function of many cellular processes, cadmium may induce sustained release of some neurotransmitters in the brain. In contrast, in different circumstances, the metal may inhibit the proper release of brain neurochemicals. As a consequence of these biochemical events, cadmium disturbs the normal function of central cholinergic, GABAergic, dopaminergic, serotonergic, glutamatergic, and opiatergic pathways (28). The water intake of the experimental groups dropped significantly during the 5 days of treatment with cadmium compared to their pre-treatment values. This finding could suggest that cadmium acted on the central nervous system, stimulating pathways that exert an inhibitory drive on water intake or, alternatively, that the metal blocked thirst-inducing pathways. Evidently, both may occur simultaneously.

A significant increase in urine output without a corresponding increase in the water intake was observed in the experimental rats during cadmium intoxication compared

to their pre-treatment values. This finding is in consonance with the report of Horiguchi *et al* (21), who observed a significant increase in the 24-h urine volume at the end of 6 and 9 months of cadmium exposure. However, this is in contrast to the findings of (29). The observed discrepancies may be due to the dose and duration of exposure in their study, which might not be enough to cause significant alteration in urine output. The significant increase in urine output suggests that cadmium may have had an adverse effect on the juxtaglomerular apparatus, which caused renin secretion to decrease. Additionally, cadmium may have caused a probable disturbance in the renin-angiotensinogen pathway resulting in a reduction in aldosterone secretion (30). A decrease in aldosterone secretion impairs the ability of the kidneys to reabsorb greater amounts of water leading to the passage of large volumes of urine. This result may lead to dehydration and a severe depletion of the major electrolytes in the body fluids of the rats. Attenuation of the cadmium–induced alteration in the urine output of rats that were treated with LIV could suggest that this drug enhanced the ability of the renal tubules to concentrate urine by facilitating the restoration of renal tissue as revealed in the photomicrographs of the kidneys.

Kidney injury due to Cd intoxication could be assessed by measuring the plasma and urinary markers of renal function, which are the biochemical hallmarks of renal tissue damage. Changes in these biochemical indices are consistent with renal impairment. Plasma creatinine and urea are used for assessing renal glomerular function. Their concentrations in the plasma depend largely on glomerular function. In renal disease, reduction in filtration rate results in elevated plasma concentrations of excretory products (31). Thus, the plasma concentrations of urea, creatinine, and uric acid increase as the filtration rate declines. In contrast, the urine concentration of excretory products depends almost entirely on tubular function. A decrease in the urine concentration of these substances is an indication of renal tubular damage (32).

In this study, the urine concentrations of creatinine and urea decreased significantly in rats that were administered Cd alone and in rats who were left for a 2-week recovery period compared to the control rats. The plasma creatinine and urea levels in these groups were significantly higher than the control rats. This finding indicates that the administration of Cd altered the glomeruli and tubular function in the rats that were administered cadmium and did not receive treatment. A similar finding was made by Goncalves et al. (33), who reported that urea and creatinine levels were increased in the serum of Cd-intoxicated rats. Furthermore, Preet and Dua (34) reported that rats exposed to cadmium experienced a significant increase in serum urea and creatinine concentrations. However, this finding is not in agreement with Horiguchi *et al.* (21), who reported that administration of Cd in rats did not alter the blood creatinine and urea levels. The lack of consistency is likely attributable to the route of exposure and dose used in their study.

Hyperuricaemia is a renal prognostic factor. Thus, the elevated plasma uric acid concentration that was observed in this study may reflect the bodily response to an increased production of endogenous reactive oxygen species because uric acid is a potent scavenger of peroxynitrite (35). The decrease in urine excretion of uric acid further confirms that the administration of Cd induced progressive tubular damage.

Significant decreases in plasma creatinine, urea, and uric acid were observed in groups treated with LIV. The restorative effect may be due to the phosphatidylcholine (PC) and vitamin E present in the drug. Vitamin E and PC have been reported to possess a membrane fluidizing effect, thereby restoring the structural integrity of the cell membrane.

The plasma glucose concentrations in rats that were administered Cd alone and rats that were left for a 2-week recovery period were significantly elevated compared to the control group. Blood glucose is commonly elevated in heavy metal toxicity and is usually linked to the inhibition of insulin release from the islets of Langerhans (36). Additionally, this finding can be linked with a reduction in glucose utilization by cells even in the presence of an elevated concentration of insulin (37) or due to disruption in glucagon secretion resulting in high glycogen breakdown (38). As the plasma concentration of glucose is increased above the renal plasma threshold, glucose appears in the urine. The higher the plasma concentration of glucose is, the greater the quantity excreted in the urine. Thus, the significant increase in urine glucose concentration that was observed in these groups may be due to higher plasma concentrations of glucose. The decrease in the plasma total protein in Cd-intoxicated rats could have resulted from changes in protein synthesis and/or metabolism. Furthermore, the increase in the total protein concentration in the urine indicates the impairment of renal function. Treatment with LIV significantly reversed the alteration caused by cadmium in plasma and urine levels of protein and glucose, suggesting its potential in restoring the structural integrity of the glomeruli and renal tubules.

Creatinine clearance is a useful measure of the glomerular filtration rate. A decrease in creatinine clearance is an indication of a marked reduction in the glomerular filtration rate and renal blood flow, resulting from an increase in the constriction of renal blood vessels or damage to the glomerular capillary endothelium. The significant decrease in creatinine clearance that was observed in the Cd-intoxicated rats seems to reflect a disruption in glomerular function. Cadmium has been reported to induce mesangial glomerular cell contraction that was evidenced by a decrease in mesangial cell surface (39). It is conceivable that even a minor reduction in the mesangial cell area considerably affects the filtering surface of the glomeruli and could explain the decreased glomerular filtration rate observed *in vivo* after toxic exposure (40). Similarly, a significant increase in urea clearance was observed in this group. However, urea clearance is not an accurate measure of the filtration rate; it varies roughly in proportion to the filtration rate in renal disease

(31). The creatinine and urea clearance in the groups treated with LIV increased significantly compared to rats that were administered Cd only. This finding is an indication of significant repair of the renal tissue as well as improved renal blood flow to the kidneys, a fact that was also corroborated by the photomicrographs of the renal tissue and the reduced level of creatinine and urea in their plasma.

Lipid peroxidation is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity of cadmium (41). Cadmium induces oxidative stress by producing hydroxyl radicals, superoxide anions, nitric oxide and hydrogen peroxide (42). A significant increase in the level of TBARS in rats that were administered cadmium only could be related to the excessive formation of free radicals, which lead to the degradation of biological macromolecules (43). In this study, Cd-intoxicated rats post-treated with LIV showed a marked decrease in the level of TBARS. This finding may be due to the presence of vitamin E in LIV. Vitamin E is an excellent scavenger of free radicals, thereby inhibiting lipid peroxidation and protein carbonylation (44). Glutathione (GSH) is a tripeptide that participates in the maintenance of cytoplasmic and membrane thiol status. It is an antioxidant, a powerful nucleophile and is critical for cellular protection by aiding in detoxification of reactive oxygen species (ROS), conjugation and excretion of toxic molecules, and control of the inflammatory cytokine cascade (45). The significant decrease in GSH activity by cadmium could be due to either increased use of GSH in the scavenging of free radicals that were produced by Cd or increased utilization of GSH for the activity of GPx forming oxidized GSH (GSSG) due to increased generation of ROS (46). Chronic pre-treatment with phosphatidylcholine partially inhibits GSH depletion in the forebrains of aged rats (47), suggesting that alterations in the phospholipid composition of the mitochondrial inner membrane and/or cytochrome oxidase activity might play a role in oxygen free radical production. Additionally, vitamin E neutralizes lipid peroxidation and unsaturated membrane lipids because of its free radical scavenging activity (48). Therefore, the significant increase in GSH activity that was observed in rats treated with LIV may be due to the ability of phosphatidylcholine and vitamin E to improve the integrity of the cell membrane by literally carrying dangerous oxidative species through the body's detoxification system, thus preventing damage to membranes.

From the results of this study, it is concluded that LIV significantly ameliorated Cd-induced kidney injury in rats. The anti-inflammatory, antioxidant, and membrane-stabilizing properties can be considered as the key factors responsible for the nephron-restorative effect of LIV. Thus, LIV represents a prospective therapeutic choice to prevent kidney injury inflicted by Cd exposure.

Conflict of Interest Statement

The authors declare that there were no conflicts of interest for this study.

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LOCALISATION AND TYPES OF COSMETIC MEDICAL TREATMENTS – CORRELATION WITH DEMOGRAPHIC CHARACTERISTICS OF SERBIAN CLIENTS

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LOKALIZACIJA I VRSTE KOZMETIČKIH MEDICINSKIH TRETMANA – POVEZANOST SA DEMOGRAFSKIM KARAKTERISTIKAMA KLIJENATA U SRBIJI

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ABSTRACT

There is a growing number of cosmetic medical treatments in the Balkan region. Yet, this trend has not been closely observed in terms of the correlation between procedure characteristics and clients' sociocultural and psychological characteristics.

The aim of this cross-sectional/retrospective research is to establish the correlation of types of cosmetic procedures with basic sociodemographic characteristics of clients in Serbia. Each of 144 study subjects underwent a cosmetic treatment (320 in total) within the first three months of 2014, while the study was being conducted. The sample included 5 male and 139 female subjects, with the age range of 17–71 (38.87±10.722).

Peaks of interventions have been detected in subjects aged 31-35 and 36-40; more frequently those were individuals with a higher level of education and their motive most commonly was of aesthetic nature. The majority of the subjects (44.44%) underwent only one intervention, while the average number of interventions per subject within the period of three months was 2.21±1.40. Face interventions were considerably higher in number than others, with a rising trend with age. The number of procedures in the area of the abdomen, breasts and thighs, rose with the increase of a body mass index. The most popular treatments included removal of stretch marks and fillers, mesotherapy and botulinum toxin.

Due to ever-growing sociocultural pressure and a modern concept of life, women often decide on cosmetic therapy at the first sign of ageing and hormonal changes, with a downward age trend especially with respect to minimally invasive procedures, as well as the most visible body parts, the face in the first place.

Keywords: *body image, cosmetic medical treatments, minimally invasive procedures, sociodemographics, Serbia.*

SAŽETAK

Broj kozmetičko-medicinskih tretmana na području Balkana sve više raste. Ipak, ovaj trend nije detaljnije ispitan u vezi sa povezanošću tipova procedura sa sociokulturnim i psihološkim karakteristikama klijenata.

Cilj ove retrospektivne studije preseka je utvrditi povezanost određenih vrsta kozmetičko-medicinskih intervencija sa osnovnim sociodemografskim karakteristikama klijenata u Srbiji. Svaki od 144 ispitanika je bio podvrgnut nekoj od kozmetičkih intervencija (320 ukupno) u periodu od prva tri meseca 2014. godine, kada je istraživanje i bilo sprovedeno. Uzorak je obuhvatio 5 ispitanika muškog i 139 ženskog pola, uzrasta od 17 do 71 godina (38.87 ± 10.722).

Najveći broj intervencija zabeležen je kod ispitanika sa oko 34. i 40. godine; najčešće kod visoko obrazovanih, a motiv za intervenciju je uglavnom bio estetske prirode. Najveći deo ispitanika (44.44%) bio je podvrgnut jednoj intervenciji, dok je prosečan broj intervencija po ispitaniku u periodu od tri meseca iznosio 2.21 ± 1.40. Najveći broj primenjenih intervencija je na licu, sa rastućim trendom sa uzrastom. Broj procedura na abdomenu, grudima i butinama raste sa povećanjem indeksa telesne mase. Najpopularniji tretmani su uklanjanje strija i fileri, mezoterapija i botoks.

Usled rastućeg sociokulturnog pritiska i modernog koncepta života, žene se sve češće odlučuju za kozmetičku terapiju već kod prvih znakova starenja i hormonalnih promena. Takođe, uočen je trend da se sve više mlađe osobe odlučuju za minimalno invazivne procedure, naročito na vidljivim delovima tela, pre svega na licu.

Ključne reči: *slika tela, kozmetičko-medicinski tretmani, minimalno invazivne procedure, sociodemografija, Srbija.*

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INTRODUCTION

Body image is regarded as a psychological representation of a personal experience one has with their body. It represents a life process that is constantly changing, a permanent process of differentiation and integration of life experiences (1). In the last few decades, the ideal of female beauty has changed many times. If we take into account the fact that beauty standards are often inapplicable to every individual because beauty cannot be measured objectively, it becomes clear why women's experiences with these changes induce stress. The constant drive to achieve the beauty ideal and the conflicting demands on what an ideal female body is lead not only to greater stress but also to further frustration and anxiety. Moreover, this drive and the accompanying conflicting demands may also result in a negative body image and other psychological problems relative to physical appearance, such as unhealthy dieting and eating disorders (2, 3). Recent studies indicate that men also suffer from negative health and psychological consequences because of body dissatisfaction (3).

The growing popularity of cosmetic medical treatments over the past decade is attributable to several factors, including the development of safer, minimally invasive procedures with less recovery time; increased media attention; and an increased willingness of people to undergo cosmetic procedures as a means to enhance their physical appearance (3, 4). The increased importance of physical appearance and the intense sociocultural pressure to achieve ideals of body image in contemporary culture combined with higher incomes among clients and lower costs for procedures further reduce clients' anxiety over cosmetic interventions (5). Cosmetic non-invasive or minimally invasive treatments, such as botulinum toxin injections, collagen injections, and laser skin resurfacing, have exceeded the traditional surgical treatments in popularity (6). Little is known, however, about the body image concerns of these clients, as they have not yet been studied in isolation (4).

In the last two years, thirteen million minimally invasive procedures have been performed in the US, an increase of 144% compared to those in the year 2000 (6). In European countries, the number of procedures varies from 100 to more than 300 thousand per year (7). A growing number of cosmetic treatments and interventions have been detected in Serbia as well. However, no comprehensive examination of the correlation between characteristics of these treatments and clients' sociocultural and psychological characteristics has been conducted. As the decision to undergo an aesthetic procedure is conditional on three components, namely, perceptive, developmental and sociocultural (8), it is essential to investigate the clients' motivations for such aesthetic interventions as well as the level of self-confidence and similar psychosocial characteristics of those clients who opt for such interventions. It is important, for example, to determine whether there are recurrent aesthetic interventions that provide short-term satisfaction with respect to personal body image, after which a recurrent and continual dissatisfaction follows, as well as an urge for repeated interventions that have no real physical foundation. In such cases, a psychological evaluation of the client prior to the intervention is necessary so that clients with certain psychological problems be advised that there are other forms of treatments available that could help them resolve such problems.

The current literature indicates that a considerable number of clients wishing to undergo cosmetic medical procedures have some psychological problem (9) and that problems such as anxiety, depression, low appearance evaluation, and body area dissatisfaction also have an impact on dissatisfaction with the performed intervention (10). Client preoperative assessments and standardised tests identify psychosocial aspects, emotional profiles and the existence of potential psychological disorders (e.g., body dysmorphic disorder), all of which represent a relative contraindication to aesthetic interventions (11). However, until there are set standards and final protocols for the preoperative selection of clients, it is vital that further information collected from doctors of all specialities within aesthetic medicine to define this process as clearly and precisely as possible. Furthermore, postoperative assessments would also be significant in terms of monitoring potential improvements of the client's psychosocial condition after cosmetic medical interventions have been performed.

The aim of this cross-sectional research is to investigate the interrelatedness between certain types of cosmetic medical treatments and some of the basic demographic characteristics of clients in Serbia. Our study provides a starting point for the selection of important demographic variables, which would serve as control variables in a follow-up study that will be investigating the relationship between the characteristics of cosmetic medical treatments and psychosocial traits.

METHODS

The sample recruited for this cross-sectional retrospective research included randomly chosen clients who underwent at least one cosmetic intervention (non-invasive procedures such as laser skin care treatments, chemical peelings or minimally invasive procedures such as fillers and botulinum toxin) at one of several aesthetic clinics in Belgrade within a testing period of three months (at the beginning of 2014).

Of 172 study subjects, 144 provided all required information and thus were included in the final sample. Hence, the final analysis included 320 conducted procedures as some of the subjects had undergone more than one procedure during the testing period[1]. Of the 5 male

[1] The calculation of an adequate sample size for estimating the proportion of the population of clients who have undergone a certain non-surgical cosmetic intervention in Serbia is a complex task. Data gathered from throughout the world on this type of intervention always include the number of conducted procedures, but they do not always include the number of people who have undergone these procedures. Second, there are no official records of the aggregate number of such procedures in Ser-

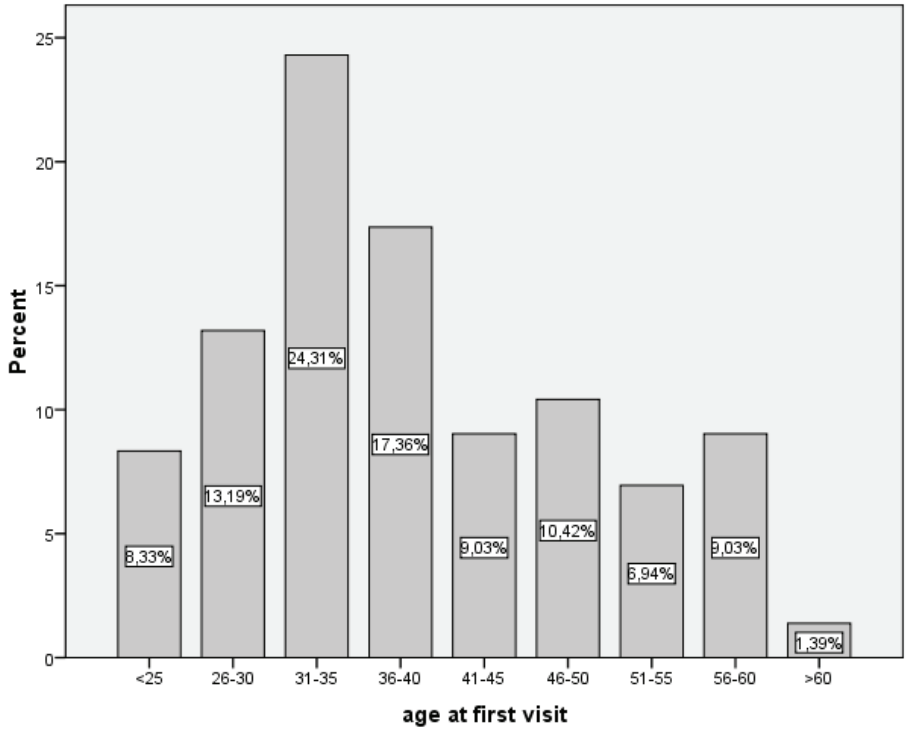

Figure 1. Distribution of the clients according to the age categories

and 139 female subjects who ranged in age from 17 to 71 (38.87±10.72), 43% had completed secondary school and 59.7% held university degrees.

All subjects provided informed consent prior to their participation in the study. The research project was approved by the Committee of the Faculty of Medical Sciences at the University of Kragujevac, where this study was conducted .

The subjects completed a list of questions regarding demographic data (sex, age, level of education, region, satisfaction with economic circumstances, knowledge of the specific intervention). Moreover, through a semi-structured interview conducted by the authors, data regarding the client's body mass index, reasons for visiting the doctor, history of chronic diseases, use of medications and other chemical substances (such as alcohol), other medical interventions, information regarding childbirth (female clients), etc. were obtained. Expert staff at the aesthetic clinics provided information about clients' surgical procedures.

Statistical analyses (descriptive and analytical) of the data were conducted using the statistical programme PASW Statistics, version 18. Correlations were determined using Pearson's coefficient of correlation. The difference in values was analysed by an χ^2-test, an independent sample t-test and ANOVA, with a significance level of 0.05.

bia. Consequently, based on existent data on the number of procedures conducted in surrounding countries (7), we estimated the number in Serbia during 2013 to be approximately 60,000. Accordingly, with an acceptable confidence interval of 3%, confidence level of 95%, and ß-.80, the appropriate number of conducted medical treatments per year would be somewhat over 1,000. Because the testing period covered three months, the appropriate number of interventions would be over 250.

RESULTS

The first finding is that there was a significant difference in sex, as male subjects constituted less than 1% of the sample. Mean values of the age variable (38.87) indicate that the sample represents the middle-age population. Peaks in interventions were most frequent with subjects between 31 and 35 and between 36 and 40 years of age (Figure 1.).

The majority of the subjects (61%) stated that the reason for their visit was aesthetic in nature, whereas 15% claimed the reason for their visit was health-related, and 24% failed to provide an explanation for their visit. Approximately two-thirds of the subjects (65.3%) had no chronic discomfort or disease, 16.6% had skin problems, 6.9% had cardiovascular issues, 7.6% suffered from respiratory issues, and 9% claimed to have endocrine issues. Furthermore, 14.6% had two or more chronic problems or diseases.

The most common source of information was electronic media (49%), followed by recommendations/suggestions of friends and acquaintances who often had already undergone some intervention (23.6%), doctor recommendations (9.4%), and print media (9.7%).

According to the BMI category, the distribution of the subjects was as follows Table 1**.** Nearly half of the subjects were currently, or had recently been, dieting (47.3%). This group significantly differed in BMI values ($t(86)=-5.278$, $p<0.000$) compared to the group that had never dieted.

Ten per cent of the sample used some form of tranquillisers or sleeping tablets, 46.1% of them smoked, and 25% consumed alcohol to some extent.

Table 1. Distribution of the clients according to the body mass index (BMI) categories

	Frequency	Valid Percent
<18.5 Underweight	10	8.5
18.5–24.9 Ideal range of BMI	85	72.0
25–29.9 Overweight	18	15.3
30–34.9 Moderately obese	3	2.5
35–39.9 Severely obese	2	1.7
M=22.57, SD=3.73; Min – 17.37; Max – 35.59		

Table 2. Distribution of cosmetic interventions according to localisation on the body

Localisation of interventions	whole face	upper face	mid face	low face	belly	breasts	gluteus	legs	other
	16.67%	9.03%	9.90%	14.11%	10.42%	6.94%	6.25%	15.97%	9.72%

Table 3. Cosmetic intervention areas according to the age categories

age (%)	face	body	extremities
<30	38.7	35.5	25.8
31-45	45.2	34.2	20.5
>45	**70.0**	22.5	7.5

Table 4. Cosmetic intervention areas according to the BMI categories

BMI categories (%)	face	body	extremities
underweight	50.0	30.0	20.0
ideal BMI	50.6	28.9	20.5
excess weight	28.0	**52.0**	20.0

Table 5. Cosmetic intervention areas according to the diet criterion

being on a diet (%)	face	body	extremities
no	54.2	31.2	14.6
yes	25.6	41.9	32.6

Table 6. Distribution of different types of cosmetic interventions according to the age categories

Intervention (%)	sample	<30	31-45	>45
Fillers	22.5	32.4	20.5	38.9
Botox	10.3	5.9	12.5	18.5
Stretch marks	23.4	44.1	30.7	5.6
Face mesotherapy	12.5	2.9	15.9	22.2
Body mesotherapy	25.9	14.7	20.5	14.8

Number of interventions

The majority of the subjects (44.44%) underwent only one intervention, 23.61% had undergone two, while others had undergone three, four or five interventions (9.72%, 11.11% and 11.11%, respectively). The average number of interventions per subject within the period of three months was 2.21 ± 1.40. No correlation was found between the number of interventions and age (r=0.10, p=0.26) or between the number of interventions and BMI (r=0.09, p=0.33). The difference in the number of interventions with regard to education exhibited border significance (t(142)=-1.95, p=0.05).

No difference was detected relative to whether they consumed alcohol, tranquillisers or sleeping tablets or relative to whether female subjects had given birth.

Localisation of interventions

If we take into account only the first visit (Table 2), the majority of the administered interventions were in the area of the face (the whole face or different face parts, most frequently lips and wrinkles around the lips). This was followed by procedures on the legs (knees, shins, thighs). If we roughly classify the interventions under face, torso and extremities, the results show that face interventions were the most frequent ($\chi^2(2)=23.29$, $p<0.01$), with 50.7% of the procedures being performed on the face in comparison to 31.2% on the torso and 18.1% on the extremities (Table 2).

Of the subjects who underwent more than one intervention during the course of the three months of observation, 34% had interventions performed on different parts of their bodies.

Because the great majority of the sample was female, we could not analyse the distribution of intervention types relative to the sex variable[2]. However, an analysis of the age categories was performed (Table 3) and revealed a significant difference ($\chi^2(4)=9.82$, $p=0.04$). For example, among the youngest age group, intervention areas were equally distributed, whereas intervention frequencies on other body parts increased significantly with an increase in age (Table 3).

The subjects who reported that the main reason for their visit was health-related (66.7%) had undergone torso interventions significantly more than persons who reported that they were seeking aesthetic improvement ($\chi^2(2)=10.20$, $p=0.02$). Of the latter group, the focus of the interventions was the face (46%).

Differences in areas of intervention were not found among those subjects with the most frequent chronic diseases ($\chi^2(6)=2.50$, $p=0.87$).

Somewhat different findings were found when analysing the body mass index category. When all subjects who had some form of excess weight were merged into one category, it was noted that this group exhibited a tendency to have more interventions performed on the torso than did other subjects ($\chi^2(6)=10.31$, $p=0.05$). More specifically, subjects with excess weight had more interventions performed on the abdominal area (27%), breasts (17%), extremities and thighs (18%), as well as the lower part of the face and neck (20%) (Table 4).

[2] Although the number of male subjects in the sample was insignificant, the sex variable was employed as a control variable in further analyses. As a result, all other data relative to demographic characteristics and types of interventions refer mainly to the female subjects.

The subjects who had dieted also underwent interventions on the torso and on the extremities more often than did subjects who had never dieted ($\chi^2(2)=8.44$, $p=0.02$). The former subjects more frequently (30%) had interventions performed on the extremities (especially thighs), followed by the abdominal area (28%), breasts (12%), and the lower part of the face and neck (14%). The most prominent types of interventions in the group of subjects who were dieting were the removal of stretch marks (42%) and body mesotherapy (26%) (Table 5).

No differences were detected with respect to the body areas on which the interventions were performed and the consumption of alcohol, tranquillisers, sleeping tablets, or smoking. However, there was a tendency for smokers to have interventions performed more frequently on the face ($p=0.06$).

Types of interventions

During the three-month observation period, 320 interventions were performed on our sample. Though most of the subjects underwent one intervention, there were those who had up to five interventions. According to the types of interventions, the most popular included the removal of stretch marks and fillers. These were followed by body mesotherapy and facial mesotherapy as well as the use of botulinum toxin (Table 6).

As we noticed some regularities indicating that some interventions were more frequently repeated than others, we analysed this phenomenon and established that botulinum toxin interventions were repeated two or more times in 30% of the subjects, fillers were repeated in 35% of the subjects, and stretch mark removal was repeated in 43% of the participants.

The results also showed significant differences in percentage relative to intervention types in three age categories ($\chi^2(8)=26.66$, $p<0.01$). The most prominent and evident differences were found with botulinum toxin and facial mesotherapy, whose numbers of interventions increased with age, while the number for stretch mark removal declined with age (Table 6).

DISCUSSION

The available evidence suggests that the likelihood of the willingness to undergo various cosmetic procedures is greater in women than it is in men (5, 12, 13) due to a greater perceived sociocultural pressure for women to live up to idealised images of physical perfection (6). This perceived pressure has resulted in a strong gender bias with up to 90% of cosmetic surgery procedures (7, 14) and 92% of minimally invasive procedures being performed on females (6). In our study, this difference is even greater in favour of the female population, a finding that may denote a distinct quality of the Serbian region, wherein aesthetic interventions for men are still regarded as unacceptable.

Nevertheless, some demographic variables of the typical client have changed in recent years, for instance, the age of clients has declined. In 2002, almost 70% of the clients who underwent cosmetic medical treatments were within the age range of 19 to 50 (4), whereas in our sample, 82.6% fell within this age range. As previously stated, peaks in interventions were noted among subjects aged between 31 and their early 40s, which is concordant with the existing data. In the study of Ishigooka and associates, for example, the most frequent interventions were reported in individuals between their late 20s and early 40s (15), a finding that supports our results.

The younger age may be explained by the occurrence of the first signs of aging, a sign that women want to erase. They also want to prevent the occurrence of new signs. Due to the perception that aesthetic beauty is imperative in today's society, individuals often seek cosmetic therapy at the very first signs of aging. The older age corresponds with the earliest hormonal changes in women (pre-menopause), at which time, a deep nasolabial fold (furrow) and wrinkles become visible even when the face is motionless (16). Additionally, the so-called frown line appears in the glabellar region, the volume of the cheekbones and lips are reduced, and the corners of the mouth droop down. Combined, these changes result in a fatigued and sad face expression that not only changes the woman's appearance but also negatively affects her emotional state due to the self-perception of an altered appearance, which is further intensified by reactions from their surroundings to the stated changes. The data suggest that the greatest number of clients seeking botulinum toxin interventions was in their early 40 sand that the majority (87.7%) were women (17).

A difference that bordered on significance was found in the number of interventions relative to the level of education. A possible interpretation of these results may be the connection between educational level and a better living status and/or potential better knowledge and an awareness of various forms and types of interventions.

There is a growing body of research that compares individuals who have undergone interventions and are exclusively driven by a physical need or by aesthetic reasons. In the current study, the percentage of those who underwent interventions for aesthetic reasons is significantly higher than the percentage of those who did so due to a physical need. Therefore, the characteristics of those who are seeking aesthetic enhancement should be further analysed in the future. For example, those seeking elective cosmetic surgery for aesthetic reasons exhibited only moderate psychosocial dysfunction, and the level of function was (negatively) related to the client's preoccupation with the abnormality rather than with either their perceived or their objective abnormality (18).

The media (music videos, television shows, reality series, magazines) are increasingly becoming a more frequent means whereby people evaluate their own physical qualities through social comparison processes (19). As there is not a sufficient number of unbiased criteria for the evaluation of physical beauty, fashion models establish, through

mass media, the parameters of physical beauty. Thus, it can be argued that the mass media have likely been instrumental in the expansion of cosmetic medicine as images of beauty and advances in cosmetic medicine are regularly promoted in the health and beauty magazines, on television shows and on Internet sites (4). Research on female subjects suggests that the relationships between depressive mood, low self-esteem, BMI and sociocultural pressures (family, media, etc.) on the one hand and body dissatisfaction on the other may be at least partially mediated by the frequency of body comparisons with the models displayed in the media (3).

Considering the frequency and dynamics of these contents in the media, it is not unusual that the media are normally not only the most frequent source of information regarding treatments, but they also establish the parameters of beauty, a fact supported by our study. However, it is important to emphasise that in the context of media, electronic media is prolific in contrast to news media.

In one study, subjects who reported favourable views of reality television shows featuring cosmetic surgery were more likely to indicate an interest in undergoing such surgery. In addition, subjects who watched a television programme about cosmetic surgery makeover wanted to change their appearance by means of cosmetic surgery more than those who were not exposed to the programme (12).

Localisation

Physical appearance is a significant part of body image because it is the primary source of information that people use to create first impressions, and it leads to further social interactions with others. Because the face is the most expressive part of the body and is in the centre of our field of vision when we communicate with other people (20), it is the most revealing and thus the most important part of body image (21). Accordingly, it is not surprising that the majority of interventions (50.7%) are performed in the area of the face. In the past, approximately 90% of clients wanted facial plastic surgery, while other body parts were of far less concern (15). Recent data from the Serbian region suggest that facial interventions remain the most popular of procedures at approximately 66%), whereas the remaining 44% are performed on other areas of the body (1).

Our results also indicated that the number of facial interventions increases with age. Because the face is vigorous and firm in young women, alterations are normally performed when clients become dissatisfied with their natural physiognomy. In older clients, body interventions are not uncommon, and the need for facial enhancements intensifies because changes resulting from the physiological process of aging become visible, changes in the papillary and reticular layers of the dermis occur, structural changes in aging skin become evident, and changes in the hypodermis are noted as the absorption and migration of fat pads occur. Though changes also occur in the muscles and the bones, they are less notable. Nonetheless, all of these changes cause wrinkles, the loss of youthful contours of the face, and an obvious decline in skin freshness.

The number of interventions performed on the body and its extremities in the younger population is considerable, which may be explained by increased obesity in this population (22), which is then followed by sagging skin and cellulite. This is further supported by our findings regarding the types of interventions, which show that the removal of stretch marks and cellulite as well as general body mesotherapy are becoming increasingly more common among the younger population. In addition, individuals with higher BMIs, as well as those who are dieting, are more likely to have interventions performed on their bodies and extremities. Nevertheless, with respect to the connection between BMI and interventions, it is important to stress that more caution be urged because being satisfied with one's own appearance is not necessarily dependent on objective BMI values. In our study, for instance, 72% of the subjects had the ideal BMI score, yet they still expressed the need to undergo specific interventions, and almost half of them expressed the need to diet or take slimming products.

With regard to repeated visits and interventions, our data, which indicate that 55% of the subjects had more than one intervention over the course of three months, are consistent with data from other countries, which report that 51% of their procedures are not first-time treatments (6). It is interesting to note that individuals who have had two or more interventions tend to choose different body parts, a finding that we intend to explore in future studies by examining these populations. Within that population, it would be particularly important to focus on those clients who initially had a deep dissatisfaction with only one part of their body but who then project this dissatisfaction onto other body parts due to certain psychological issues, such as body dysmorphic disorder, social anxiety, etc., despite the success of the initial treatment (9).

Types of interventions

With regards to the frequency of certain interventions, our data partially differ from the data reported by other countries. In the US, for example (6), botulinum toxin is a highly preferred intervention, with 47% of subjects opting for this method of cosmetic enhancement, compared to only 10% of the subjects in our study. Similar to the US, the average percentage worldwide of those electing botulinum toxin intervention is approximately 43% (7). Fillers are the second most frequent treatment, with 16% of those seeking enhancement in the US choosing fillers, 26% worldwide opting for fillers, and 22% of our sample preferring fillers. Body mesotherapy is estimated to be approximately 24% in the US compared to 31% in our sample and approximately 30% worldwide. Not surprisingly, ratings for youthful appearance and facial attractiveness are highly correlated, though they both decline with age, particularly among women (23).

This decline in their importance seems to influence the decision for minimally invasive cosmetic behaviours, such as botulinum toxin, fillers and mesotherapy, which are becoming known as anti-aging treatments.

Certain interventions, however, reinforce the repetitive nature of their use, which is why 40% of the responses in studies involving clients who underwent botulinum toxin interventions elected to undergo more frequent administrations (17). In the current study, botulinum toxin was administered two or more times in 30% of the subjects within the three-month period, fillers were applied in 35% of the cases and stretch marks were removed in 43% of the subjects, thus confirming the aforementioned statements regarding the repetitive nature of certain types of interventions. Thus, it is concluded that future studies should seek to identify a more refined solution.

CONCLUSION

It is widely known that those judged to be attractive are also more likely to be rated as intelligent, amusing, confident, sexy, strong, friendly and successful in contrast to those who are considered to be unattractive (24). That is why there has been an unusual increase in interventions aimed at improving physical appearance. The rise in cosmetic medical treatments, which is expected to continue in our region, emphasises the need for subsequent studies to examine the psychological characteristics of clients who are seeking such procedures and the resultant psychological outcomes of the interventions. This will be the subject of the follow-up study that will pivot on a more thorough investigation into the interrelatedness of the characteristics of cosmetic medical treatments and certain psychosocial traits based on the importance of extracted demographic variables identified in the present study.

The aesthetic client of the present time has changed greatly. At a younger age, they want only non- or minimally invasive procedures to retain a good figure. Moreover, once they have been treated successfully and gained confidence, this aesthetic client will likely continue to be a surgical candidate in the future at the same medical institution and with the same doctor (25).

As methodological problems from previous research have limited the validity and generalisability of the findings, there is a need for methodologically sound investigations. Accordingly, it is important to note the limitations of the current study, specifically, sample size and cross-sectional design. Future studies should include reliable and valid measures, pre- and post-treatment assessments, and appropriate control or comparison groups, which would result in a sufficient number of reliable criteria to serve as a basis for a potential psychological evaluation in medical aesthetic clinics. Such evaluations would be analogous to the taking of case histories and the detection of side effects, thereby promoting the safe medical treatment of the client.

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DOBUTAMINE STRESS ECHOCARDIOGRAPHY IN PATIENTS WITH DILATED CARDIOMYOPATHY

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DOBUTAMIN STRES EHOKARDIOGRAFIJA KOD BOLESNIKA SA DILATACIONOM KARDIOMIOPATIJOM

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ABSTRACT

A clear distinction between two of the most common forms of dilated cardiomyopathy is very important due to their different prediction and therapeutic approaches. Dobutamine stress echocardiography appears to be a noninvasive selection method due to its clear differentiation potential. Major factors influence test interpretation, resulting in a wide interval of diagnostic accuracy for this test. Fraction flow reserve (FFR) is a novel invasive method for estimating coronary artery stenosis responsible for myocardium ischaemia. Decisions about lesion significance in coronary blood vessels have thus far been based on angiographic estimations, but this approach is being replaced by FFR measurements, which serve as a new gold standard and involve a noninvasive test. The goal of this study was to clearly differentiate two forms of dilated cardiomyopathies through analysis of the segmented mobility of the left ventricular wall. Fifty patients were analysed: 20 with ischaemic dilated cardiomyopathy, which was confirmed not only through coronary angiography but also functionally through FFR measurement, and 30 patients with nonischaemic dilated cardiomyopathy, which was confirmed by coronary angiography. A standard dobutamine stress echocardiography protocol was implemented. A positive dobutamine stress echocardiography test was defined as the presence of emerging incidents in segment contractility or worsening of existing incidents in at least one segment. Statistically relevant differences in the movement dynamics of a number of differently characterised segments during the observed time intervals (ANOVA p=0.000) was noted in both groups of patients, as was variation in the index value of the summarized mobility of the left chamber wall. In patients with ischaemic cardiomyopathies, regional contractility worsened at the maximum dose of dobutamine; in contrast, this feature slightly improved in nonischaemic cardiomyopathy patients. The results indicate that by analysing segmental motion, these two forms of dilated cardiomyopathies can be differentiated with high sensitivity (Sn=90%) and specificity (Sp=98%), which can be interpreted as concrete evidence of truly ischaemic lesions in coronary blood vessels.

Keywords: *dobutamine; echocardiography; cardiomyopathies; FFR*

SAŽETAK

Jasno odvajanje dva najčešća oblika dilatacionih kardiomiopatija veoma je važno zbog različite prognoze i terapijskog pristupa. Dobutamin stres ehokardiografija se čini neinvazivnom metodom izbora za njihovo jasno diferenciranje. Veliki uticaj operatera i interpretaciji testa doveo je do širokog intervala dijagnostičke tačnosti ovog testa. Frakciona rezerva protoka (FFR) je nova invazivna metoda za procenu stenoze koronarnih arterija odgovornih za ishemiju miokarda. Odluka o značajnosti lezija na koronarnim krvnim sudovima na osnovu do sada neprikosnovene angiografske procene, zamenjena je FFR merenjem - novim zlatnim standardom prema kome se mogu tumačiti i rezultati do sada korišćenih neinvazivnih testova. Studija je imala za cilj da kroz analizu segmentne pokretljivosti zida leve komore napravi jasnu razliku između ova dva oblika dilatacionih kardiomiopatija. Analizirano je 50 bolesnika od kojih 20 sa ishemijskom dilatacionom kardiomiopatijom što je dokazano ne samo angiografski već i funkcionalno, FFR merenjem. 30 bolesnika imalo je neishemijsku dilatacionu kardiomiopatiju, što je potvrđeno koronarografijom. Sproveden je standardni dobutamin stres ehokardiografski protokol. Pozitivan dobutamin stres ehokardiografski test je definisan kao pojava novonastalih ispada u segmentnoj kontraktilnosti, ili pogoršanje postojeće u najmanje jednom segmentu. Uočena je statistički značajna razlika u dinamici kretanja broja različito okarakterisanih segmenata, u posmatranim vremenima merenja (ANOVA p=0,000), kod obe grupe bolesnika, kao i vrednosti indeksa zbirne pokretljivosti zida leve komore. U grupi sa ishemijskim kardiomiopatijama regionalna kontraktilnost se pogoršala na maksimalnoj dozi dobutamina, za razliku od neishemijskih kardiomiopatija, gde se blago poboljšala. Dobijeni rezultati ukazuju da se analizom segmentne pokretljivosti, mogu diferencirati ova dva oblika dilatacionih kardiomiopatija, i to sa visokom senzitivnošću (Sn= 90%) i specifičnošću (Sp=98%), što se može tumačiti pouzdanim dokazom o stvarno ishemijskim lezijama na koronarnim krvnim sudovima.

Ključne reči: *dobutamin; ehokardiografija; kardiomiopatije, FFR*

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ABBREVIATIONS

ANOVA - analysis of variance/analiza varijanse
Sp - specificity/specifičnost
Sn - sensitivity/senzitivnost
EDD - end-diastolic diameter/enddijastolni dijametar
EF - ejection fraction/ejekciona frkcija
LK - left chamber/leva komora
IM - myocardial infarction/infarkt miokarda
WMSI - wall motion score index/indeks zbira pokretljivosti zida
DobAtro SET - dobutamin atropin stres ehokardiografski test
SD - standard deviation/standardna devijacija
TP - true positives/stvarno pozitivna
TN - true negatives/stvarno negativna
FP - false positives/lažno pozitivna
FN - false negatives/lažno negativna

INTRODUCTION

The ability of numerous non-invasive tests to detect coronary diseases of patients with dilated cardiomyopathy has been evaluated (1). Determining the presence of coronary disease in a patient is important not only for therapy but also for prognosis. Echocardiography is widely used in researching dilated cardiomyopathies. Unfortunately, regional fall-out of wall mobility in nonischaemic dilated cardiomyopathies and global dysfunction of patients with ischaemic cardiomyopathies frequently prevents the identification of coronary disease.

Several non-invasive techniques have been used to differentiate these two forms of dilated cardiomyopathy, but their differentiation has been limited due to numerous controversial results (2). The work of Shapr and associates was the first to demonstrate the higher sensitivity and specificity of dobutamine stress echocardiography test in patients with dilated cardiomyopathy (3). This report and the majority of studies (4) have been dominated by patients with ischaemic cardiomyopathy and data on myocardial infarction (MI). Few researchers have focused their work on segmented contractility in dobutamine stress echocardiography tests in patients with primary dilated cardiomyopathy (3,5,6). Wide intervals of sensitivity, specificity and diagnostic accuracy can be generated due to inaccurate interpretations of coronary results. Interpreting angiographic stenosis from a 50% or even 75% lumen diameter as significant without functional assessment through FFR appears to be unacceptable today (7,8). In our research, we have concluded that the normal response of the left chamber to an elevated dose of dobutamine in the absence of coronary disease is an increase in contractility. However, if significant coronary disease is present, contractility should initially increase after dobutamine infusion, and if the dose of dobutamine is high, contractility should significantly worsen due to a provocation of myocardium ischaemia. The goal of our study was to define a pattern for differentiating the most severe forms of dysfunction and dilatation of the left chamber (LC) of unknown cause during the dobutamine stress echocardiography test, based on characterisation of segmental contractility before and during dobutamine infusion in patients with ischaemic and nonischaemic (primary) dilated cardiomyopathy.

MATERIAL AND METHODS

During the period 2010-2012, the Clinic for Cardiology KC Kragujevac performed dobutamine stress echocardiography in 50 patients with dilated cardiomyopathy, with the addition of atropine in 15 patients. All patients were subjected to a coronary angiography procedure, and, for patients with stenosis

> 50%, FFR measurements were performed. Significant stenosis was indicated by a measurement value <0.80.

Analysis of studied population. The study included patients with dilated cardiomyopathy without histories of myocardial infarction. To be included, patients needed to have the following: optimal ultrasound images, end-diastolic diameter (EDD) ≥ 6.0 cm (M-mode), ejection fraction (EF) <40% (Simpson method). Patients were excluded based on the existence or a history of the following: congestive heart failure, unstable angina, significant ventricular arrhythmias, LV aneurysm, severe valvular disease appliances and any contraindications to dobutamine infusion or the administration of atropine (7).

DobAtro SET protocol. The protocols for DobAtro SET vary depending on the facility in which the test is performed, with differences in the dobutamine dose (20–40 mg/kg/min), atropine dose (0–2 mg) and administration time of various dobutamine doses (2-8 min). The most widely used protocol for DobAtro SET, which is applied in our practice and was used in this work, include the following: after a baseline echocardiographic study, dobutamine is administered intravenously at an initial dose of 5 μg/kg/min for 3 minutes and is then increased to 10 μg/kg/min, 20 μg/kg/min, 30 μg/kg/min, and up to a maximum of 40 mg/kg/min over three minute intervals at each dose. If the submaximal frequency was not achieved (calculated by the formulas 220 - age x 0.85 for men and 200 - age x 0.85 for women), we administered atropine at a dose of 0.25 mg to a maximum dose of 2 mg. Patient echocardiograms were continuously monitored, and the results were recorded on three-channel electrocardiograph devices after the end of each dobutamine dosing interval and 6 min after the test. The echocardiograms were continuously monitored by videotape recordings of the final minute of each stage and 5 min after the test. Longitudinal and transverse sections of the parasternal view and the apical view with four or

two cavities were recorded. The echocardiographic studies were performed on an ultrasonic device Vivid 7 GE3.5 MHz and recorded in AVI format on CDs. Each stage of the test was digitised directly (online) with four sections using the digitalising system NovaMicrosonics.

Interpretation of echocardiographic results. Segmental myocardial contractility was assessed according to the recommendations of the American Society of Echocardiography, based on which the left ventricle is divided into 16 segments. The left ventricular wall motion sum index (WMSI) was calculated for each stage of the test by adding together the points of each segment. The contractility of each segment was determined semiquantitatively by scoring from 1 to 4, wherein normal contractility is assessed as 1 (> 5 mm endocardial movement), hypokinesia as 2 (> 5 mm endocardial movement), akinesia as 3 (lack of movement or endocardial movement < 2 mm) and dyskinesia as 4 (paradoxical outward movement in systole). A negative DobAtro SET was defined as uniformly increasing mobility of walls and systolic thickening with telesystolic reduction of the left ventricular volume. A positive DobAtro SET was defined as the occurrence of failure emerging in segmental contractility or worsening failure in at least one segment. Reading of the results was performed by two independent echocardiographers who were not familiar with the coronary angiographic findings.

Stress electrocardiography. Before the test, and after each stage of the test, electrocardiographic recordings were made on a three-channel electrocardiograph. The EKGs were interpreted as normal, ischaemic or non-diagnostic. Ischaemic changes were defined by the presence of ST segment depression > 0.1 mV or > 0.2 mV in the leads, with ST abnormalities at rest. The electrocardiograms were interpreted as non-diagnostic if blockage of the left branches was present after digitalis therapy or ST depression > 2 mm was observed at rest.

Coronary angiography. Visual assessment of stenosis diameter expressed in % was performed by two experienced angiographists. Significant coronary stenosis with a > 50% reduction in the absolute lumen diameter of the main epicardial arteries or their main branches was the indication for FFR measurements. A measured FFR value < 0.80 indicated the presence of significant coronary stenosis. Patients with verified significant contraction of 1 main coronary artery were considered to have ischaemic dilated cardiomyopathy.

Statistic data analysis. The following descriptive statistics methods were used to describe the general characteristics of patients in the observed group: absolute numbers and proportions, central tendency measures (average value) and variability measures (standard deviation). Student's t-tests were used to compare the average observed numeric values between two groups. When a characteristic's distribution prevented the application of parametric statistics, a Mann-Whitney test was used. Two-factor variance analysis was used to compare the average number of segments for certain categories of wall mobility between the two groups in every observation period. Based on the test results and given diagnosis, test sensitivity and specificity were determined, as were positive and negative predictive values.

RESULTS

Clinical, electrocardiographical and basic echocardiographical data. The data obtained for the tested groups are shown in Table 1. No statistically significant differences in the anthropometric values, echocardiographical or echocardiographical data were observed between groups. A statistically considerably higher frequency of angina pectoris was observed in patients with ischaemic cardiomyopathy. Smoking and inheritance were the dominant risk factors in the ischaemic and nonischaemic dilated cardiomyopathy groups, respectively.

Results of the stress test and side effects. When comparing patients with ischaemic and nonischaemic dilated cardiomyopathy, no statistically significant differences were found in the average dobutamine dose, atropine dose, achieved submaximal frequency, systolic blood pressure or double product. We also did not observe any considerable differences in the appearance of chest pain, dyspnoea or ST segment modifications. Chest pain was registered in 4 and 3 patients with ischaemic and nonischaemic dilated cardiomyopathy, respectively. Dyspnoea was reported in 3 patients with nonischaemic dilated cardiomyopathy, compared to 2 patients in the other group. Modifications in the ST segment were not observed in nonischaemic dilated cardiomyopathy patients but were found in 2 patients from the first group (Table 2).

Results of echocardiographic monitoring of LK wall mobility. The segment LK classification results were tested using two-factor and repeated measures variance analysis at rest, after a low dose of dobutamine, and after the dobutamine dosing tests. Analysis was performed separately for the ischaemic and nonischaemic dilated cardiomyopathy groups (Table 3). In both tested groups, the LK function was significantly reduced. Regional dyssynergy was significantly dilated in both groups. After a low dose of dobutamine, contractility was improved in both tested groups, with an increasing of number of segments identified as normokinetic versus segments with problematic kinetics. With a high dose of dobutamine, an evident increase in the number of segments with irregular kinetics was observed in patients with ischaemic dilated cardiomyopathy, whereas individuals from the other group exhibited further increases in the number of segments with normal kinetics. In regards to segment movement dynamics, an intergroup analysis (Bonferroni t-test) indicated a lack of statistically significant differences in the average number of normal segments between tested groups (p=0.084). Additionally, no distinction between these two groups in terms of the average number of normal segments was found after a low dobutamine dose treatment (p=0.067). At peak do-

Table 1. Clinical, electrocardiographic and baseline echocardiographic data

Observed parameters		Ischemic heart disease	Cardiomyopathies	Differentiation significance
Average age (X±SD) (in years)		55.3±8.54	50.33±10.56	Not significant
Wight (X±SD) (in kilograms)		78.95±9.04	83.47±15.22	Not significant
Height (X±SD) (in cm)		172.05±6.85	175.17±7.49	Not significant
BMI		26.75±2.70	27.27±4.17	Not significant
Sex (n (%))	male	20 (100%)	25 (83.3%)	Not significant
	female	0 (0%)	5 (16.7%)	
NYHA (n (%))	class I	1 (5%)	2 (6.7%)	Not significant
	ccass II	14 (70%)	13 (43.3%)	
	class III	5 (25%)	14 (46.7%)	
	class IV	0 (0%)	1 (3.3%)	
Angina (n (%))	yes	12 (60%)	8 (26.7%)	Significant
	no	8 (40%)	22 (73.3%)	
Hypertension (n (%))	yes	10 (50%)	12 (40%)	Not significant
	no	10 (50%)	18 (60%)	
Diabetes mellitus (n (%))	yes	4 (20%)	4 (13.3%)	Not significant
	no	16 (80%)	26(86.7%)	
Left bandle branch block (n (%))	yes	4 (20%)	9 (30%)	Not significant
	no	16 (80%)	21 (70%)	
Ejection fraction		21.85±5.77	20.13±4.94	Not significant
End diastolic dijameter EDD (mm)		67.10±4.13	70.80±5.83	Significant
Mitral regrgitation (n (%))	yes	10 (50%)	16 (53.3%)	Not significant
	no	10 (50%)	14 (46.7%)	
Family history of disease (n (%))	yes	4 (20%)	15 (50%)	Significant
	no	16 (80%)	15 (50%)	
Smoking (n (%))	yes	15 (75%)	12 (40%)	Significant
	no	5 (25%)	18 (60%)	
Hypercholesterolemia (n (%))	yes	2 (10%)	0 (%)	Not significant
	no	18 (90%)	30 (100%)	
Atrial fibrillation (n (%))	yes	6 (30%)	6 (20%)	Not significant
	no	14 (70%)	24 (80%)	
Sinus rhythm (n (%))	yes	14 (70%)	24 (80%)	Not significant
	no	6 (30%)	6 (20%)	

butamine doses (p=0.008) and after testing (p=0.039), statistically significant differences in the average number of normal segments were observed between patients with ischaemic and nonischaemic dilated cardiomyopathy. The average number of hypokinetic segments differed at statistically significant levels between the 2 tested patient groups at rest (p=0.03) and after testing (p=0.018), whereas, at low (p=0.0114) and peak dobutamine doses (p=0.505), so such differences were observed in the number of hypokinetic segments. For akinetic segments, significant differences in the average number of relevant segments were found at only peak doses of dobutamine (p=0.048).

LK wall collective mobility index (WMSI) values. The WMSI values were monitored for the two groups of patients at rest and after a low dose of dobutamine, peak dobutamine dose, and completion of testing. Two-factor repeated measures analysis of variance was used to compare the obtained index values. Variation in the WMSI values in this study resulted from differences in heart disease derivation, so we analysed the WMSI measurement time (at rest, at low doses of dobutamine, at peak dobutamine doses and after the test) and individual differences between patients. In this model, the measurement time was used as an intrasubject factor, and the difference be-

tween ischaemic and nonischaemic dilated cardiomyopathy patients served as an intersubject factors. This analysis indicated a difference in the observed measurement times and in the dynamics of changes in WMSI values between observed groups at given measurement times. Significant differences were observed in the dynamic movement value of the calculated WMSI values, which were obtained by measuring parameters of wall mobility segment analysis before, during and after the test, between the two patient groups at given measurement times (two-way repeated measures ANOVA, measuring time *patients group; p=0.000). Differences between the WMSI average values were observed at various measurement times (two-way repeated measures ANOVA, measuring time; p=0.000) (Table 4, Graph 1). After an almost uniform change in the WMSI after a low dobutamine dose, the high dose of dobutamine provoked ischaemia in and worsened the kinetics of the group with ischaemic dilated cardiomyopathy. After the test, WMSI values were registered. We divided our patients into four groups ac-

Table 2. Stress test results and side effects

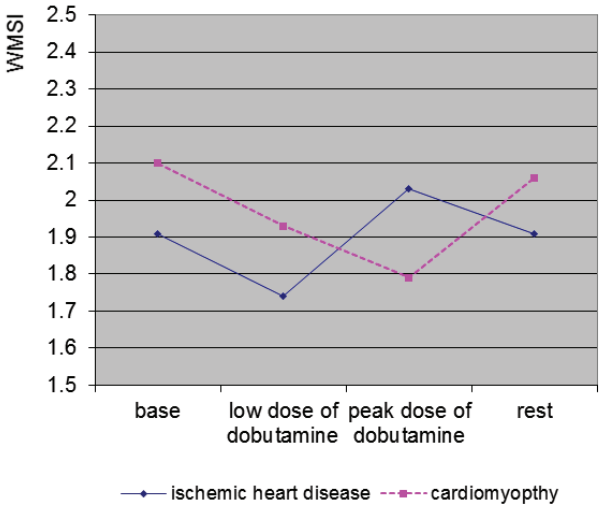
Observed parameters		Ischemic heart disease	Cardiomyopathies	Differentiation significance
Average doses of dobutamine (X±SD) (u μg/kg/min)		37.5±5.5	37.33±4.5	Not significant
Average doses of atropine (X±SD) (u mg)		0.53±0.21	0.5±0.2	Not significant
Atropine (n (%))	**yes**	8 (40%)	7 (23.3%)	Not significant
	no	12 (60%)	23 (76.7%)	
Achieved maximal frequency (n (%))	**yes**	16 (80%)	23 (73.3%)	Not significant
	no	4 (20%)	7 (26.7%)	
Maximal frequency (X±SD)		128.53±23.94	137.85±22.24	Not significant
Maximal values od systolic pressure(X±SD)		132.83±21.72	143±13.8	Not significant
Frequency x systolic pressure/1000		17.29±5.1	19.78±3.61	Not significant
Chest pain (n (%))	**yes**	4 (20%)	3 (10%)	Not significant
	no	16 (80%)	27 (90%)	
Dispnea (n (%))	**yes**	3 (15%)	2 (6.7%)	Not significant
	no	17 (75%)	28 (93.3%)	
ST segment changes (n (%))	**yes**	2 (10%)	0 (0%)	Not significant
	no	18 (90%)	30 (100%)	

Table 3. Grading of left ventricular segments at stress test

Observed parameters	Base	Measuring time			
		Low dose of dobutamine	Peak dose of dobutamine	Rest	
Ischemic heart disease	**Normal segments**	4.25±4.04	6.1±3.85	3.55±3.5	4.05±3.78
	Hypokinetics segments	9.25±4.68	7.65±3.91	8.05±3.82	9.25±3.74
	Akinetics segments	2.8±2.17	2.15±1.95	4.4±3.15	2.7±2.18
Cardiomyopathies	**Normal segments**	1.33±1.88	4.27±3.05	6.07±2.91	1.7±2.31
	Hypokinetics segments	11.7±3.09	9.37±3.55	7.3±3.9	11.67±3.18
	Akinetics segments	2.97±3.06	2.83±2.96	2.63±2.85	2.83±3.01

Table 4. The value of WMSI at patients with ischemic and nonischemic cardiomyopathy

Posmatrane grupe ispitanika	Vrednosti WMSI u posmatranim vremenima merenja (X±SD)			
	Base	Low dose of dobutamine	Peak dose of dobutamine	Rest
Ischemic heart disease	1.91±0.3	1.74±0.29	2.03±0.35	1.91±0.31
Cardiomyopathies	2.1±0.25	1.93±0.26	1.79±0.28	2.06±0.28



Graph 1. WMSI at the patients with ischemic and nonischemic dilated cardiomyopathy

cording to their stress test results: group 1 for truly positive results (ischaemic heart disease and positive test, TP-true positives), group 2 for truly negative results (patients with nonischaemic dilated cardiomyopathy and negative test, TN-true negatives), group 3 for false positive results (nonischaemic dilated cardiomyopathy and positive test, FP-false positives) and group 4 for false negative results (ischaemic heart disease and negative test, FN-false negatives). After creating 2x2 contingency tables, we calculated the following rates: TPR=TP/TP+FN and TNR=TN/TN+FP. Sensitivity and specificity are widely accepted synonyms for TPR and TNR. Test sensitivity is an indicator of the test's ability to detect patients with ischaemic disease; in our case, the test sensitivity was 90% (CI=85-100%). Test specificity, which reflects the test's ability to identify healthy patients, was 98% (CI=96%-100%).

DISCUSSION

Differentiating between ischaemic and nonischaemic dilated cardiomyopathy is highly important (10,11,12,13), but the clinical aspect of these conditions can be very complicated. Ischaemia is likely the cause of cardiomyopathy in patients with myocardium infarct (IM) or aneurism LK. Nevertheless, some patients with ischaemic cardiomyopathy do not exhibit anamnestic or electrocardiographic signs of IM nor anamnestic data indicating chest pain but demonstrate more diffuse rather than regional hypocontractility of segments in ultrasound (14,15). In contrast, many patients with nonischaemic dilated cardiomyopathy often experience angina pain, and these patients' electrocardiograms contain features that match the signs of IM (11). The main advantage of our research is the absolute confirmation of ischaemia in patients with dilated cardiomyopathy by FFR measurements, compared with other research that used only coronary graph results as an indicator of ischaemic disease aetiology, without reliable confirmation about the existence of ischaemia (7,8). In our research, we clearly demonstrated that in patients with LK dysfunction of unknown cause, regional dyssynergy upon receiving a peak dose of dobutamine clearly indicates an ischaemic aetiology of disease. In a carefully chosen group of patients, we confirmed that dobutamine stress echocardiography can differentiate two forms of dilated cardiomyopathies. We noticed that for nonischaemic dilated cardiomyopathies, contractility responses to dobutamine were not characterised by global hyperkinesia but rather mild and moderate improvements in contractility, which often limited to partial LK segments. Currently, other techniques are used to differentiate these two forms of dilated cardiomyopathies such as talium-201 scintigraphy at rest. However, loading and infusion of dipyridamole does not offer final solutions, whereas the use of positron emission tomography is limited by its price. New techniques and myocardium acquisition have not solved the problem of low applicability thus far (16, 17). The interpretation of dyssynergy at rest is a well-known technique, due to infarct scarring, but often these large or real aneurysms can be caused by myocarditis. Mild to moderate dyssynergy is commonly observed with nonischaemic dilated cardiomyopathies and can be caused by hypoperfusion as a result of inadequate microcirculation (18, 19, 20). In our study, basal hypocontractility in the ischaemic dilated cardiomyopathy group was less homogeneous than in the other group. In our research, we did not clearly separate necrotic myocardium from mixed myocardium, according to patient responses to dobutamine.

Electrocardiographic and haemodynamic data were not useful in our study for differentiating the two forms of cardiomyopathy. The response to a low dose of dobutamine was similar in both groups due to a favourable response from the largest number of hypokinetic segments. Nevertheless, this improvement in ischaemic cardiomyopathy spread to certain akinetic segments, which probably were part of the hibernating myocardium, whereas other segments remained dyssynergic. For a high dose of dobutamine in nonischaemic dilated cardiomyopathy patients, hypo- and akinetic segments maintained their previous improvement in kinetics, compared with the ischaemic disease group that exhibited a worsening in kinetics, with and without the presence of previous dyssynergy. The WMSI values also differed between tested groups at high doses of dobutamine, which was previously reported by Sharp *et al.* and Vign *et al.* (21, 3, 6). In contrast to the aforementioned studies, we registered a significant increase in the WMSI when increasing from a low to high dose of dobutamine, with an especially effectual response in the nonischaemic dilated cardiomyopathy group, whereas the ischaemic diseases group exhibited different responses. Variations in the tested groups and different dobutamine infusion protocols (dobutamine dose, duration of each stage) can partially

explain these discrepancies. In some undefined cases, intertwined factors of both forms of cardiomyopathies can be assumed to be responsible for false negative and false positive results (22, 23). Extensive collaterals and inability to achieve adequate loads for this group of patients can explain the decreased test sensitivity. Patients with nonischaemic diseases often exhibit weak and limited responses in certain segments during dobutamine infusion. Registering a worsening of LK function for a high dose of dobutamine in the absence of coronary disease is likely the effect of the progressive phase of the myopathic process or simply a decreased coronary reserve (24,25,26).

Limitations during the research. This study included several limitations. The number of patients was relatively small. We did not use relatively new technical modalities to improve endocardium visualisation. We did not consider right ventricle reactions to the infusion of dobutamine or right ventricle dimensions, based on the opinion that the left ventricle's function is preserved in ischaemic but not in nonischaemic dilated cardiomyopathies. The aetiology of dilated cardiomyopathy in single vessel coronary disease is disputable, as is the interpretation of results in these cases.

CONCLUSION

Dobutamine-atropine stress echocardiography is a comfortable patient testing method with very mild and rare side effects. The dynamics of LV segments in patients with ischaemic dilated cardiomyopathy exhibited significant differences during the individual stages of the test as a consequence of the test's ability to provoke ischaemia. The dynamics of LV segments in patients with nonischaemic (primary) dilated cardiomyopathy reflects the positive inotropic effect of dobutamine in healthy coronary vessels. Changes in the WMSI value during the test are a clear predictor of ischaemic heart disease in patients with severe LV dysfunction of unknown origin. The sensitivity of the test was 90%, and the specificity of the test was 98%, with the identification of actual ischaemic lesions in coronary blood vessels confirmed by FFR measurement.

Finally, patients with severe LV dysfunction are all different, and the possibility of coronary artery disease in patients with primary dilated cardiomyopathy should be noted.

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CHARACTERISTICS OF PREGNANCY, DELIVERY AND THE POSTPARTUM PERIOD IN PREGNANT WOMEN DIAGNOSED WITH GESTATIONAL DIABETES MELLITUS

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KARAKTERISTIKE TRUDNOĆE, PORODJAJA I POSTPARTALNOG PERIODA TRUDNICA SA DIJAGNOZOM GESTACIJSKOG DIJABETES MELITUSA

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ABSTRACT

Gestational diabetes mellitus refers to both transient diabetes that arises during pregnancy and is restored postpartum as well as forms of the disease that arise for the first time during pregnancy and persistently exhibit insulin-dependence (type 1) after childbirth. The basis for the development of gestational diabetes is the existence of insulin resistance.

Our target population was pregnant women between 20 and 46 years of age who were diagnosed with gestational diabetes (after the 24th week of pregnancy) and who were treated at the Department of Pathology of Pregnancy, Clinical Centre Kragujevac. During the research period, data were collected from 95 pregnant women with diagnosed gestational diabetes. In 3 women, the pregnancy ended in intrauterine foetal demise, and the study was continued with 92 subjects. This is a cross-sectional, retrospective and observational study.

The average age of the examinees in our sample is 31.6 years. A total of 77.89% of the examinees achieved normoglycaemia exclusively via a hygienic dietary regimen. However, 27.2% of the subjects exhibited comorbidities in addition to gestational diabetes, which further complicated the pregnancy. A total of 70.7% examinees delivered between the 37th and 40th week of gestation. Vaginal delivery was dominant, with episiotomy in almost half the cases. The average body weight of newborns from pregnancies complicated by gestational diabetes was 3587.07 grams, which is very close to the macrosomia limit of 4000 grams.

The timely detection of gestational diabetes and an adequate treatment of pregnant women can prevent the occurrence of foetal macrosomia as the primary complication of these pregnancies. Pregnancy complicated by gestational diabetes is not necessarily an indication for a Caesarean section.

Keywords: *pregnancy, gestational diabetes, delivery*

SAŽETAK

Gestacijski dijabetes melitus odnosi se na tipove dijabetesa nastale u trudnoći sa prolaznim karakterom postpartalno, ali i na oblike ove bolesti koji se prvi put otkrivaju u trudnoći, a nastavljaju da perzistiraju i nakon porođaja kao insulin zavisni tipovi (tip 1).

Ciljana populacija bile su trudnice starosti između 20 i 46 godina sa dijagnostikovanim gestacijskim dijabetesom (nakon 24. nedelje trudnoće) lečene na odeljenju patologije trudnoće KC Kragujevac. U periodu ispitivanja podaci su prikupnjeni od 95 trudnica sa dijagnozom gestacijskog dijabetesa. Zanemareni su podaci prikupljeni od 3 trudnice sa intrauterinom smrti ploda, a studija je nastavljena sa 92 trudnice koje imaju dijagnozu gestacijskog dijabetes melitusa. Studija koju smo sproveli je studija preseka, retrospektivna i opservaciona.

Prosečna starost ispitanica u uzorku iznosi 31.6 godina. Većina trudnica su bile prvorotke. 77.89% trudnica je stanje normoglikemije ostarivalo higijensko dijetetskim režimom. Prateće dijagnoze koje su trudnoću komplikovanu gestacijskim dijabetesom dodatno komplikovale imalo je 27.2% trudnica. 70.7% trudnica se porodilo između 37. i 40. nedelje gestacije. Dominirao je vaginalni način porođaja uz epiziotomiju kod skoro polovine ispitanica.

Pravovremeno otkrivanje gestacijskog dijabetesa i adekvatno lečenje trudnica sprečiće nastanak makrozomije fetusa kao osnovne komplikacije ovih trudnoće. Trudnoća komplikovana gestacijskim dijabetesom nije sama po sebi indikacija za carski rez.

Ključne reči: *trudnoća, gestacijski dijabetes, porođaj*

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INTRODUCTION

Metzger defines gestational diabetes mellitus (GDM) as "any degree of glucose intolerance with onset or first recognition during pregnancy"(1). This definition does not encompass cases of undiagnosed diabetes in which the disease was present before pregnancy. For this reason, the boundary between comorbidities associated with diabetes during pregnancy and gestational diabetes is blurred (2). Gestational diabetes mellitus refers to those types of diabetes that arise during pregnancy with transient character postpartum, but it also refers to the forms of disease that arise for the first time during pregnancy but continue to persist even after childbirth as insulin-dependent types (type 1). Gestational diabetes mellitus refers to both transient diabetes that arises during pregnancy and is restored postpartum as well as forms of the disease that arise for the first time during pregnancy and persistently exhibit type 1 insulin-dependence after childbirth (3).

Positive correlations between GDM and obesity, as well as glucose-intolerance and diabetes mellitus type 2, have led to an increase in the prevalence of this disease on a global level (4).Although the prevalence of the disease varies by nationality and ethnicity (1-28%), recent studies have shown that it is taking on an epidemic character (5). The latest IDF (*International Diabetes Federation*) findings show that one out of six pregnant women (16.8%) has some type of hyperglycaemia and that 16% of these cases are due to insulin-dependent (type 1) or noninsulin-dependent (type 2) diabetes mellitus; the remaining 84% of cases are due to gestational diabetes (6).

While both general and gestational diabetes are predominantly categorized as a disorder of carbohydrate metabolism, the mechanisms of this condition are complex, with lipid and protein metabolisms being affected as well (3). The basis for the development of gestational diabetes is a state of insulin resistance (7). It contributes to weight gain during pregnancy due to fat accumulation and increased calorie intake as well as reduced physical activity (4-7). The other pathophysiological aspects contributing to gestational diabetes relate to altered hormonal expressions. The placental hormones are the primary drivers of gestational diabetes, which is supported by the fact that insulin resistance diminishes after placental expulsion (8).

Human placental lactogen, which is secreted during pregnancy, reduces glucose utilization and intensifies lipolysis (8). This causes an increased level of free fatty acids, which contributes to insulin resistance (8-9). Insulin resistance results in further exhaustion of beta cells in the islets of Langerhans, thus reducing their productive capacity (9, 10).

The placenta develops during the pre-embryonic and embryonic periods. Therefore, manifestation of gestational diabetes rarely occurs during the first trimester of pregnancy (11). Manifestations during the second and third trimesters (usually after the 24th week of pregnancy) are evident due to the production of human placental lactogen, which is the insulin antagonist (the antagonist of the growth hormone, cortisol). Additionally, oestrogen and progesterone also play an indisputable role (11, 12). Prior to placental signalling, developmental changes occur in the mother's body, and hyperglycaemia and hypoinsulinaemia are manifestations of these placental signalling processes (12).

As a result of insulin resistance, glucose passes the placental barrier and causes hyperinsulinemia in the foetus. As insulin is the main growth factor during the foetal period, hyperinsulinemia causes accelerated growth of the foetus and subsequent macrosomia (9, 11).

Research Goals:

- To determine whether a correlation exists between age and parity of subjects and incidence of gestational diabetes
- To determine whether establishing normoglycaemia in subjects can be achieved primarily through a hygienic-dietary regimen
- To determine the influence of comorbidities on pregnancy outcomes in women with gestational diabetes
- To determine whether vaginal delivery is dominant in pregnancies with gestational diabetes
- To determine whether a correlation exists between foetal macrosomia and gestational diabetes and to determine the effectiveness of treatmentTo demonstrate whether gestational diabetes persists into the postpartum period in up to 5% of cases

MATERIALS AND METHODS

The research we have conducted is a cross-sectional, retrospective and observational study. The data were collected by labour, newborn, and postpartum chart reviews. After the input of appropriate data, we processed the data in the SPSS program, version 20.

We recruited pregnant women between 20 and 46 years of age who were diagnosed with gestational diabetes (after the 24th week of pregnancy), who were treated at the Department of Pathology of Pregnancy, Clinical Centre (CC), Kragujevac, and who gave birth at the Maternity Ward, CC Kragujevac, from January 1st 2013 through June 30th 2015.

For didactic reasons, the data were divided into four categories: general information of the examinees as well as data related to the prepartum, intrapartum, and postpartum periods.

The general information included the age of the subjects, the length of the pregnancy, the outcome of the pregnancy and whether the conception was done naturally or by artificial insemination (IVF/ET – *in vitro fertilization/ embryo transfer*). Pregnant women were stratified into four groups that analysed parity: primipara, secundipara, tripara, and multipara (more than three childbirths).

During the prepartum period, the diagnosis and treatment of diabetes is emphasized at doctors' examinations because it greatly contributes to pregnancy risk. Based on the method of treatment, the subjects were divided into

two groups: those who achieved normoglycaemia by a hygienic-dietary regimen (HDR) and those who were concomitantly treated with insulin therapy. The World Health Organization (WHO) recommends that the cut-off for hyperglycaemia in blood to be set at 6.1 mmol/L. The subjects were further divided into those with or without the following comorbidities: thrombophilia, high blood pressure, placental abruption, and a multiples pregnancy.

The intrapartum period includes the period of childbirth. The time of delivery was analysed (preterm delivery – before 36.6 weeks of pregnancy, term – from 37.0 to 39.6 weeks of pregnancy, postterm – after 40.0 weeks of pregnancy). Additionally, the method of delivery (spontaneous vaginal delivery, vaginal delivery with episiotomy, and delivery by Caesarean section) and the Apgar scores (1-9) were assessed as well as the average body weight, height, and head circumference of the foetus.

The postpartum period includes follow-up through 40 days after childbirth, with the intent to detect persistent hyperglycaemia in the subjects. Subjects were divided into two groups: those who had persistent hyperglycaemia and those whose hyperglycaemia had resolved.

RESULTS

During the study period, the data from 95 gestational diabetic pregnant subjects were collected. Ninety-two women (96.84%) carried their pregnancy to live childbirth, and for 3 women, the pregnancy ended in intrauterine foetal demise (3.16%). The data were then analysed to include the remaining 92 subjects as the complete dataset (representing 100% of the subjects).The average age of subjects was 31.6 years old, with ages ranging from 20-46 years. Natural conception occurred in 90 cases, while two cases were impregnated using IVF/ET methods.

Fifty (54.3%) subjects were primipara, 32 (34.8%) subjects were secundipara, 6 (6.5%) subjects were tripara, and 4 (4.3%) subjects or were multipara (as shown in Tables 1 and 2).

Seventy-four (77.89%) of the examinees achieved normoglycaemia by a hygienic-dietary regimen, while for 18 (22.11%) of the examinees, insulin therapy was necessary to return the glycaemia to balanced levels (Table 1).

Twenty-five (27.2%) of the subjects exhibited additional complications due to comorbidities. The most frequent diagnosis was pre-pregnancy or pregnancy-induced hypertension (17 women [18.48%]), thrombophilia (12 women [13.04%]), and bleeding immediately prior to childbirth or placental abruption (3 women [3.26%]). There was only one case of a multiples pregnancy (1.09% of the study population). In almost 30% of the cases, the pregnancy was complicated by more than one comorbidity (Table 3).

In regard to the intrapartum period, 65 (70.7%) examinees delivered between the 37th and 40th week of gestation. Preterm delivery (before the 37th week) occurred in 10 (10.9%) of women examinees, while postterm pregnancy was present in 17 women (18.5%) (Table 4).

Table 1. Descriptive table*

	Number of examinees	%
primipara	50	54.3
multipara (>one childbirth)	42	45.7
hygienic-dietary regimen	74	77.89
+ insulin therapy	18	22.11
without accompanying diagnosis	67	72.8
with accompanying diagnosis	25	27.2
full term delivery	65	70.7
pre- or postterm delivery	27	29.3
vaginal delivery	58	63
Caesarean section	34	37

Table 2. Parity of research examinees

parity of research examinees		
Parity	number of examinees	%
Primipara	50	54.3
Secundipara	32	34.8
Tripara	6	6.5
Multipara	4	4.3
Total	92	100

Table 3. Accompanying diagnosis which complicate the pregnancy

accompanying diagnosis which complicate the pregnancy				
Accompanying diagnosis	Examinees			
	Not present		67	72.8%
	Present	hypertension	25	27.2%
		thrombophilia	12	13.04%
		abruption	3	3.26%
		multiple pregnancy	1	1.09%
		multiple diagnosis	27	29.8%

Table 4. Time of labour in terms of gestational age

Time of labour		
	number examinees	%
preterm	10	10.9
full term	65	70.7
postterm	17	18.5

Table 5. Delivery

Delivery		
	number of examinees	%
spontaneous	17	18.5
episiotomy	41	44.6
Caesarean section	34	37.0

The modes of delivery (Table 5) had the following characteristics: 41 (44.6%) women had a vaginal delivery, with episiotomy in almost half the cases; 17 (18.5%) women had

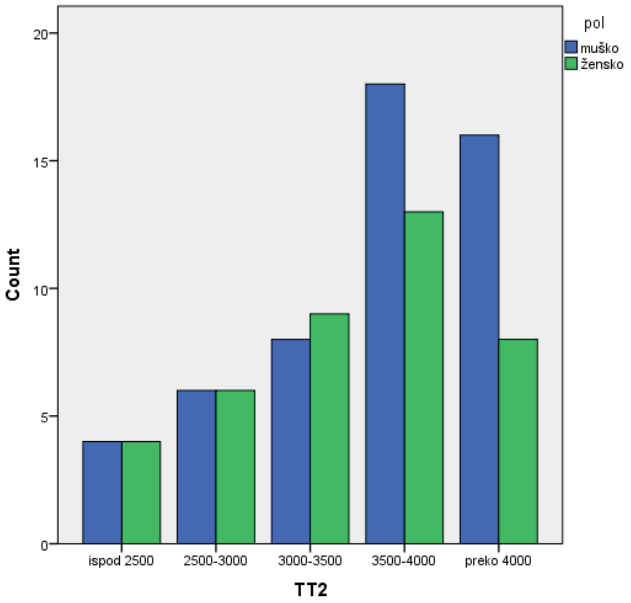

Table 2. Parity of research examinees

a spontaneous vaginal delivery; and 34 (37.0%) women had a Caesarean section.

The average body weight of newborns from pregnancies complicated by gestational diabetes was 3587.07 grams, which is very close to the macrosomia limit of 4000 grams. The largest newborn weighed 4950 grams. Despite this finding, the Apgar scores in the first minute were relatively high (8.63) and did not significantly change during the fifth minute. The average length of newborns was 55.1 cm, with an average head circumference of 34.72 cm. A total of 56.5% of the newborns were male, and 43.5% were female, as shown in Graph 1.

An analysis of the data indicated that elevated glycaemia measurements during the 12-hour glycaemic profile (tested with 75 mg of glucose) occurred in 4 (4.34%) of the cases.

DISCUSSION

Analysis of these results indicated that the age of the subject did not predict the emergence of gestational diabetes. Contrary to expectations, the average age of examinees in our sample was 31.6 years, which was closer to the age of our youngest examinee (20 years) than our oldest examinee (46 years). More than 50% of examinees were recruited from the primipara category, which is contrary to previous reports indicating that gestational diabetes is more frequent from multipara subjects (2, 4).

Seventy-four (77.89%) examinees achieved normoglycaemia exclusively by following a hygienic-dietary regimen. These results support previous studies. For example, *Crowther et al.* demonstrated that normalization of glycaemia in pregnant women with gestational diabetes was successfully achieved by the hygienic-dietary regimen in 75-80% of the cases (12).

Almost a quarter of examinees had an accompanying comorbidity that further increased pregnancy risk. The dominant co-diagnosis was hypertension, and more than one third of examinees (29.8%) had at least two or more accompanying diagnoses. Analysing the data, it was determined that surgical delivery was required for the majority of subjects with comorbidities.

A total of 70.7% of women had a full-term pregnancy, and most of the deliveries were vaginal with an episiotomy. It is important to note that gestational diabetes, although it contributes to foetal macrosomia (12), is not necessarily an indication for Caesarean section. The main indications for Caesarean section were the following:

- Malpresentation of the foetus
- Previous Caesarean sections
- Comorbidities in mothers
- Placental abruption
- Artificial insemination (13)

The above listed indications for a surgical delivery are the same as those for a normoglycaemic pregnancy. The frequency of surgical delivery in pregnant women with gestational diabetes was greater than the usual percentage of Caesarean sections. This increased rate may be caused by the frequency of accompanying diseases, comorbidities, and complications from diabetes.

The body weight of newborns serves as a proxy for whether gestational diabetes was treated adequately during pregnancy. Gestational diabetic pregnancies specifically result in giving birth to macrosomic newborns, which can be defined as either 4000 g or babies that are too large for their given gestational age (i.e., over the 90th percentile) (6, 12). Early detection of gestational diabetes and adequate treatment of pregnant women can prevent the occurrence foetal macrosomia.

In this study, 25 pregnant women who were diagnosed with gestational diabetes gave birth to children whose body weight was over 4000 g. This statistic comprises 27.12%, which is slightly higher than the body weight averages reported in previous studies. For example, two papers found that approximately 20% of macrosomic newborns are from gestational diabetic pregnancies (4, 12).

Interestingly, a more detailed analysis of the anamnesis of patients who gave birth to macrosomic babies found that they had difficulty regulating glycaemia levels during pregnancy using standard therapeutic procedures. These data suggest that maternal hyperglycaemia and subsequent foetal hyperinsulinemia was a longstanding issue.

In these data, no statistically relevant differences were found in the body weight, head circumference or sex of the newborns. Additionally, the Apgar score of 8.63 showed that this population did not deviate significantly in comparison with newborns from uncomplicated pregnancies.

Gilmartin found that in 3-5% of gestational diabetic pregnancies, the condition persists into the postpartum period (4). For this study, a test with 75 mg of glucose was given. The results indicated that one or more measure-

ments of glycaemia within the parameters of a 12-hour glycaemic profile were elevated in 4 (4.34%) of the subjects.

CONCLUSION

The results from the first aim of our study were contradictory to the previous literature. We did not find a positive correlation between the age of the subjects and the occurrence of gestational diabetes. Additionally, the largest percentage of examinees in our research were recruited from the primipara category.

Our findings suggest that in two-thirds of cases, normoglycaemia can be achieved in pregnancies complicated by gestational diabetes when a subject adheres to a hygienic-dietary regimen, which is similar to previous results. In our patient population, the physiological values of glycaemia were achieved by modified nutrition and moderate physical activity.

Pregnancy complicated by gestational diabetes is not necessarily an indication for a Caesarean section. In this study population, the frequency of surgical delivery in pregnant women with gestational diabetes was greater than the reference distribution from the general population. In our population, the main causes for these Caesarean sections were accompanying diagnoses, comorbidities and diabetes complications.

Timely detection of gestational diabetes and adequate treatment of pregnant women can prevent the occurrence of foetal macrosomia as the primary complication resulting from these pregnancies. The data analysed in our research show a slightly greater frequency of macrosomic newborns compared to the reference literature, even with adequate therapy.

Persistence of gestational diabetes after childbirth occurs in 3-5% of cases. These data are congruent with the results of our research.

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THE ANALYSIS OF RISK FACTORS AND CLINICAL-DEMOGRAPHIC CHARACTERISTICS OF PATIENTS WITH CLOSTRIDIUM DIFICILLE INFECTION AS WELL AS THE OUTCOME OF THEIR TREATMENT

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ANALIZA FAKTORA RIZIKA I KLINIČKO-DEMOGRAFSKIH KARAKTERISTIKA KOD BOLESNIKA SA CLOSTRIDIUM DIFFICILE INFEKCIJOM KAO I ISHOD NJIHOVOG LEČENJA

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ABSTRACT

Pseudomembranous colitis is a frequent nosocomial infection associated with significant morbidity and mortality. Clostridium difficile infection incidence most frequently increases due to unreasonable antibiotic use and the appearance of new hypervirulent bacterial strains, which leads to prolonged hospitalization and an increase in the total cost of hospital treatment.

This is a retrospective design study conducted at Clinical Centre Kragujevac from January to December 2014. The patient data were obtained from the protocol of the Virological Laboratory and from medical documentation. All statistical analyses were performed using the computer program SPSS. The descriptive statistical data are expressed as percentage values. Continuous variables are expressed as the arithmetic mean with the standard deviation.

Clostridium difficile infection occurred more frequently with elderly patients (123 patients were over 65 years old). Out of 154 patients on antibiotic treatment, 110 patients were treated with a combination of two or more antibiotics from different pharmacological groups. The most represented antibiotics were from the cephalosporin (71.4%) and quinolone (46.3%) groups. A total of 85.8% of the patients used proton pump inhibitors and H2 blockers.

Our results describe the clinical and demographic characteristics of patients with diagnosed Clostridium difficile infection. The most prevalent characteristics (age, antibiotic therapy, PPI and H2 blocker use), which other researchers have also mentioned as risk factors, were present in our study as well.

Keywords: *Clostridium difficile; diarrhoea; antibiotics; proton pump inhibitors; metronidazole; vancomycin*

SAŽETAK

Pseudomembranozni kolitis je česta nozokomijalna infekcija koja je udružena sa značajnim morbiditetom i mortalitetom. Incidenca Clostridium difficile infekcije najčešće se povećava neracionalnom primenom antibiotika i pojavom novog hipervirulentnog soja bakterije što dovodi do produžene hospitalizacije i povećanja ukupne cene bolničkog lečenja.

Istraživanje je retrospektivnog dizajna, spovedeno je u Klinčkom centru Kragujevac u periodu od januara do decembra 2014. godine. Podaci o pacijentima uzeti su iz protokola Virusološke laboratorije i medicinske dokumentacije. Sve statističke analize su urađene u kompjuterskom programu SPSS. Deskriptivni statistički podaci su izraženi u procentima. Kontinuirane varijable su izražene srednjim vrednostima uz podatak o standardnom odstupanju.

Clostridium difficile infekcija češće se javlja kod pacijenata starije životne dobi (123 pacijenta preko 65 godina). Od 154 pacijenta koji su tretirani antibiotskom terapijom, 110 pacijenata je lečeno kombinacijom dva ili više antibiotika iz različite farmakološke grupe. Najviše zastupljeni antibiotici su bili iz grupe cefalosporina (71,4%) i hinolona (46,3%). Kod 85,8 % pacijenata primenjivani su inhibitori protonske pumpe i H2 blokatori.

Naši rezultati obuhvataju kliničko-demografske karakteristike pacijenata sa dijagnostikovanom Clostridium difficile infekcijom. U najvećem procentu su zastupljene one karakteristike (starost, antibiotska terapija, primena IPP i H2 blokatore) koje su i drugi istraživači naveli kao faktore rizika.

Ključne reči: *Clostridium difficile; dijareja; antibiotik; inhibitori protonske pumpe; metronidazol; vankomicin*

ABBREVIATIONS

IPPs – proton pump inhibitors;
SPSS – Service Provisioning System Software;
ATC – Anatomical Therapeutic Chemical;

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INTRODUCTION

Pseudomembranous colitis is a frequent nosocomial infection associated with significant morbidity and mortality rates. The most frequent colitis-causing pathogen in hospitalized patients is anaerobic, Gram-positive, sporogenous *Clostridium difficile* bacillus. The clinical spectrum of the disease varies from mild, watery diarrhoea to fulminant pseudomembranous colitis with complications (1).

A great number of studies have shown that exposure to antibiotics is considered the most significant risk factor for the onset of *Clostridium difficile* infection. Old age; surgical interventions on the gastrointestinal tract; enteral feeding; prolonged hospitalization, especially at intensive care units; chemotherapy; and comorbidity presence can also contribute to the onset of infection (2-4).

With regard to proton pump inhibitor utilization and its association with the onset of *Clostridium difficile* infection, clinical study data are contradictory. Some clinical studies indicate that there is no significant difference in *Clostridium difficile* infection incidence during the use of proton pump inhibitors (PPIs) (5), while other studies indicate that there is a three times greater risk of morbidity after PPI utilization (6, 7).

The pseudomembranous colitis incidence has increased worldwide due to unreasonable antibiotics utilization and the appearance of new hypervirulent strains of the bacterium. This increase leads to prolonged patient hospitalization and increases the total cost of hospital treatment. The estimated treatment costs are approximately 3 billion € per year (8).

The aim of this study was to determine the clinical and demographic characteristics of patients with clostridial infection as well as the outcome of their treatment.

MATERIALS AND METHODS

This is a retrospective study conducted at Clinical Centre Kragujevac from January to December 2014. A total of 169 patients diagnosed with *Clostridium difficile* infection were included. The patient data were obtained from the protocol of the Virological Laboratory, where stool analysis was performed using an enzyme immunoassay test (EIA). Further research included only the patients with positive tests, while the patients with negative tests or incomplete medical documentation were excluded from the study. The following data were collected:

- Socio-demographic characteristics of the patients;
- The patients' health conditions (acute or chronic diseases);
- Results of diagnostic procedures and laboratory analyses;
- Surgical and medicamental observation.

All statistical analyses were performed in the SPSS computer program (version 18). For each continuous variable, the distribution normality was determined, and then the values were expressed as the arithmetic mean with standard deviation. Descriptive statistical data are expressed as percentage values.

RESULTS

The research included 95 male (56.2%) and 74 female (43.8%) patients. The average age of the examinees was 70 (69.9±12.6). The youngest examinee was 27, and the oldest was 95 years old. The results demonstrated that *Clostridium difficile* infection occurred more frequently in elderly patients (123 patients were over 65 years old).

The average hospitalization length was 24.7±16.3 days. The infection occurred more frequently in patients hospitalized in internal medicine units (cardiology 16%, pulmonology 14.8%, neurology 11.8%). In the intensive care unit, during 2014, 20 patients (11.8%) were registered with the above-mentioned symptoms. Diarrheic syndrome developed after 15 days of hospitalization (14.7±10.4). The patients formed three to four stools a day on average.

Most of the patients were on antibiotic treatment (91.12%). For 11 patients (6.5%), there were no data about antibiotic consumption, while 4 patients (2.4%) were not receiving antibiotic treatment during or immediately before hospitalization. Most of the patients were receiving a combination of two or more antibiotics from a different group in the ATC Classification System (Table 1).

Within the group of those receiving combined antibiotic therapy, 48 patients (43.6%) were treated with cephalosporins and quinolones, 38 (34.5%) patients were treated with cephalosporins, and 10 (9.1%) patients used quinolones as one of the antibiotics in their treatment. A total of 14 patients (12.7%) on combined antibiotic therapy did not use antibiotics from either the cephalosporin or the quinolone group.

Antibiotics from the cephalosporin (71.4%) and quinolone (46.3%) groups were most the highly used for antibiotic therapy. Third-generation cephalosporins and levofloxacin were the most frequently administered (Table 2).

Table 1. Pharmacotherapeutic groups of antibiotics administered to patients with *Clostridium difficile* infection

ATC	Pharmacotherapeutic groups of antibiotics	Patients (N°)	Patients (%)
J01C	Penicillins	1	0.7
J01D	Cephalosporins	19	12.9
J01DH	Carbapenems	4	2.7
J01G	Aminoglycosides	2	1.4
J01M	Quinolones	10	6.8
J01A	Tetracyclines	1	0.7
J01	CAT*	110	74.8
Total		147	100

*A combination of two and/or more antibiotics from a different pharmacological group.
∫ For 7 patients the administered antibiotic is not known.

Table 2. The presence of antibiotics from cephalosporin and quinolone groups

Pharmacotherapeutic groups of antibiotics	ATC	Antibiotics	Patients (N°)	Patients (%)	Patients (%)©
Cephalosporins	J01DB	1st generation	8	7.6	4.7
	J01DC	2nd generation	7	6.7	4.1
	J01DD	3rd generation	73	69.5	43.2
	J01DE	4th generation	3	2.9	1.8
	J01D	Combination£	14	13.3	8.3
	Total		105	100	62.1
Quinolones	J01MA12	Levofloxacin	36	52.9	15.0
J01MA02		Ciprofloxacin	28	41.2	11.7
	J01MA	Combination¥	4	5.9	1.7
	Total		68	100	28.3

£ A combination of two or more antibiotics from the cephalosporin group.
¥ A combination of levofloxacin and ciprofloxacin.
© The percentage of incidence out of the total number of patients (169).

The average length of antibiotics use (for 125 patients) was 16.2±11.5 days.

PPIs were used by 47 (27.8%) patients and H2 blockers were used by 55 (32.5%) patients. Both drugs were used by 43 (25.4%) patients, while 24 patients did not use any of the drugs from the given pharmacological groups.

A total of 155 patients (91.72%) were treated with antibiotics to eliminate *Clostridium difficile* bacillus. In eradication therapy, metronidazole alone or a combination of metronidazole and vancomycin were the most frequently used (Table 3).

Out of 58 patients on combined eradication therapy, 45 patients (77.6%) were first treated with metronidazole and then vancomycin, while 13 patients (22.4%) simultaneously used both antibiotics.

Pulmonography was performed on 129 patients. Out of that number, pleural effusion occurred in 38 patients (29.5%).

The presence of free fluid was visualized by ultrasound in 23 patients (35.4%), although echogram of the abdomen was performed on 65 patients.

Disease relapse occurred in 19 patients (11.2%). A total of 32 (18.9%) patients passed away, mostly due to comorbidity.

DISCUSSION

The results of the study show a statistically significant connection between the onset of clostridial infection and the patient's age. Previous studies have shown that the infection develops more frequently in elderly patients (2), and elderly patients composed 72.8% of the examinees included in our study.

Hospitalization for longer than fifteen days is considered one of the risk factors for developing *Clostridium difficile* infection (3, 9). Prolonged treatment, especially in intensive care units, increases the risk by nearly two-fold (3). The average hospitalization length in our study was longer than 15 days (24.7±16.3), and the intensive care unit was one of the most frequent departments (11.8%) where the infection developed. However, the department structure's significance for clostridial infection onset cannot be observed as an isolated statistic variable considering that there are differences between departments in regard to antibiotics consumption and the inpatients' age.

Antibiotics use increases the onset incidence of *Clostridium difficile* infection by about seven times (10). The highest risk for the development of clostridial infection is the use of clindamycin, quinolone, cephalosporin, monobactam and carbapenem, which have risk factors that are similar to that of multiple antibiotics utilization, as well as their long-lasting use (10, 11). Meta-analysis conducted on 30184 patients during 2013 showed that cephalosporin and quinolone use increased the risk by five to six times (10). Macrolides, trimethoprim-sulfamethoxazole and penicillins have less influence on the infection onset (10, 11). In most cases, our patients were treated with combined antibiotics therapy (Table 1.). The most frequently applied antibiotics were 3rd-generation cephalosporins and antibiotics from the quinolone group (Table 2), which are, according to studies, the most influential in triggering *Clostridium difficile* infection. The average length of antibiotics utilization was approximately 16 days.

With regard to the significance of PPI use in the onset of *Clostridium difficile* infection, data obtained from clinical studies are contradictory. A meta-analysis that included 39 studies showed a statistically significant association

Table 3. Eradication therapy of *Clostridium difficile* infection

Antibiotics	Patients (N°)	Patients (%)
Metronidazole tbl.	58	34.9
Vancomycin amp.	39	23.5
Combined eradication therapy ∂	58	34.9
Without eradication therapy	11	6.6

∫ For 3 patients (1.78%) the data about eradication therapy are missing.
∂ Metronidazole (amp. and tbl.) and vancomycin (amp.).

between PPI use and the development of *Clostridium difficile* infection (6). According to studies, the incidence of the disease is two to three times higher in patients treated with PPIs than in patients who were not treated with PPIs (6, 12), while the use of H2 blockers increases this risk by two-fold (12).

Studies conducted during 2011 and 2012 did not show an influence of PPI use on the onset of this infection (5). In our study, only 14.2% of the examinees received neither PPIs nor H2 blockers.

Recurrent infection is expressed by repeated manifestations of disease symptoms 14 to 60 days after a previous episode of infection. Relapse after initial antibiotic therapy is manifested in 20-30% of patients (13). Feldman and associates established that infection relapse is the result of reinfection by new bacterial strains (14). Repeated infection occurred in 11.2% of our patients. Some patients with recurring disease were probably cured at regional hospitals, so the total number of recurrent infections is probably higher.

The mortality rate for *Clostridium difficile* infection in numerous studies varies from 13% to 27% (15, 16). Comorbidity of congestive heart diseases, chronic pulmonary diseases, renal insufficiency, malignant neoplasms, inflammatory bowel diseases (Crohn's disease, ulcerative colitis), and diabetes mellitus are all associated with worse outcomes and a higher mortality rate among hospitalized patients with *Clostridium difficile* infection (17, 18). Our results show that mortality was mostly caused by comorbidity.

The treatment of *Clostridium difficile* infection depends on the severity of clinical conditions and includes metronidazole and/or vancomycin use along with other substitution and symptomatic therapy. A meta-analysis (1218 patients) has indicated that metronidazole and vancomycin provide similar cure rates in patients in mild clinical condition, while vancomycin is more effective at treating patients in severe clinical condition (19). In severe clinical condition cases, 97% of the patients treated with vancomycin were cured, in comparison to 76% of those treated with metronidazole (20). Combined eradication therapy and the independent use of metronidazole were administered for the highest number of patients (Table 3). However, because this was a retrospective study design and the data about the severity of the clinical condition were inaccessible, it cannot be confirmed whether the appropriate treatment protocol was followed. Patients who were not on combined eradication therapy during hospitalization either passed away immediately after being diagnosed or their treatment was continued at authorized health institutions.

CONCLUSION

Clostridium difficile is a very significant pathogen causing hospital-acquired diarrhoea. The disease affects mostly elderly, hospitalized patients with comorbidities. It appears most frequently after cephalosporin and fluoroquionolone use. Treatment depends on the severity of the clinical condition and relies on metronidazole and/or vancomycin utilization along with other substitution and symptomatic therapy.

Our study included only patients with diagnosed *Clostridium difficile* infection. The presented results include the clinical and demographic characteristics of these patients. The most prevalent characteristics (age, antibiotic therapy, PPI and H2 blocker use), which other researchers have also mentioned as risk factors, are present in our study as well.

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MECHANISMS OF INTRACELLULAR CHLAMYDIAE SURVIVAL

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MEHANIZMI INTRACELULARNOG PREŽIVLJAVANJA HLAMIDIJA

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ABSTRACT

Chlamydiae are Gram-negative, non-motile, obligate intracellular, and spherically shaped bacteria with a diameter of 0.2-1.5 μm. Chlamydiae are present in several different morphological forms: the elementary body, the reticular body, and in the last several years, there has been the observation of a third form known as the persistent or atypical form. The intracellular localization of Chlamydia provides a unique replication cycle that occurs inside a membrane-surrounded vacuole in the host cell cytoplasm and is significantly different from the method of multiplication of other microorganisms. Chlamydiae are capable of manipulating different signalling pathways inside the infected cell, thus avoiding the host immune response. This ensures intracellular multiplication, survival, and long-term persistence of Chlamydiae. There are two basic means of achieving this persistence: inhibition of apoptosis and manipulation of NF-κB (nuclear factor kappa B)-mediated signals in the host.

Keywords: *Chlamydia, invasion, inclusion, intracellular survival, persistence*

SAŽETAK

Hlamidije su Gram-negativne, nepokretne, obligatno intracelularne bakterije, sferičnog oblika, prečnika od 0,2-1,5 μm. Pojavljuju se u više različitih morfoloških formi: elementarno telo, retikularno telo, a u poslednjih nekoliko godina uočava se postojanje i treće forme označene kao perzistentna ili atipična forma. Zahvaljujući intracelularnoj lokalizaciji, hlamidije imaju jedinstven replikativni ciklus koji se znatno razlikuje od ostalih načina umnožavanja mikroorganizama, a odvija se unutar vakuole oivičene membranom u citoplazmi ćelije domaćina. Hlamidije su unutar inficirane ćelije u stanju da manipulišu različitim signalnim putevima i da na taj način izbegavaju imunski odgovor domaćina, obezbeđujući sebi umnožavanje i dugotrajnu perzistenciju. Proučena su dva osnovna načina na koji ove bakterije to mogu: inhibicija apoptoze i manipulisanje NF-κB (nuklearni faktor kapa V) posredovanim signalima.

Ključne reči: *Hlamidija, invazija, inkluzija, unutarćelijsko preživljavanje, perzistencija*

GENERAL CHARACTERISTICS AND MORPHOLOGY

Chlamydia trachomatis (CT) are strictly intracellular bacteria that target primarily cylindrical epithelial cells. These cells are found on the surfaces of the conjunctiva, urethra, endocervix, endometrium, and ovarian tube, thus explaining the localization of diseases caused by CT (1). Most Chlamydia infections remain undiagnosed because they are often asymptomatic and last for a long time. Consequently, this results in unrecognized and untreated infections that can be serious and difficult to treat (2). Bacteria within the *Chlamydiaceae* family are similar to viruses in their size and intracellular localization. On the other hand, they are considered bacteria due to some characteristics, such as the existence of inner and outer membranes, simultaneous existence of both DNA and RNA, prokaryotic ribosomes, lipids and nucleic acids, sensitivity to many antibiotics, and capability of visualization with light microscopy (3). Chlamydiae are Gram-negative, non-motile, obligate intracellular bacteria, spherical in shape, with a diameter of 0.2-1.5 μm. The Chlamydia genome is characterized by double-helix DNA with an approximate size of 106 bp. All human serotypes of *Chlamydia trachomatis* have a common plasmid of 7.5 kbp (4), which is highly conserved and has a role in pathogenesis, thus it can be used for identification purposes (5). Chlamydiae are present in

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two morphologically and functionally different forms. The metabolically inactive, infective form is called the basic or elementary body (EB), and the metabolically active, non-infectious form is called the gridded or reticular body (RB). Both represent evolutionary forms of chlamydiae adaptation to extracellular and intracellular living conditions (6). The latest research notes to existence of a third morphological form known as the persistent form.

The Chlamydia cell wall is similar to the cell walls of Gram-negative bacteria. It consists of inner and outer cytoplasmic membranes with penicillin-binding proteins but without a peptidoglycan layer in between (3). The LPS in Chlamydia can be located in the inclusion body inside the inclusion membrane, in the cytoplasm and surface of the host cell, or in the surrounding infected cells. LPS is important in the pathogenesis of chlamydial infections and in the exposure of infected cells to the host immune system (7). The major outer membrane protein (MOMP) represents a type-specific antigen that determines the chlamydial type and serotype and functions as a porin and an adhesin (8). The second major protein family is known as the polymorphic outer membrane protein (POMP) of as yet unknown biological function (7). Two cell wall proteins, OmcA and OmcB, are rich in cysteine and can interact with other proteins. Additionally, the OmcB protein participates in the adhesion process of chlamydia to a host cell in the early stages of interaction (9). The inclusion proteins, IncA, IncB, and IncC, have several functions, such as inclusion development, avoidance of lysosomal fusion, signalling of EB-RB-EB reorganization, etc. (10). Special spike-like structures on the surface of the chlamydiae elementary and reticular bodies have been observed by electron microscopy. Numerous studies note that these structures serve as channels between the host cell and parasites.

THE LIFE CYCLE OF CHLAMYDIAE

The infectious cycle starts when the EB establishes contact with the surface of the host cell. A large number of chlamydial proteins (i.e., MOMP, OmcB, PmpD, cysteine-rich proteins) function as adhesins. Adhesion occurs through glycosaminoglycans that act as "bridges" between receptors on the bacteria and receptors on the host cell (11). There are two described means of chlamydiae entry into the host cell: receptor-mediated endocytosis and microfilament-depended phagocytosis. After infection, the EB becomes embedded inside a membrane connected to a vacuole called the inclusion. The EB differentiates into the metabolically active form called the RB that undergoes repeated cycles of binary fission and eventually secondary differentiation back to the EB form (7). Both EB and RB forms possess type III secretion systems (T3SS) that are envelope-spanning nano-machines conserved among diverse Gram-negative bacterial pathogens. T3SS translocate virulence effector proteins directly into host cells, where they subvert cellular processes to promote bacterial entry, survival, and replication (12). Primary differentiation from the EB into the RB includes changes in the structure of the outer membrane, with the breaking of disulfide bonds between MOMP and other proteins of the outer membrane (13). The decrease in the number of disulfide bonds results in increased membrane permeability, easier transport of nutritive material, and increased metabolic activity, while simultaneously contributing to mechanic and osmotic sensitivity of the RT (14). Treatment of the EB with dithiotreitol results in a reduction of disulfide bonds, an increase in permeability and metabolic activity, and a decrease in osmolarity and infectivity (13). In contrast, the compact electronically dense nucleotide structure of the EB is preserved, which indicates that the mere reorganization of membrane structure is not enough to induce differentiation. DNA is loosely packed in the RB. In relation to this, changes in structural organization of nucleotides represent a crucial moment in the developmental cycle of Chlamydia. Decondensation of the chlamydial chromosome is the most important step in the activation of transcription and translation. The differentiation of the RB into the EB is followed by reincorporation of MOMP and other proteins of the outer membrane as the infectious cycle progresses (13). Lastly, the host cell lyses releasing EBs that infect surrounding cells (7).

THE PERSISTENT OR ALTERED DEVELOPMENTAL CYCLE of *C. trachomatis*

The term "persistent infection" represents the absence of visible development, suggesting the presence of Chlamydia in a form that is different from typical intracellular morphological forms (Table 1, Figure 1). This altered developmental cycle correlates with a decrease in metabolic activity, which limits growth and multiplication and postpones the differentiation into EB forms (13). Chlamydiae induce the secretion of interferon-γ (IFNγ), which completely inhibits bacterial development (7). Low concentrations of IFNγ induce the development of morphologically aberrant forms of chlamydia (13). The levels of chlamydial MOMP decrease with low concentrations of IFNγ resulting in the maintenance of a chronic infection and accumulation of high quantities of chlamydial heat shock protein 60 (HSP60) in infected host cells (15). The persistent forms of Chlamydia are not only morphologically atypical but also express different key chlamydial antigens. This is concurrent with a reduction in the synthesis of chlamydial MOMP and LPS and an increase in the synthesis of HSP60.

Table 1. Basic mechanisms of intracellular survival of chlamydiae

Mechanism	References
Persistence	13,15
Apoptosis inhibition	51,52,53,54,55,56,57,58
Modulation of NF-kB signalling	59,60,61,62,63,64,65

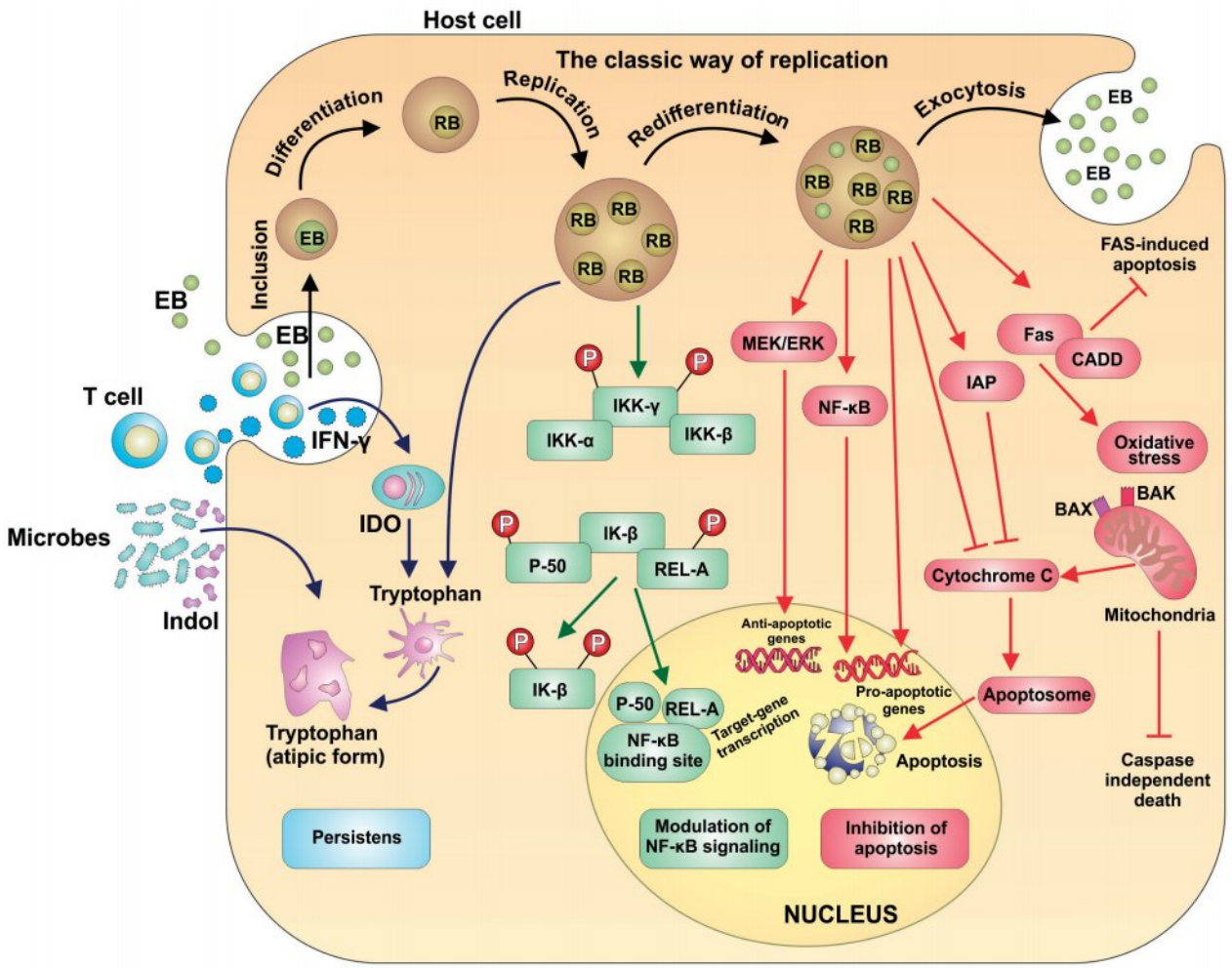

Figure 1. Developmental cycle and intracellular survival mechanisms of Chlamydia: black - classical replication pathway; blue - persistent developmental cycle; green - intracellular survival via modulation of NF-κB signalling; red - intracellular survival via inhibition of apoptosis.

MECHANISMS OF INTRACELLULAR SURVIVAL

Different bacterial adhesins and ligands mediate the invasion of many cell lines, and their mode of "contribution" depends on the type of cell and type of Chlamydia involved in the process. Well defined adhesins are glycosaminoglycan (GAG), MOMP, OmcB, and PmpD (16). The adhesion of chlamydiae is a two-step process, which entails an initial reversible interaction between the EB and the target cell via heparan sulfate proteoglycans (HSPG). Following this initial interaction there is an irreversible adhesion step via a secondary receptor of high affinity (17). Alongside heparan sulfate, mannose-6-phosphate-receptors and oestrogen receptors are other surface receptors used by chlamydiae to enter the target cells (18). A chlamydial protein, known as Tarp, translocates during entry and facilitates the invasion and differentiation of EB to RB (19, 20), while Rab GTPs act as regulators that enable the formation of the inclusion harbouring bacteria (21).

Following adhesion, chlamydiae reorganize the host cell cytoskeleton via induction and activation of the Rho family of GTPs (22). Entry of Chlamydiae into non-phagocytic cells is mediated by small GTPase-dependent reorganization of the actin cytoskeleton (17, 23-26). Activation of Rac1 results in the recruitment of the actin regulators WAVE2, Abi-1, and Arp2/3, which are necessary for *C. trachomatis*-induced actin reorganization (27). Both chlamydial and host proteins may function synergistically to promote invasion. After entering the cell, the EB is located inside a membrane-surrounded vacuole known as the inclusion. The newly formed inclusion moves along micro tubes to the peri-Golgi space, thus preventing fusion with lysosomes (22). A pH>6 within the inclusions indicates that there has been no fusion with lysosomes (28). This complex set of interactions between the chlamydial inclusions and cell internal pathways is important in acquiring essential nutrients, such as amino-acids, lipids and iron, while at the same time limiting the capability of recognition by the host immune system.

After entry into the host cell, the newly formed chlamydial inclusion is transported alongside micro tubes to the microtube-organization centre on a dynein–dependent or dynein–independent fashion mediated by Src kinase (29, 30). Recent studies have shown that the inclusion membrane is not homogeneous and that micro domains are made of inclusion proteins (Inc), active Src kinases, and

cholesterol combined with centrosomes and dynein (31). The inclusion forms a dynein-dependent relation with the centrosome during the cell cycle. These findings are interesting in terms of a possible connection between chlamydiae and HPV-combined cervical cancer (32). Numerous studies have shown a close relation between chlamydial inclusions and the Golgi apparatus from which bacteria take exocytotic vesicles with sphingomyelin and cholesterol via a Brefeldin A–sensitive manner (33). Sphingomyelin is necessary for growth and stability of the inclusion membrane but not for the replication process of chlamydiae (34). On the other hand, ceramide transfer protein (CERT), a cytosolic protein that transports ceramide from the endoplasmic reticulum to the trans–Golgi region, is recruited from the inclusion membrane through an interaction with the inclusion membrane protein IncD and it is involved in acquiring sphingomyelin (35). Host cell glycerophospholipids, such as phosphatidylinositol and phosphatidylcholine, are also taken over in the process, which entails the activation of phospholipase A2 located in the cytosol (36). In addition to the Golgi apparatus, the inclusion interacts with other cellular organelles, such as the multivesicular bodies, which are also a significant source of sphingolipids and cholesterol, as well as lipid droplets that translocate into the inclusion lumen and serve as a source of neutral lipids (37-39). The entire set of chlamydial proteins show tropism towards lipid droplets, which are lipid storage organelles. Lipid droplets pass through the inclusion membrane and are in close connection with the chlamydia RB. These interactions facilitate nutrient acquisition necessary for replication of bacterial cells, as well as expansion and stability of the inclusion membrane (31). The metabolically inert EB undergoes morphological changes and is reorganized into the RB (23) while the disulfide bonds between the MOMP and other outer membrane proteins break apart. Chromatin is released from a condensed structure, and transcription becomes more intense. The RB becomes metabolically active and divides via binary division inside host cell endosomes. Following a growth and division period, the RB is once again reorganized into the EB. The differentiation of the RB into the EB is associated with the reincorporation of MOMP and other outer membrane proteins as the infectious cycle progresses. All intracellular pathogens must eventually exit the host cell (40, 41). *C. trachomatis* has evolved at least two, possibly three, distinct mechanisms of host-cell egress, lysis, and extrusion (42), in addition to a non-lytic exocytosis-like mechanism (43). The extrusion mechanism is believed to be dependent on actin polymerization, N-WASP, Rho GTPase, and myosin II as determined by the use of specific inhibitors for each of their activities (42). The recruitment of the actin coat to the inclusion prior to extrusion appears to be a sporadic and dynamic event relying on a combination of both bacterial and host factors (43).

Cells of the innate immune system express receptors important for the recognition of microorganisms. Some of these receptors are located on the cell surface, while others are located in the cytoplasm or endoplasmic reticulum. As with most bacteria, chlamydia infections are detected by host pattern recognition receptors (PRRs) that recognize chlamydial LPS via Toll-like receptor 4 (TLR4) (44-47) and Hsp60 via TLR2 and TLR4 (48). Signals from TLRs, which are specific for different bacterial antigens (49), enable the production of cytokines and enzymes involved in a variety of antimicrobial functions. Chlamydiae are capable of manipulating these signalling pathways and prevent the initiation of the innate immune response (50). There are two primary ways of achieving prevention: inhibition of apoptosis and manipulation of NF-κB-mediated signals (Table 1).

The effects of Chlamydiae on the apoptotic signalling programme are complex (Figure 1). Chlamydiae inhibit apoptosis primarily via the inhibition of mitochondrial cytochrome C releasing, thus preventing early death of the host cell (51, 52). The Bcl-2 family of proteins regulates the release of mitochondrial cytochrome C. Chlamydiae induce the degradation of BH3-only Bcl-2 family proteins. (52). However, the cleavage of BH3-only proteins in cell lines engineered to express active recombinant CPAF occurs with different kinetics from canonical substrates and is prevented by the proteasome-specific inhibitor MG-132. This suggests that degradation of BH3-only proteins occurs via a proteasome-dependent mechanism indirectly influenced by CPAF (53). Although its anti-apoptotic role is unclear, CPAF is considered a central immune regulatory protein. Other potential anti-apoptotic mechanisms include the stabilization of inhibitor of apoptosis (IAP) proteins (54) and the sequestration of pro-apoptotic phosphorylated BAD and protein kinase Cδ (PKCδ) at the chlamydial inclusion (55, 56). The increased expression of the anti-apoptotic protein Mcl-1 in infected cells has also been linked to the activation of Raf/MEK/ERK (57), a signalling cascade that affects inflammatory responses (58).

The interference with NF-κB signalling is crucial in the modulation of the host immunity by chlamydial (59, 60). The NF-κB subunits RelA (p65) and p50 form a heterodimer complex that translocates into the nucleus and acts as a transcription activator (Figure 1). During chlamydial infection, proteolysis of RelA occurs with the participation of *Chlamydia trachomatis* Tsp-like protease (Ct441), thus blocking translocation of NF-κB (60, 61). Chlamydia may also block NF-κB activation by regulating ubiquitin-mediated protein degradation. Nuclear translocation depends on the degradation of the inhibitor IκBα via ubiquitin-mediated proteolysis during the canonical NF-κB activation pathway (62). Ectopically expressed ChlaDub1 binds to IκBα and inhibits its ubiquitination. This in turn suppresses degradation of IκBα and subsequent activation of NF-κB (63). Although CPAF is an extensively characterized protease with numerous potential substrates relevant to innate immunity (64), a recent report suggests that several proteins are targeted by CPAF, including the NF-κB p65/RelA subunit, RFX5, Bim, and Puma (discussed below), all of which may not be bona fide CPAF substrates in in vivo settings (65).

CONCLUSIONS

Taken together, there is still much to learn from using a combination of structural, cellular, and molecular approaches to study the critical early interactions between *C. trachomatis* and host cells. Although the developmental cycle of chlamydiae is well studied, the signals that start the conversion of EB into RB and vice versa are still unknown. The biology and means of intracellular survival of chlamydiae are still not completely understood, thus there is a need for all methods of research to understand how these intracellular bacteria survive extremely well within an infected cell. The exact mechanism of control and regulation of chlamydial intracellular development is still unknown and therefore, should be a focus of present and future studies.

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GASTROINTESTINAL NON-INFECTIOUS COMPLICATIONS IN PATIENTS ON PERITONEAL DIALYSIS

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NEINFEKTIVNE GASTROINTESTINALNE KOMPLIKACIJE KOD BOLESNIKA NA PERITONEUMSKOJ DIJALIZI

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ABSTRACT

Gastrointestinal complications are common among patients on peritoneal dialysis. Risk factors for the development of gastrointestinal complications in this patient population include: toxic effects of uremic toxins, frequent use of nonsteroidal anti-inflammatory drugs, Helicobacter pylori infection, angiodysplasia, increased intra-abdominal pressure, use of bioincompatible solution for peritoneal dialysis, increased glucose in solutions for peritoneal dialysis, secondary hyperparathyroidism (hypercalcemia), a disorder of lipid metabolism (hypertriglyceridemia), and the duration of peritoneal dialysis treatment. The most important non-infectious gastrointestinal complications in patients on peritoneal dialysis are: gastrointestinal bleeding, herniation and leaking of the dialysate from the abdomen (increased intra-abdominal pressure), impaired lung function (intra-abdominal hypertension), acute pancreatitis, and encapsulating sclerosis of the peritoneum. Intra-abdominal hypertension is defined as IAP ≥ 12 mmHg. Pouring the peritoneal dialysis solution leads to increased intra-abdominal pressure, which results in the development of hernias, pleuro-peritoneal dialysate leakage (hydrothorax), and restrictive pulmonary dysfunction. Risk factors for the development of acute pancreatitis in this patient population include: uraemia, secondary hyperparathyroidism with hypercalcemia, hypertriglyceridemia, features of the peritoneal dialysis solution (osmolarity, acidity, glucose, chemical irritation, and calcium in the solution for peritoneal dialysis lead to "local hypercalcemia"), toxic substances from the dialysate, the bags and tubing, and peritonitis and treatment of peritonitis with antibiotics and anticoagulants. Encapsulating sclerosis of the peritoneum is rare and is the most serious complication of long-term peritoneal dialysis. It is characterized by thickening of the peritoneum, including cancer, and signs and symptoms of obstructive ileus. Diagnosis is based on clinical, laboratory and radiological parameters. Encapsulating sclerosis of the peritoneum can be indicated by an AR-CA-125 concentra-

SAŽETAK

Gastrointestinalne komplikacije se često javljaju kod bolesnika koji se leče peritoneumskom dijalizom. U faktore rizika za razvoj gastrointestinalnih komplikacija u ovoj populaciji bolesnika spadaju: toksično dejstvo uremijskih toksina, česta upotreba nesteroidnih antiinflamatornih lekova, infekcija helikobakterom pilori, angiodisplazija, povećan intraabdominalni pritisak, bioinkompatibilni rastvor za peritoneumsku dijalizu, povećan sadržaj glukoze u rastvorima za peritoneumsku dijalizu, sekundarni hiperparatireoidizam (hiperkalciemija), poremećaj metabolizma lipida (hipertrigliceridemija), dužina lečenja peritoneumskom dijalizom. Najznačajnije neinfektivne gastrointestinalne komplikacije kod bolesnika koji se leče peritoneumskom dijalizom su: gastrointestinalno krvarenje, hernije i oticanje dijalizata iz abdomena (povećan intraabdominalni pritisak), poremećaj funkcije pluća (intraabdominalna hipertenzija), akutni pankreatitis i inkapsulirajuća skleroza peritoneuma. Intraabdominalna hipertenzija se definiše kao IAP ≥ 12 mmHg. Ulivanje rastvora za peritoneumsku dijalizu dovodi do povećanja intraabdominalnog pritiska, a to za posledicu ima razvoj hernija i pleuro-peritoneumsko oticanje dijalizata (hidrotoraks), restriktivni poremećaj funkcije pluća i hernije. U faktore rizika za razvoj akutnog pankreatitisa u ovoj populaciji bolesnika spadaju: uremija, sekundarni hiperparatireoidizam sa hiperkalciemijom, hipertrigliceridemija, karakteristike rastvora za peritoneumsku dijalizu (osmolarnost, kiselost, sadržaj glukoze, hemijska iritacija, kalcijum u rastvoru za peritoneumsku dijalizu dovodi do "lokalne hiperkalciemije"), toksične supstancije iz dijalizata, kesa i cevčica, peritonitis i lečenje peritonitisa antibioticima i antikoagulantnom terapijom (i.p. primena antibiotika i heparina). Inkapsulirajuća skleroza peritoneuma je retka, najozbiljnija komplikacija dugogodišnjeg lečenja peritoneumskom dijalizom, koja se karakteriše zadebljanjem peritoneuma, obuhvatanjem creva, simptomima i znacima opstruktivnog ileusa. Dijagnoza se postavlja na osnovu kliničkih, laboratorijskih i radioloških parametara. Koncentracija AR-CA-125 manja od 33 U/min

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tion of less than 33 U/min and a concentration of AR-IL-6 greater than 350 pg/min in the effluent of patients with ultrafiltration weakness. Treatment consists of stopping peritoneal dialysis, using anti-inflammatory (corticosteroids) and anticicatricial drugs (tamoxifen), while surgical treatment includes enterolysis and adhesiolysis.

Keywords: *gastrointestinal complications, peritoneal dialysis*

i AR-IL-6 veća od 350 pg/min u efluentu kod bolesnika kod kojih postoji slabost ultrafiltracije ukazuje na inkapsulirajuću sklerozu peritoneuma. Lečenje se sastoji u prestanku lečenja bolesnika peritoneumskom dijalizom, primeni antizapaljenskih (kortikosteroidi) i antiožiljnih lekova (tamoksifen), dok hirurško lečenje uključuje enterolizu i adheziolizu.

Ključne reči: *gastrointestinalne komplikacije, peritoneumska dijaliza*

INTRODUCTION

Gastrointestinal complications are common among patients suffering from chronic kidney disease, and uraemia and dialysis (hemodialysis, peritoneal dialysis) are risk factors for gastrointestinal complications in this patient population (1). The prevalence of gastrointestinal symptoms in patients suffering from chronic kidney disease, including patients treated with haemodialysis and peritoneal dialysis, is estimated to be 70-80% (1). The prevalence of gastrointestinal symptoms and complications increases during the time a patient is treated with dialysis (1).

Gastrointestinal symptoms

Depending on the presence or absence of organic disease in the gastrointestinal system, patient symptoms may be organic (associated with lesions of the gastrointestinal tract), and/or functional (such as psychological factors, visceral hypersensitivity, or altered mobility or motility of the gastrointestinal tract) (1). There are six different groups of functional gastrointestinal symptoms: oesophageal, gastroduodenal, bowel syndrome, functional abdominal pain, biliary, and anorectal (1). The most common gastrointestinal symptoms in patients on peritoneal dialysis are sickness, nausea, vomiting, abdominal pain, constipation and diarrhoea. Irritable bowel syndrome is also highly prevalent in this population of patients (11-33%) (1).

Gastroparesis, or prolonged gastric emptying, frequently occurs in patients suffering from chronic kidney disease and patients on peritoneal dialysis. Etiopathogenesis is not completely clear; the main symptoms are nausea, vomiting, loss of appetite and anorexia (1).

The prevalence of constipation is 10-20% in the general population, 29% in patients on peritoneal dialysis, and 63% in patients on haemodialysis. The main causes of constipation are reduced physical activity, reduced intake of foods rich in fibre, the use of a phosphate binder, and the presence of a number of co-morbidities, such as diabetes mellitus and cerebrovascular disease(1).

Gastrointestinal complications

Gastrointestinal bleeding

Gastrointestinal bleeding is a common complication in patients suffering from chronic kidney disease and in patients treated with renal replacement therapies (hemodialysis, peritoneal dialysis). Gastrointestinal bleeding can be from the upper and/or lower gastrointestinal tract. The main causes of bleeding from the upper gastrointestinal tract are mucosal erosions (toxic effects of uremic toxins and/or nonsteroidal anti-inflammatory drugs), ulcers of the stomach or duodenum (uremic toxins, nonsteroidal anti-inflammatory drugs, Helicobacter pylori infection) and angiodysplasia. The prevalence of Helicobacter pylori (*Helicobacter pylori*) is high and varies between 49-66%. Helicobacter pylori infection is diagnosed by a urea breath test, which has a reduced sensitivity and specificity in this patient population. Triple therapy (proton pump blockers, clarithromycin, amoxicillin or metronidazole) is used to treat the infection, and oesophagogastroscopy is used to assess the treatment. The main causes of bleeding from the lower gastrointestinal tract are angiodysplasia, diverticulosis (32%) and colon cancer. Colon diverticulosis and diverticulitis are common in patients with polycystic kidney disease in the general population (1). Depending on the clinical course, the gastrointestinal bleeding may be acute (hematemesis, melena, and/or rectorrhagia) or chronic (a positive stool test for occult blood) (1). Acute bleeding from the upper gastrointestinal tract is more common in patients with chronic kidney disease than in the general population (21 cases/1000 patients/year) (1). Acute bleeding from the upper gastrointestinal tract is a significant cause of mortality in these patients; it is responsible for 3-7% of all deaths in patients with end-stage chronic kidney disease (1). Among patients suffering from chronic kidney disease, the prevalence of positive occult blood tests is 19% (1). Angiodysplasia is the most common cause of bleeding in the upper and lower gastrointestinal tracts. The use of antiplatelet and anticoagulant therapy increases the risk of gastrointestinal bleeding in patients with angiodysplasia and chronic kidney disease (1).

Intra-abdominal hypertension/abdominal compartment syndrome

Normal intra-abdominal pressure (IAP) is defined as 5-7 mmHg and is 9-12 mmHg in obese patients (9). Intra-abdominal hypertension (IAH) is defined as an IAP value ≥ 12 mmHg (9, 10). Depending on the level of IAP, there are four categories of IAH: grade I: IAP = 12-15 mmHg,

grade II: IAP = 16-20 mmHg, grade III: 21-25 mmHg and grade IV: IAP 25 mmHg (9, 10). Abdominal compartment syndrome (ACS) is defined as an IAP of > 20 mmHg and is associated with the dysfunction of a new organ or organ system with or without APP < 60 mmHg. Abdominal pressure perfusion (APP) = MAP - IAP (9, 10). ACS can be primary (intra-abdominal cause) or secondary (extra-abdominal cause). IAP values of 12-20 mmHg affect the function of organs, including the kidneys (9, 10). According to the *WSACS* (*World Society of the Abdominal Compartment Syndrome*) conditions associated with IAH and ACS include conditions with increased intra-abdominal volume (dilatation of the gastrointestinal tract, gastroparesis, stomach distention, ileus, volvulus, pseudo-obstruction of the colon, a tumour mass in intra-abdominal or retroperitoneal areas, ascites or haemoperitoneum, pneumoperitoneum (for example, during laparoscopic surgery), and peritoneal dialysis (peritoneal dialysis solution)) and conditions due to reduced elasticity of the abdominal wall (abdominal surgery, especially with tight, solid closing of the abdomen, bleeding into the wall of the abdomen, and surgical correction of large abdominal hernias), or a combination of both pathological conditions (obesity, sepsis, severe sepsis and septic shock, severe acute pancreatitis, complicated intra-abdominal infections, massive infusion therapy (resuscitation), and large burns) (9, 10). In patients with severe sepsis and septic shock, the incidence of IAH and ACS is 51-76% and 33%, respectively, while in patients with acute pancreatitis, the incidence of IAH and ACS is 59-84% and 25-56%, respectively (9). IAH is a risk factor for adverse outcomes in patients in intensive care units (9, 10).

The main clinical effects of IAH and ACS in patients who suffer from kidney failure are acute renal failure and increased pressure in the intrathoracic cavity (increased central venous pressure). The cut-off value of IAP for the development of acute renal failure is 12 mmHg (RIFLE classification for acute renal failure) (9). Due to the decreased venous return of blood from the abdomen (IAH), filling of the heart is decreased (cardiac preload), which results in reduced displacement of blood (reduced stroke volume/reduced cardiac output) and renal hypoperfusion (9, 10). Increased intrathoracic pressure leads to an increase in central venous pressure, which results in renal compartment syndrome (increased pressure in the venous system of the kidney and in the renal parenchyma) and reduced GFR (9, 10).

The main clinical consequences of increased IAP in patients on peritoneal dialysis are reduced compliance of the lungs (increased intrathoracic pressure), leaking of the peritoneal dialysis solution from the abdomen into the pleural space (hydrothorax) and the occurrence of hernias in weak areas of the abdominal wall (9, 10).

Leaking of the dialysate from the abdomen is classified as early (occurring within the first 30 days of placement of the catheter for peritoneal dialysis) and late (within the first year of catheter placement). Early dialysate leaks are designated as pericatheter dialysate leaks (dialysate flowing beside the catheter). Late dialysate leaks (into the abdominal wall, genital organs, or pleural space) are clinically manifested by increased body weight, swelling, and feelings of suffocation. There is also poor drainage of the dialysate and ultrafiltration weakness with late dialysate leaks (14). Risk factors for dialysate leakage are catheter placement technique, the catheter design, the period between the catheter placement and the initiation of peritoneal dialysis, the condition of the abdominal wall, and the condition of the diaphragm (14). Using the optimal technique to place a catheter for peritoneal dialysis, delaying initiation of peritoneal dialysis (10-14 days after placement of the catheter), gradually increasing the volume of the solution for peritoneal dialysis, and preserving the integrity of the diaphragm all prevent the development of dialysate leakage (14). Dialysate leaking from the abdomen into the pleural space results in the development of hydrothorax. Hydrothorax (transudate) is a rare complication of peritoneal dialysis that occurs as a result of dialysate leaking from the abdomen into the pleural space because of diaphragm defects or the transport of dialysate through the lymphatic vessels of the diaphragm (14, 15). The prevalence of hydrothorax in patients on peritoneal dialysis ranges from 1.6-6.0% (15). It occurs most often after the start of PD therapy. The right pleural space is predominantly affected, and the early clinical manifestations include feelings of suffocation, pleural pain, and decreased ultrafiltration (15). The diagnosis of peritoneal-pleural dialysate leakage can be aided by radiography of the heart and lungs (detecting pleural effusion), spirometry (identifying restrictive pulmonary dysfunction, pulmonary function tests to indicate a decreased vital capacity and reduced total lung capacity), thoracentesis (distinguishing transudates from exudates, indicating fluid peritoneal dialysis), computed tomography (CT) and nuclear magnetic resonance (NMR) peritoneography (detecting place and causes of dialysate leakage), scintigraphy peritoneum - Tc-99m DTPA (detecting leakage of dialysate) and video-assisted thoracoscopic surgery (VATS) (detecting defects of the diaphragm) (15, 16).

For the diagnosis of pleural effusion associated with peritoneal dialysis, we determine the concentration of glucose in the pleural fluid. A concentration of glucose in pleural fluid that is greater than 16.5 mmol/ l (> 300 mg/dl) indicates the presence of peritoneal dialysis solution (15). The cut-off value for the diagnosis of pleural effusions caused by peritoneal dialysis is a pleural liquid-serum concentration gradient greater than 2.77 mmol/ l (50 mg/ dl) (15). The treatment of pleural effusion associated with peritoneal dialysis (peritoneal-pleural dialysate leaking) is termination of PD therapy (4-6 weeks after cessation of peritoneal dialysis there is resolution of the effusion), video-assisted thoracoscopic surgery and talc pleurodesis (tetracycline and fibrin gel are equally used for pleurodesis) (16, 17, 18).

The treatment of IAH and ACS consists of placing a nasogastric tube in critically ill patients with pancreatitis, peritonitis, abdominal trauma and those who are postoperative (9, 10, 11). In patients with ileus (abdominal distension), prokinetic agents are applied, such as metoclopramide or erythromycin (9, 10, 11). In the case of ascites, paracentesis is used (9, 10, 11). In patients with marked hypervolemia (accumulation of fluid in the bowel wall, mesentery, retroperitoneum, abdominal cavity, or the abdominal wall), positive fluid balance (controlled infusion therapy), loop diuretics, and dialysis (ultrafiltration) are used (9, 10, 11). If reduction of IAP does not occur in patients after the administration of medication, surgical decompression of the abdomen is indicated if the IAP > 25 mmHg (APP < 50 mmHg (APP should be maintained at values > 60 mmHg)) and if there is new organ system failure (9, 10, 11).

Acute pancreatitis

Acute pancreatitis is an acute inflammatory disease of the pancreas caused by intracellular activation of pancreatic digestive enzymes (1, 5). The decomposition of pancreatic tissue stimulates a systemic activation of the coagulation, fibrinolytic, and complement systems, and the release of cytokines and reactive oxygen species leads to severe manifestations of systemic diseases, such as shock, acute renal failure, acute respiratory distress syndrome, and adult respiratory distress syndrome (5). The incidence of acute pancreatitis has dramatically increased in the last two decades. In the United States, severe acute pancreatitis is responsible for more than 200,000 hospitalizations annually (13). In 80% of cases, acute pancreatitis is mild; approximately 20% of patients develop a severe form of acute pancreatitis with local and systemic complications. The mortality rate of severe acute pancreatitis is high and amounts to approximately 30% (13).

Pancreatitis associated with cholelithiasis occurs in 45% of patients, and alcohol is responsible for acute pancreatitis in 35% of cases (5). In 10% of patients, there is idiopathic acute pancreatitis (unclear cause). Alcoholic pancreatitis is more common in people younger than 40 years of age. Cholelithiasis-associated pancreatitis is more common in women and those aged between 50 and 60 years (3:1 ratio). Hypercalcemia is also an important cause of acute pancreatitis in patients who are suffering from hyperparathyroidism (5).

The exocrine pancreas secretes 1500-2000 ml of fluid, 150-200 mmol $HCO3^-$/day and inactive precursors of amylolytic (amylase), lipolytic (lipase) and proteolytic (proelastase, chymotripsynogen) digestive enzymes. Trypsin is necessary to activate these digestive enzymes. Trypsin is created through the conversion of trypsinogen by the action of enterokinase, which is secreted by the mucosa of the duodenum (5). Several important factors in the development of acute pancreatitis are damage to the mechanisms that block the activation of proteolytic enzymes; permanently increased concentrations of calcium in the serum; increased pressure in the pancreatic channel (obstruction of the flow of pancreatic secretion due to edema, stones, spasms of the ampulla of Vater sphincter, disruption of small ductules of the pancreatic duct, and the spread of pancreatic juices into the pancreatic parenchyma); the return of duodenal contents in pancreatic duct (duodenal pancreatic reflux); the activation of trypsinogen under the influence of enterokinase and hypersecretion due to increased stimulation of muscarinic receptors associated with poisoning by organic phosphates or a scorpion bite (5). The destruction and degradation of the pancreatic tissue leads to a systemic activation of the coagulation system, the fibrinolytic system and the complement system. This results in the release of cytokines (TNFα, IL-1, IL-6, IL-8, and platelet-activating factor, PAF), and the reactive metabolite oxygen, which leads to systemic manifestations of the pancreatitis, such as a shock (increased permeability of the capillary walls, vasodilation, decreased contractility of the myocardium), acute renal failure, and acute respiratory distress syndrome (ARDS) (5).

There are two forms of acute pancreatitis: oedematous interstitial pancreatitis and necrotizing pancreatitis. Depending on the natural flow, there are two phases of acute pancreatitis: an early phase (in the first one to two weeks) and a late phase (in the following few weeks and months) (12). The majority of patients (80-90%) have acute interstitial oedematous pancreatitis (a diffusely enlarged pancreas) without the presence of necrosis in the parenchyma of the pancreas or in the peripancreatic tissue (12). Peripancreatic fluid collection, excluding pancreatic necrosis, can occur with this type of pancreatitis (12). With necrotizing pancreatitis there is necrosis of pancreatic parenchyma tissue and/or peripancreatic tissue. The presence of parenchymal tissue necrosis indicates a more severe form of acute pancreatitis. A CT scan of the abdomen with contrast is the gold standard for the diagnosis of pancreatic and peripancreatic necrosis in the first weeks of the disease (12). The necrotizing form of acute pancreatitis is often associated with infection, which is diagnosed on the basis of symptoms and signs of sepsis and laboratory parameters (CRP, procalcitonin). Percutaneous aspiration of necrotic pancreatic tissue with a thin needle is used to diagnose and determine the cause of the infection (bacteria or fungi). Infection can also occur secondarily after percutaneous, endoscopic, or operative intervention and is associated with increased morbidity and mortality (12).

The clinical picture depends on the type, stage and severity of acute pancreatitis. Epigastric abdominal pain, which spreads in the upper abdomen area below the ribs, is the dominant symptom of acute pancreatitis. The pain can be moderate or severe, constant or sporadic. Nausea and vomiting occurs in over 90% of patients. Other symptoms include bloating of the stomach, blueness in the umbilical area because of haemoperitoneum (*Cullen's sign*), and bluish-red, purple, or brown discoloration of the skin on

Table 1. *Ranson/Imrie* prognostic criteria for acute pancreatitis

At the reception or at the time of diagnosis
Age > 55 years
The number of leukocytes > 15.0 x 10^9/l
Hyperglycemia > 10 mmol/l
LDH in serum > 600 U/l
AST in serum > 100 U/l
During the first 48 hours of hospitalisation
Drop in hematocrit of > 10%
Hypocalcemia < 2.0 mmol/l
Increasing of urea for > 1.8 mmol/l
Sequestration of fluid > 4.0 liters
Hypoalbuminemia < 32 g/l
Hypoxemia < 60 mmHg (FiO2 0.2l)

Three or more positive criteria indicate severe acute pancreatitis.

the lateral areas of the abdomen (*Grey-Turner's sign*) (5). During the early stages of acute pancreatitis (approximately 1-2 weeks), systemic manifestations (acute renal failure, acute respiratory distress syndrome, and circulatory insufficiency) are associated with a systemic inflammatory response (SIRS) and anti-inflammatory syndrome (CARS) (12). The late stage can last several weeks or months and is characterized by systemic symptoms and signs of inflammation, local and systemic complications and/or transition to persistent organ failure (12).

After the diagnosis of acute pancreatitis, it is important to estimate its severity to establish the treatment and prognosis (12). There are three stages of severity in acute pancreatitis: mild (85%), moderately severe and severe. The definition of the severity of acute pancreatitis is based on the presence or absence of organ failure and local and systemic complications (12). Mild acute pancreatitis is characterized by the absence of organ failure and local/systemic complications (12). Moderately severe acute pancreatitis is defined as transient organ failure (organ failure that recovers within 48 hours) and/or the presence of local or systemic complications (12). Severe acute pancreatitis is characterized by the persistent failure of one or more organ systems (failure lasts longer than 48 h) (12).

The *Ranson/Imrie* score is used to assess the severity of acute pancreatitis (table 1) (5).

Persistent organ failure is defined as organ failure that lasts longer than 48 hours. For the detection of renal organ failure, the modified *Marshall System Score* is used. This score assesses the function of the three organ systems that are most affected in acute pancreatitis: the respiratory and cardiovascular systems and the kidneys (urinary system) (Table 2) (12).

Persistent organ failure is defined as a score ≥ 2 for a period longer than 48 hours for a single organ system (12). Transient organ failure is also important for the classification of moderately severe acute pancreatitis and is defined as a score ≥ 2 for at least 1 of the three body systems that is present for a period no longer than 48 hours (12).

The local complications include acute peripancreatic collection of fluid (APEC), pancreatic pseudocyst, acute necrotic collection (ANC) and limited necrosis or WON (*walled-off necrosis*) (12). APEC occurs in the acute phase of interstitial oedematous acute pancreatitis. It is not associated with necrotizing acute pancreatitis. If it persists for longer than 4 weeks it becomes a pseudocyst of the pancreas (12). A pancreatic pseudocyst is encapsulated, clearly limited by a wall or homogenous collection of liquid, and occurs 4 weeks after the beginning of interstitial oedematous pancreatitis (12). ANC is present in the first four weeks after the beginning of disease (intrapancreatic, extrapancreatic). It occurs as a result of pancreatic necrosis or necrosis of peripancreatic tissue, and it contains varying amounts of liquid and solid necrotic material and has no encapsulating wall (12). MRI and ultrasound are better for the detection of solid material within the cyst or necrotic cavity (12). Acute necrotic collection can be sterile but can also lead to its infection (13).

ANC is not considered a pancreatic pseudocyst because it contains solid material associated with tissue necrosis (12). After maturation, ANC becomes clearly limited by a wall - WON (thickened wall of the reactive tissue) (12). This develops at least 4 weeks after the beginning of necrotizing pancreatitis. There can be a lot of ANC and WON (12). Local complications prolong hospitalization, require interventions, and are important for the definition of moderately severe acute pancreatitis. Persistent or permanent abdominal pain, secondary or repetitive increases in the concentration of amylase or lipase serum levels, or-

Table 2. Modified Marshall Scoring System

Organ system	Score				
	0	1	2	3	4
Respiratory: PaO_2/FiO_2	> 400	301-400	201-300	101-200	≤ 101
Kidney: creatinine µmol/l	< 134	134-169	170-310	311-439	> 439
KV system: systolic blood pressure, *mmHg*	> 90	< 90 (in reply to fluid resuscitation)	< 90 (lack of response to fluid resuscitation)	< 90 pH < 7.3	< 90 pH < 7.2

gan failure or fever require prompt use of an abdomen CT scan with contrast in order to detect any complications (12). Systemic complications include functional disorder of the lungs, cardiovascular system and the kidney. These complications are the foundation for systemic inflammatory response syndrome (SIRS), which can occur in acute pancreatitis.

Patients with severe acute pancreatitis have a greatly increased risk of death (30-50%). If there is pancreatic or peripancreatic necrosis, the mortality rate increases to 80% (12).

The conventional diagnostic test for acute pancreatitis is measuring the concentration of amylase activity in the serum. Values that are at least 3 times greater than the upper limit of normal indicate acute pancreatitis (5). In patients with acute pancreatitis, amylase concentration in the serum increases within 2-3 hours, peaks within 12-24 h, and returns to normal after 3-5 days (5). The normal concentration of amylase in urine is 10-300 IU/l; values greater than 750 IU/L indicate acute pancreatitis. A concentration of serum lipase greater than 2 times the upper normal limit indicates acute pancreatitis. Lipase concentration in serum increases within 4-8 h, peaks after 24 hours, and normalizes after a week (5). In patients with acute pancreatitis, amylase concentration in the fluid of the peritoneum is more than 50.000 IU/l (5). Additional tests for acute pancreatitis include abdominal ultrasound, CT of the abdomen with contrast (the gold standard for the diagnosis of pancreatic necrosis and peripancreatic collection) and endoscopic retrograde cholangiopancreatography (ERCP) (acute pancreatitis caused by a small stone in the ampulla of Vater) (5). Based on the findings of the abdominal CT scan, severe acute pancreatitis is categorized into five stages, 0-4. The degree of pancreatic necrosis is also evaluated on the basis of an abdomen CT scan with contrast and defined by four categories. Based on these two scoring systems, the severity score of the CT index is calculated. An index score greater than 7 indicates a high morbidity and mortality in patients with acute pancreatitis (13).

All patients with severe acute pancreatitis and failure of at least one organ system (circulation, kidney, or lung) require admission to the Intensive Care Unit. The treatment consists of supportive therapy; infusion therapy provides an optimal haemodynamic and electrolyte status, the pancreatic and enteric stimulation is blocked by placing a nasogastric tube, and antibiotics are administered prophylactically (imipenem 500 mg IV every 8 h for 7-10 days, plus IV Fluconazole 400 mg/day) (13).

A significant complication of severe acute pancreatitis is acute renal failure (renal hypoperfusion, IAH: oliguria occurs when IAP ≥ 15 mmHg, and anuria occurs when IAP ≥ 30 mmHg). Fractional excretion of sodium (FE_{Na+}) > 1%, the concentration of sodium in the urine sample (U_{Na+}) > 40 mmol / l and fractional excretion of urea (FE of urea) ≥ 35% point to the development of acute tubular necrosis in patients with severe acute pancreatitis (5, 7). Treatment of acute renal failure in patients with severe acute pancreatitis includes the treatment of acute pancreatitis, IAH, ACS, and dialysis support therapy (5, 7). Continuous veno-venous-hemofiltration (CVVHF) is used as a nonrenal indication for the reduction of a systemic inflammatory response. High-volume CVVHF (HVHF) is used in the early stages of acute pancreatitis (within 72 h), and the test results show that it substantially reduces the concentration of inflammatory cytokines in the serum (TNFα, IL-1, IL-2, IL-6) (5, 7). With patients with severe acute pancreatitis, acute renal impairment and haemodynamic instability, continuous veno-venous haemodiafiltration (CVVHDF) is used (standard dose filtration rate: 20 ml/kg/h, high dose of filtration: 35 ml/kg/h). In patients who are haemodynamically stable, intermittent dialysis modalities, such as slow low-efficiency everyday dialysis (SLEDD) (6 times a week, 3 x per week) or standard haemodialysis (3 x week), can be administered. The ultrafiltration dose should be adjusted individually for each patient depending on their clinical condition (5, 7).

Acute pancreatitis in patients on peritoneal dialysis

Acute pancreatitis is an acute inflammatory disease of the pancreas that is not very common among patients on peritoneal dialysis. The incidence of acute pancreatitis in patients on peritoneal dialysis is 7/241 patients/year (1). Risk factors that increase the incidence of acute pancreatitis in this patient population include uraemia, secondary hyperparathyroidism with hypercalcemia, hypertriglyceridemia, different features of the solution used for peritoneal dialysis (osmolarity, acidity, glucose, chemical irritation, and calcium in solution for peritoneal dialysis leads to "local hypercalcemia"), the toxic substances from the dialysate, the bags and tubing, peritonitis and treatment of peritonitis with antibiotics and anticoagulants (i.e. applying of antibiotics and heparin) (table 3) (1, 5, 8).

The main symptoms of acute pancreatitis in patients on peritoneal dialysis are acute pain in the abdomen (100% of patients), nausea, vomiting (in 73% and 67% of patients), bloating of the stomach (7% of patients), and haemorrhagic effluent (approximately 20% of patients) (1, 5, 8).

Table 3. The most common risk factors for the development of acute pancreatitis in patients on peritoneal dialysis

Risk factors	The number of cases N(%)
Biliary lithiasis	10 (13.3)
Alcohol	4 (6.2)
Medications	8 (11.6)
Hypercalcemia	26 (34.7)
Hyperlipidemia	32 (43.2)
Hyperparathyroidism	25 (51.0)
Trauma/Surgery/Transplantation	5 (10)
Infection	2 (6.1)
Idiopathic	20 (27.4%)

The diagnosis of acute pancreatitis in patients on peritoneal dialysis is difficult because of the reduced sensitivity of laboratory parameters (biochemical tests include amylase and lipase in the serum) and because it overlaps with other syndromes, such as peritonitis. Serum amylase tests have been shown to have reduced sensitivity in patients on peritoneal dialysis with long-term icodextrin exchanges, because icodextrin reduces the activity and concentration of amylase in the serum. The concentration of amylase in the serum was found to be normal in 12.8% of acute pancreatitis episodes (8). Measuring the concentration of lipase in the serum has a higher diagnostic value for acute pancreatitis among patients on peritoneal dialysis who are using 7.5% solution of icodextrin (5, 6, 8). Increased concentration of amylase in the effluent of patients with acute abdominal pain being treated by peritoneal dialysis may indicate the development of acute pancreatitis in the absence of acute peritonitis (1, 5, 8). In addition to laboratory testing, the two most important examinations of the morphology of the pancreas are abdominal ultrasound and CT (1, 5, 8).

The majority of patients is treated conservatively by supportive therapy. The development of complications of acute pancreatitis, such as pancreatic abscess or necrosis, requires laparotomy (8). The mortality rate of patients on peritoneal dialysis for acute pancreatitis is 31.5% (8).

Encapsulating sclerosis of the peritoneum

Encapsulating sclerosis of the peritoneum (EPS) is rare and is the most serious complication of long-term peritoneal dialysis. EPS is characterized by thickening of the peritoneum, seizing of the small intestine, and the symptoms and signs of obstructive ileus (1, 2, 3). The prevalence of EPS in patients on peritoneal dialysis ranges from 0.5-7.3% and increases to 15.2% in patients receiving peritoneal dialysis treatment for more than 15 years (1, 2, 3).

Risk factors for the development of EPS include recurrent episodes of peritonitis, the absence of residual renal function, exposure of the peritoneum to solutions with a high concentration of glucose over a longer period of time, bioincompatible solutions for peritoneal dialysis (glucose, glucose degradation products, lactate in acid solution), treatment cessation of patients with peritoneal dialysis (switching to haemodialysis), the post-transplantation period in patients who were treated by peritoneal dialysis (use of blocking calcineurin), and trauma to the peritoneum (surgery) (1, 2, 3).

The pathogenesis of EPS is not fully understood. Increased production of transforming growth factor beta (TGFβ) and endothelial growth factor (VEGF) in fibroblasts of the peritoneum and neovascularization (formation of new blood vessels in the thickened peritoneum) have important roles in the thickening of the peritoneum (2, 3).

The symptoms and signs that indicate EPS include abdominal pain, abdominal bloating, nausea, vomiting, anorexia, constipation, loss of body weight, loss of ultrafiltration capacity (ultrafiltration weakness of the peritoneum), high transport characteristics of the peritoneum and intermittent bowel obstruction (small bowel obstruction) (1, 2, 3).

A high index of clinical suspicion is necessary to diagnose EPS. Diagnosis is based on clinical and radiological findings (abdominal ultrasound and CT). A CT scan of the abdomen can reveal thickening of the peritoneum, adhesions of the intestinal loops, signs of bowel obstruction and the presence of fluid collection in the abdomen (1, 2, 3). Treatment includes cessation of peritoneal dialysis, application of the anticicatricial drugs (tamoxifen), corticosteroids and immunosuppressive agents, nutritional support and surgical enterolysis and adhesiolysis (1, 2). Tamoxifen blocks the formation of TGFβ in the fibroblasts of the peritoneum and is administered at a dose of 10-80 mg/day (generally 40 mg/day). It is well tolerated; potential side effects are nausea, weakness, endometrial cancer and deep vein thrombosis (1, 2). Corticosteroids are used at a dose of 0.5-1.0 mg/kg/day (maximum daily dose of 80-100 mg), with a gradual dose reduction for 4-5 months. In addition to corticosteroids, patients receive azathioprine at a dose of 50 mg/day for 2-3 months (1, 2). In most clinical studies, corticosteroids have been administered at a dose of 0.5 mg/kg/day in combination with azathioprine at a dose of 1.5 mg/kg/day for 4 weeks. After treating the symptoms of the gastrointestinal tract, the dose of azathioprine is reduced to 75 mg/day, and the corticosteroid is reduced to 20 mg/day for several months (2). Corticosteroid doses of 50 mg/day can be applied in combination with mycophenolate mofetil at a dosage of 500 mg 2 x 1 during the two months (2).

In addition to the optimization of anti-inflammatory and anticicatricial therapy, trials of EPS are focused on the early detection of the inflammatory phase of EPS. Currently this is done through determination of the concentrations of CA-125, IL-6, TGFβ, and MMP-2 in the effluent. These biomarkers are associated with inflammation, remodelling of tissue, damage of the peritoneal membrane and increased transport through the peritoneal membrane (2). In patients with ultrafiltration weakness, an effluent concentration of less than 33 U/min of AR-CA-125 and greater than 350 pg/min of AR-IL-6 has a sensitivity of 70% and specificity of 89% in diagnosing EPS (2). Well-controlled clinical trials should confirm the importance of CA-125 and IL-6 concentrations in the effluent for diagnosing the inflammatory stage of EPS (2).

For patients with an increased risk of EPS, it is necessary to use prophylaxis, which involves tamoxifen 20-40 mg/day and low doses of corticosteroids (0.5 mg/kg/day) (2).

Regardless of the treatment, the mortality rate is high, between 20-93%, and in patients who are on peritoneal dialysis for more than 15 years, the mortality rate is almost 100% (1, 2).

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ADENOSARCOMA MULLERI ASSOCIATED WITH TAMOXIFEN USE AFTER BREAST CANCER THERAPY: A CASE REPORT

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MILEROV ADENOSARKOM UTERUSA - UDRUŽENOST SA UPOTREBOM TAMOKSIFENA NAKON LEČENJA KARCINOMA DOJKE-PRIKAZ SLUČAJA

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ABSTRACT

The term 'mixed Müllerian tumour' applies to uterine tumours composed of epithelial and mesenchymal elements of Müllerian origin. These neoplasms are classified into adenomyomas, adenofibromas, adenosarcomas, and carcinosarcomas (malignant Müllerian mixed tumours) based on whether the epithelial and stromal elements are benign or malignant. Adenosarcomas are low-grade neoplasms classified halfway along the spectrum of mixed Müllerian tumours, with adenofibromas at one end and carcinosarcomas (malignant Müllerian mixed tumours) at the other. Adenosarcoma is a mixed Müllerian tumour composed of benign-appearing but neoplastic glandular elements and a sarcomatous stroma, which is usually low grade. Histologically, there are heterologous mesenchymal elements (usually rhabdomyosarcoma, but also cartilage, fat, and other elements) in 20–25% of cases.

We have observed that some women with these tumours have received tamoxifen treatment for breast cancer or have a history of radiation therapy.

We herein report the case of a 46-year-old patient who was hospitalized at OGC CC Kragujevac because of excessive bleeding from the uterus. The patient had undergone right mastectomy three years earlier for breast cancer. After surgery, she had received Nolvadex (tamoxifen) treatment. Exploratory curettage was performed, and then, a classic abdominal hysterectomy with bilateral adnexectomy was completed. The histopathological findings indicated adenosarcoma Mülleri; therefore, the patient received postoperative radiation therapy according to our current protocol.

Keywords: *adenofibroma; adenosarcoma; carcinosarcoma; malignant Müllerian mixed tumour;*

SAŽETAK

Termin mešani Milerov se odnosi na tumore uterusa sastavljene od epitelijalnih i mezenhimalnih elemenata porekla Milerovih kanala. Ove neoplazme su klasifikovane u adenomiome, adenofibrome, adenosarkome i karcinosarkome (maligni Milerovi mešani tumori), zavisno od toga da li su epitelijalne i stromalne komponente benigne ili maligne.

Adenosarkomi su neoplazme niskog gradusa zrelosti klasifikovane negde na pola spektra mešanih milerovih tumora, sa adenofibromima na jednoj i karcinosarkomima (malignim Milerovim mešanim tumorima) na drugoj strani. Adenosarkom je mešani Milerov tumor sastavljen od neoplastičnih glandularnih elemenata ali benignog izgleda i sarkomatozne strome, koja je obično niskog gradusa.

Često ga dijagnostikujemo kod pacijentkinja koje su primale terapiju Tamoxifenom za lečenje karcinoma dojke ili su imale zračnu terapiju. U vreme dijagnoze, većina tumora je ograničena samo na uterus (Stadijum I). U histološkoj slici heterologi mezenhimalni elementi (obično rabdomiosarkom, ali takođe i hrskavica, masno tkivo i drugi elementi) su prisutni u oko 20-25% slučajeva.

Prikazana je četrdesetšestogodišnja pacijentkinja koja je hospitalizovana u GAK KC Kragujevac zbog obilnog krvarenja iz materice, a pre tri godine joj je odstranjena desna dojka zbog karcinoma nakon čega je bila na terapiji Nolvadexom/Tamoxifenom. Nakon eksplorativne kiretaže a zatim i klasične abdominalne histerektomije sa obostranom adnexectomijom dijagnostikovan je Milerov adenosarkom te je pacijentkinja primila i postoperativnu zračnu terapiju prema važećim protokolima.

Ključne reči: *adenofibrom; adenosarkom; karcinosarkom; maligni Milerov mešani tumor;*

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INTRODUCTION

Uterus Müllerian adenosarcoma is a rare gynaecological malignant tumour.

It is composed of epithelial and mesenchymal elements of Müllerian origin. This rare tumour, which represents 2-5% of all uterine tumours, i.e., approximately 8% of all uterine sarcomas, usually originates from the endometrium and grows as a polypoid mass inside the cavum (1). The uterine body is most often affected, but this tumour is also common on the cervix and ovaries and, more rarely, in the vagina and fallopian tubes. Its origin can also be the peritoneal surface or even outside of the genital system, such as in the intestines (2).

The histological findings are characterized by benign, sometimes mildly atypical glandular epithelial elements associated with malignant stromal changes, which are characterized by specific structures of "periglandular cuffing" with increased cellularity and intraglandular polyps. The stroma is usually characterized by increased cellularity around epithelial elements, which leads to the formation of a cambium layer. The stromal component is commonly of low morphological grade and consists of endometrial stroma or fibroblasts (that are hormone receptor- and CD10-positive) (3). It may occasionally be of high grade, and this represents an unidentified sarcoma (4). Adenosarcomas of the uterus are generally neoplasms of low grade, are capable of recidivism after polypectomy or hysterectomy and are only very rarely metastatic. The two most important negative prognostic factors, which are occasionally present, are deep miometric invasion with a predominant sarcomatous component and high morphological grade, which is followed by the loss of hormone receptors and CD10. Adenosarcoma can be mistaken for various lesions, and the main differential diagnosis is adenofibroma, which by definition has a morphologically benign component (5).

The main symptom of these lesions is abnormal vaginal bleeding, pain in the lower abdomen, a palpable tumour mass in the lower abdomen, unusual urinary symptoms, increased and prolonged vaginal discharge, and sometimes the presence of polypoid tissue that can be seen in a dilated cervical canal (14). These tumours have a tendency to appear in postmenopausal patients with an average age of 66 years. The average time interval from the first symptoms to the final diagnosis is approximately 13 months. The risk factors are similar to those of uterine adenosarcoma and include obesity, oestrogen therapy, radiation exposure, nulliparity, and potentially tamoxifen exposure (7). The prognosis is much better in the early phase and in younger patients, although these are often incorrectly treated due to an incorrect diagnosis (6). The usual treatment is surgical resection, which consists of total abdominal hysterectomy with bilateral salpingo-oophorectomy, along with the application of broad-spectrum antibiotics, followed by postoperative radiation of the pelvis (15, 16, 17).

Stage I	Carcinoma is limited to the body of the uterus
Stage II	Carcinoma affects the body and the cervix of the uterus
Stage III	Carcinoma has spread outside of the uterus but not outside of the small pelvis
Stage IV	Carcinoma has spread outside of the small pelvis and affects the bladder or rectal mucosa

Table 1. The tumour staging

Adenosarcomas are low-grade neoplasms, which are classified somewhere in the middle of the mixed Müllerian tumours, with adenofibromas on one side and carcinosarcomas (malign mixed Müllerian tumours) on the other. Adenosarcoma generally has a worse prognosis than endometrial carcinoma, with a five-year mortality rate in the range of 5% up to 40%. However, the total mortality rate is less than 20% (18, 19). The key prognostic factor is the stage of the disease at the time of the diagnosis (Table 1)(15).

Lifelong tracking of these patients is necessary because of the high risk of recurrence, mainly in the cases with myometrial invasion (12).

Adenosarcoma Mulleri is a metaplastic carcinoma that represents a subtype of uterine sarcoma. The subsidiary oncological therapy is similar to that applied for cases of highly malignant uterine sarcoma, such as leiomyosarcomas and unidentified sarcomas (17, 19). It has been shown that most uterine adenosarcomas are monoclonal neoplasms and are actually metaplastic carcinomas. The behaviour of the uterine adenosarcomas is more like that of the endometrioid type of endometrial adenocarcinoma and the aggressive subtypes of uterine carcinoma. The sarcomatic component derives from carcinomatous elements, which are the driving forces. It is currently unclear whether adenosarcoma begins as a carcinoma in transition through sarcomatous transdifferentiation or as a sarcoma in transition through epithelial transdifferentiation (13).

There is increasing evidence in the literature that this tumour is associated with tamoxifen therapy for the treatment of breast cancer (8, 9, 10). At the time of the diagnosis, most of the tumours are limited to the uterus (Stage I) (11).

Prolonged oestrogen or androgen exposure can lead to the development of mesenchymal and mixed epithelial-mesenchymal uterine tumours.

There are both homologous and heterologous types of adenosarcoma. In patients with the homologous type, the sarcoma component is in the uterine tissue, such as the endometrium, fibroid tissue or lean muscle tissue. The heterologous type is characterized by elements of rhabdomyosarcoma, chondrosarcoma or osteosarcoma (14).

It has been noted that in the adenosarcoma cases, PI3K signalling mutations are common, which are generally present before the tumour transdifferentiation and metastasis, providing considerable support for applying treatments targeting PI3K signalling in most cases of uterine adenosarcoma (4, 6).

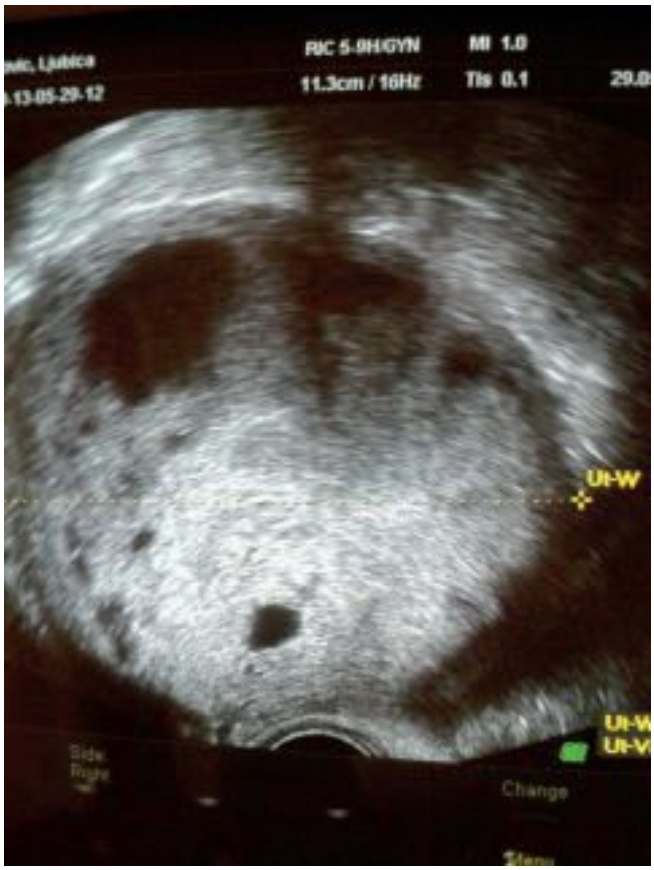

Image 1. The US presentation of the uterus (transverse section), with a hypoechoic tumour mass that filled the entire uterine cavum

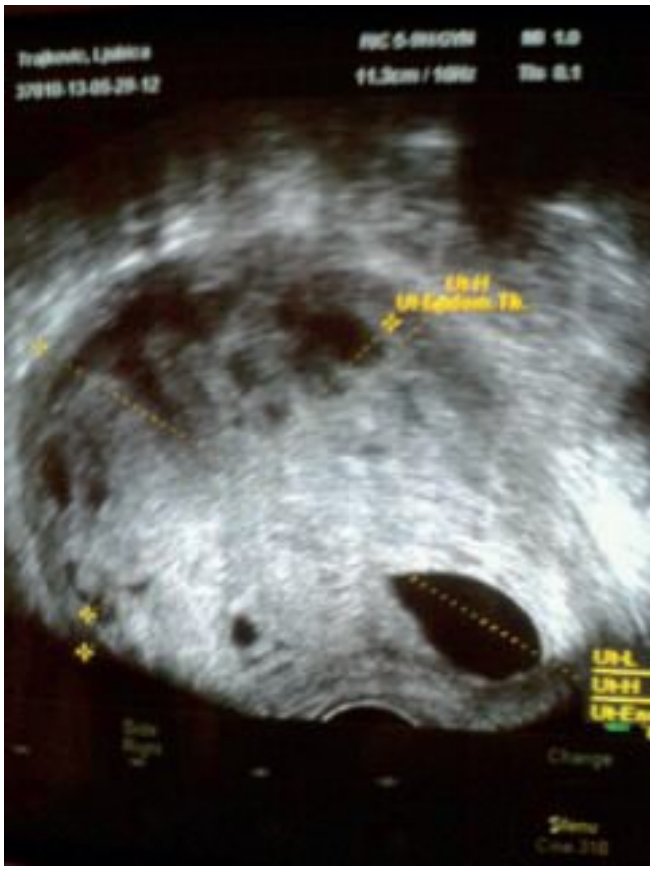

Image 2. The US presentation of the uterus (longitudinal section), with the tumour mass in the cavum, which had a thickness of 75 mm, while the myometrium had a thickness of 6 mm.

CASE STUDY

We herein report the case of a 46-year-old patient who was hospitalized at OGC CC Kragujevac because of excessive bleeding from the uterus. The patient reported having stopped menstruation three years earlier, had regular and normal periods prior to that, had undergone one C-section and had terminated one pregnancy. She was suffering from Raynaud syndrome, and her right breast had been removed three years earlier because of breast cancer. After the mastectomy, she was administered Nolvadex (tamoxifen) therapy.

Irregular bleeding had appeared one month prior, which was treated by exploratory curettage of the uterus. Eighteen days later, the patient was sent to OGC CC Kragujevac for the same symptom (without any histopathological information).

The patient was thoroughly examined in our department. The results of the lab analysis were Le, 7.5; Er, 4.28; HGB, 136; Hct, 0.394; Plt, 269; PTs, 10.3; INR, 0.906; **PT%, 122.3**; Fib, 1.908; **APTT, 22.9**; **glucose, 9.3;** urea, 4.1; creatinine, 62; K, 4.2; Na, 139; urine, without irregularities; Troponin I, 0.002; CEA, 0.7; **AFP, 9.39; CA125, 59;** CA19-9, 7.1; B-HCG, 0.60; TSH, 1.54 and fT4, 11.86. She was blood group O and Rh +.

The ultrasound (US) findings showed that the uterus was in the AVF and had dimensions of 121 x 91 x 91 mm with the cavum filled with hyperechoic contents and a thickness of 75 mm. Of note, the described changes did not penetrate the myometrium, which had a thickness of 6 mm. Neither of the ovaries had any pathological changes. The Pouch of Douglas was empty.

Following repeated exploratory curettage, the material obtained was sent for a histopathological analysis, but the pathologists were unable to determine an exact diagnosis. The material was therefore sent to Belgrade for a histopathological analysis.

The results of the Kreitman test showed a malignant tumour with short, round and somewhat elongated cells, a basophilic cytoplasm, irregular cells, and pseudobeam-like fields. The was clear chondroid differentiation. There were individual, partly klef-like, glandular benign structures inside the stroma, which were ER+/PR+. The immunophenotype of the tumour was CD10+/-/Desmin- or only focally/Actin-pan- or only focally /SMA-/CD99- or focally /C-kit- or focally /S-100-Er-/Pr-/Myogenin and MyoD1 only in one group of nuclei. The mitotic count was 8/10 HPF; the Ki 67 index was high at 40%.

Based on these findings, we concluded that the patient had a malign tumour with predominant sarcomatous stroma, benign endometrial glands, and imaging findings characteristic of adenosarcoma Mülleri with elements of heterologous differentiation (chondroid and rhabdomyoblastic). We eventually concluded that the tumour was an

endometrial sarcoma with heterologous differentiation that was associated with glandular proliferation. The patient was treated with intensive antibiotic therapy. After consulting with pathologists, it was decided that the patient should undergo hysterectomy with bilateral adnexectomy (salpingo-oophorectomy).

After obtaining the patient's consent, the surgery was performed. During the operation, we noted that size of the uterus was large, equivalent to that of two fists, with a smooth and neat surface and without macroscopically noticeable pathological changes on the serosa. The fallopian tubes were clear though the whole distance, the ovaries were of normal size, appearance and position. The omentum had a normal appearance and size, without any noticeable pathological changes. The peritoneum of the small pelvis was normal, with no accumulation of fluid in the abdomen, or any other pathological changes.

Classical hysterectomy with bilateral adnexectomy was performed. The tissues were sent for a histopathological analysis. The abdominal wall was closed layer by layer, with the skin sewn with individual seams.

The results of the histopathological analysis were Adenosarcoma Mulleri-heterologous type. The sarcoma component comprised 80% of the tumour, with chondrosarcoma elements, as well as a 10% leiomyoma component and a field of endometrial sarcoma, with a low degree of malignancy. Areas of bleeding and necrosis were present in the tumour tissue, and there were signs of blood and lymphatic vessel invasion; the tumour mass had infiltrated more than two-thirds of the muscular uterine wall. The pathological stage of the tumour was pT1c Nx Mx: FIGO stage Ic.

The other findings of note were moderate hyperplasia of the squamous epithelia of the ectocervix, low-degree chronic cervical inflammation, a nabothian cyst, chronic salpingitis, a cystic follicle and corpora albicantia ovariarum.

The surgery and postoperative recovery were unremarkable. The patient was released from the clinic and sent to the Department of Oncology at the Radiology and Oncology Insitut of Serbia.

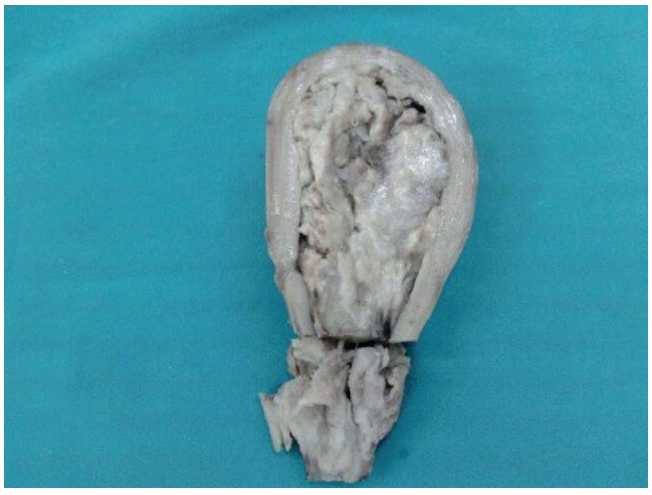

Image 3. A histopathological uterine preparation (longitudinal section) showing that the tumour mass filled the cavum

There, the patient received postoperative radiotherapy of the small pelvis with TD 46 Gy in 22 fractions (after taking into consideration the last dose received after Ro castration) with the "Izocentre techniqie" in combination with brachytherapy for a TD of 24 Gy (4 x 600 cGy via a vaginal cylinder).

The described therapy was subjectively and haematologically well received, with the only side effect being haemorrhoidal discomfort. Radiotherapy was administered at the planned dosages.

During her hospitalization in the Oncology and Radiology Institute of Serbia, abdominal and pelvic CT scans were performed, as well as US studies of the neck, abdomen, pelvis and ingvinum. The

CT scans showed pleural adhesions in the lung base. No abnormalities were observed in the liver, gallbladder, pancreas, kidneys or adrenal glands. The retroperitoneal and parailiac bilateral lymph nodes were all smaller than 10 mm in diameter. There were differentiated nodular formations of up to 5 mm in diameter, which corresponded to the mesentery lymph nodes. The urinary bladder showed no intraluminal vegetation. There has thus far been no evidence of recurrence. The ischiorectal region was fossa free, and the bilateral inguinal lymph nodes were all 10 mm or smaller in size.

The US studies showed a homogenic parahilar zone with many repaired parenchymal tissue and structural changes possibly resulting from the applied TH. There was no ascites present. The biliary tract was free from pathological changes. Tracking the patient's liver condition is mandatory, and she will continue to be monitored. The pancreas, spleen, adrenal glands and urinary tract were all free from pathological changes. The postoperative scar was regular, clear, and free from signs of recidives. The rectum was normal. There were no pathological changes observed in the para aortocaval, parailiac, supraclavicular or spinal lymph nodes. The bilateral inguinal area had no pathological lymph nodes but did have reactive lymph nodes with a lipomatose appearance, which had a diameter up to 12 mm.

The patient is regularly seen every three months for a follow-up examination. There have thus far been no signs of relapse. The gynaecological findings are normal. The last US findings of the abdomen and the small pelvis were normal. The results of the laboratory analysis were also within the normal limits. She continues to receive adjuvant therapy with Nolvadex. The values of Ca15-3 have been within the normal range.

DISCUSSION

Uterine adenosarcomas are relatively rare tumours, although their incidence appears to have increased in recent years. It is likely that this increase is due to a better un-

derstanding of the different anatomical aspects of uterine sarcomas (especially after the introduction of immunohistochemical staining) and possibly due to the exposure of patients to predisposing factors, including pelvic irradiation and the use of tamoxifen for the treatment of breast cancer (20).

Although the full aetiology remains unknown, at least three possible risk factors are currently under consideration. These risk factors include pelvic irradiation, as some cases have been reported in patients with a history of previous pelvic irradiation (21); hyperestrogenism, where the tumours would have developed as a result of prolonged unbalanced oestrogen stimulation or a long period of oral contraceptive use (22); and tamoxifen treatment, because some cases have been reported in patients previous treated with tamoxifen for breast cancer (23).

Patients with uterine endometrial adenosarcomas have generally been postmenopausal, with the median age at presentation previously reported to be 58 years (24). The most common symptom is abnormal vaginal bleeding (71%), spotting, menorrhagia or metrorrhagia, as in our patient. These tumours can present as a pelvic mass (37%), uterine polyp (22%), or an enlarged uterus (22%) (25). Pain and the presence of a foul-smelling vaginal discharge or symptoms of pelvic pressure have also been reported. Deep myometrial invasion, as a predictor of aggressive behaviour, and sarcomatous overgrowth are the two most important predictors of a poor prognosis (2). Myometrial invasion is found in 15% of cases, but deep myometrial invasion is generally found only in 5% of cases (24). The presence of heterologous elements, especially rhabdomyosarcoma, may reflect a more clinically aggressive tumour. The immunophenotype of these tumours is similar to that of an undifferentiated uterine sarcoma, which usually have poor expression of the cell differentiation markers, the oestrogen receptor, progesterone receptor and CD10 (26). Mullerian adenosarcoma can be easily distinguished from adenofibroma (where both the epithelial and stromal components are benign) using the criteria defined as unique to adenosarcoma, such as a marked degree of atypia of mesenchymal cells, a histologically malignant element, the presence of myometrial invasion, and two or more mitotic figures per 10 HPF (27).

Nevertheless, these features are not always present. Thus, they are less applicable for the categorization of the degree of malignancy.

When treating these tumours, most authors recommend total abdominal hysterectomy usually accompanied by bilateral salpingo-oophorectomy (28). There is currently no consensus among gynaecologists on the value of staging by lymphadenectomy during primary surgery.

Generally, patients with tumours invading more than halfway through the myometrium, such as our patient, or with two or more unfavourable factors present have a high likelihood of recurrence and might benefit from high-dose pelvic radiation with or without aggressive chemotherapy (29).

CONCLUSION

The best approach for the management of uterine adenosarcomas has yet to be defined; surgery, chemotherapy, radiotherapy, and careful follow-up have all been used. In our case, surgery and postoperative radiotherapy with close follow-up seems to have been an appropriate course of action.

CONFLICT OF INTEREST

The authors declare no financial or commercial conflicts of interest.

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DYNAMIC NATURE OF POSTPARTAL CAROTID ARTERY DISSECTION

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DINAMIČKA PRIRODA POSTPARTALNE KAROTIDNE DISEKCIJE

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ABSTRACT

Craniocervical carotid artery dissection (CCAD) is an important cause of stroke in young adults, but it has rarely been reported as a cause of stroke in puerperium.

We report the case of a 27-year-old female with a history of migraine who presented with unilateral left headache, transient episodes of dysphasia and right hemiparesis 30 days after vaginal delivery. The first symptoms started six days after the prolonged childbirth. The first magnetic resonance angiography revealed dissection in the supraclinoid and a cavernous segment of the left Internal carotid artery (ICA). We followed up the patient for two years and she had an unstable course. During this time, she had occlusion of the supraclinoid segment of the left ICA, with caudal extension on the extracranial segment and recanalisation one month later. Two months later, she had intracranial extension with dissection of the left anterior cerebral artery. During this time, she suffered from two strokes with minimal neurological impairment and good clinical recovery.

The pathophysiology of CCAD appears to be multifactorial. Vessel wall injury related to the Valsalva manoeuvre during labour, as well as hemodynamic and hormonal changes of the vessel wall related to pregnancy in a patient with a history of migraine, may be causes of postpartum spontaneous craniocervical artery dissection in healthy women.

Keywords: *CCAD, stroke, migraine, pregnancy, postpartum, collateral circulation, MRI, DSA*

SAŽETAK

Kraniocervikalna disekcija karotidne arterije (KDKA) je važan uzrok moždanog udara kod mladih, ali se retko javlja kao uzrok moždanog udara u puerperijumu.

Prikazaćemo slučaj 27 godina stare osobe ženskog pola, koja je hospitalizovana zbog unilateralne glavobolje leve polovine glave, tranzitorne disfazije i desne hemipareze, 30 dana nakon porođaja. Prvi simptomi su nastali šest dana nakon prolongiranog porođaja. Prva angiografija magnetnom rezonancom pokazala je disekciju supraklinoidnog i kavernoznog segmenta leve karotidne arterije. Pacijentkinja je praćena dve godine i imala je nestabilan tok bolesti. Tokom tog vremena imala je okluziju supraklinoidnog segmenta leve karotidne arterije, širenje disekcije u kaudalnom smeru na ekstrakranijalni segment i rekanalizaciju mesec dana kasnije. Nakon dva meseca disekcija se proširila intrakranijalno na levu prednju moždanu arteriju. Imala je dva moždana udara, minimalno neurološko pogoršanje i dobar klinički ishod.

Patofiziologija KDKA je multifaktorijalna. Povreda zida krvnog suda izazvna Valsalva manevrom tokom porođaja, kao i hemodinamski i hormonski izazvane promene zida u trudnoći, kod pacijenkinja sa migrenom, mogu biti uzrok postpartalne kraniocervikalne karotidne disekcije kod zdravih žena.

Ključne reči: *KDKA, moždani udar, puerperijum, migrena, kolateralni protok, MR, DSA*

ABBREVIATIONS

CCAD - craniocervical carotid artery dissection
CTA - computed tomography angiography
DSA - digital selective angiography
ICA - internal carotid artery
MRA - magnetic resonance angiography

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INTRODUCTION

Pregnancy and the postpartum period are associated with an increased risk of ischemic stroke. The incidence of stroke is 14/100.000 deliveries (1). The relative risk of ischemic stroke during pregnancy is 0.7 but increases to 8.7 during the first four months after childbirth (2). Potential explanations for this increase in reported incidence of stroke include a change in intravascular volume, an effect of hormonal changes during pregnancy on the cerebral arteries, acute lesions of cerebral vessels during delivery, and an abnormality of blood coagulation that increases the risk of arterial thrombosis. An unusual cause of ischemic stroke is extracranial or intracranial artery dissection. Lesions of the arterial vessels supplying the brain due to the dissection are a very rare event in the postpartum period. In women younger than 50 years of age, craniocervical artery dissections (CCAD) occur within 6 weeks after delivery in 6% of cases (3). We report a case of the CCAD with a complete occlusion of the dissected vessel at the intracranial segment that resulted in an ischemic stroke. At the same time, an extension of intramural hematoma in both the proximal and distal directions was observed. The ischemic stroke was followed by nearly complete recovery. This case report includes a review of the literature on postpartal craniocervical artery dissection.

CASE REPORT

A 27-year-old female had a family history of migraine and cluster headaches. She had an uneventful pregnancy that was complicated only by labour, which was performed under epidural anaesthesia and lasted for 20 hours. On the sixth day after childbirth, she complained of a severe headache that was localised unilaterally. She also noted occasional clumsiness in her right hand and was anxious that she could drop the baby. To treat her headache, she used common analgesics several times daily. She described her pain as throbbing and located retro-orbitally on the left, and she noted that this headache was different from her usual migraine attacks. After her discharge from hospital, she experienced several headache attacks, but the pain intensity had decreased. She also felt an intermittent weakness in her right hand but thought that this was due to her tiredness. Five weeks after labour, she experienced repeated headaches with increased pain intensity, as well as transient episodes of drowsiness, incomprehensible speech, and weakness in her right hand.

On the admission day, the neurological examination, ECG, blood pressure, and routine blood and biochemical parameters were normal. The carotid duplex ultrasound (MyLab 50, XVision, Esaote, Genoa) showed a reduction in the diffuse vessel diameter in the left internal carotid

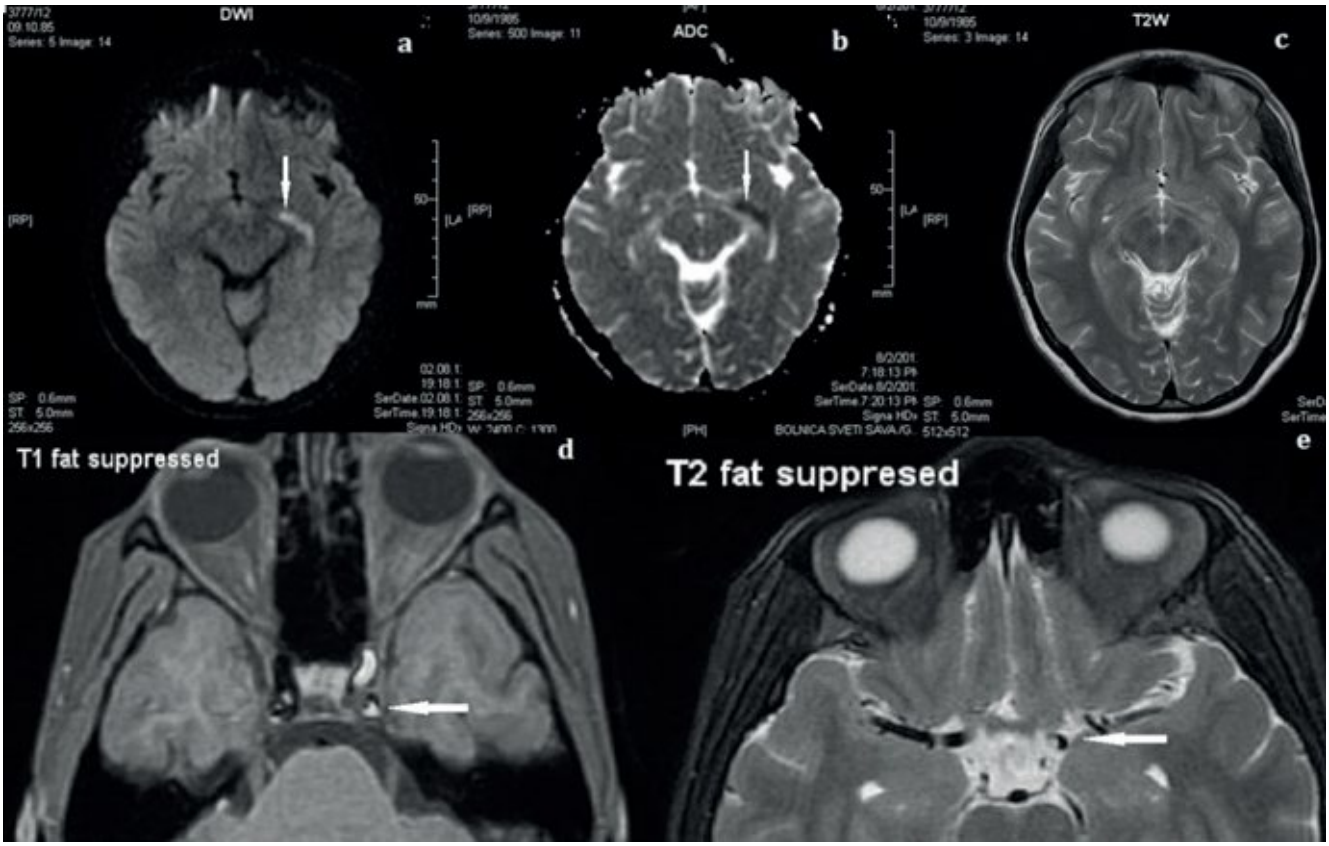

Fig 1. Magnetic resonance images on the admission

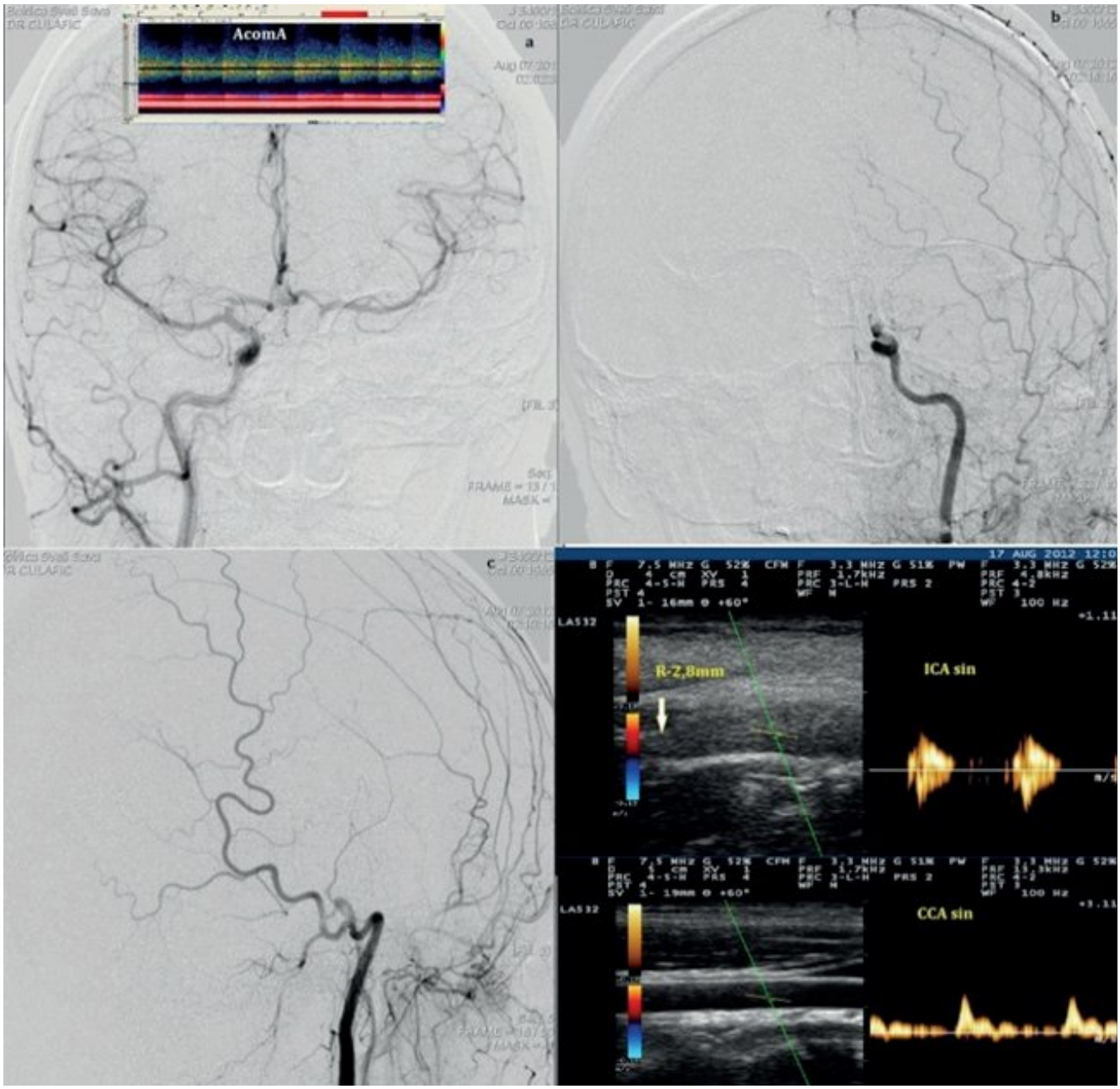

Fig 2. Digital subtractional angiography images with the left approach (a), and the right approach (b, c), color Doppler images of the left common carotid artery and ICA (d).

artery (ICA) compared with the right side. There were no atherosclerotic lesions and disturbances in blood flow velocities. The intima-media thickness (IMT) measured 0,6 mm. The vessel lumen diameters were as follows: right common carotid artery (CCA) - 6.1 mm; left CCA-5.6 mm; right ICA-4.5 mm, left ICA-3.5 mm. The measured peak systolic velocity (PSV) on the right ICA was 140 cm/sec, and it was 60 cm/sec on the left ICA. All magnetic resonance imaging (MRI) examinations were performed on a 1.5-Tesla system (MR Signa, HDx, 1,5T, GE, Milwaukee). MRI with diffusion weighted images (DWI) of the brain showed a hyperacute ischaemic lesion in the left parahippocampal region (Fig. 1a, b, c). Magnetic resonance angiography (MRA) with 3D TOF sequences and with axial plane T1 and T2 fat suppressed images presented dissection in the supraclinoid and cavernous segment in the left ICA with a hyperintense T1W intramural hematoma that was asymmetrically surrounded by a narrowed vessel lumen with high-grade stenosis in this segment (Fig. 1e, f). As a variant, an accessory right anterior cerebral artery (ACA) was observed in the A2 segment.

The patient took acetylsalicylic acid 100 mg daily and bromocriptine to stop postpartal milk production. On the sixth day after admission, she had had a series of three transient ischaemic attacks (TIA) lasting up to two minutes that were characterised by aphasia and weakness of

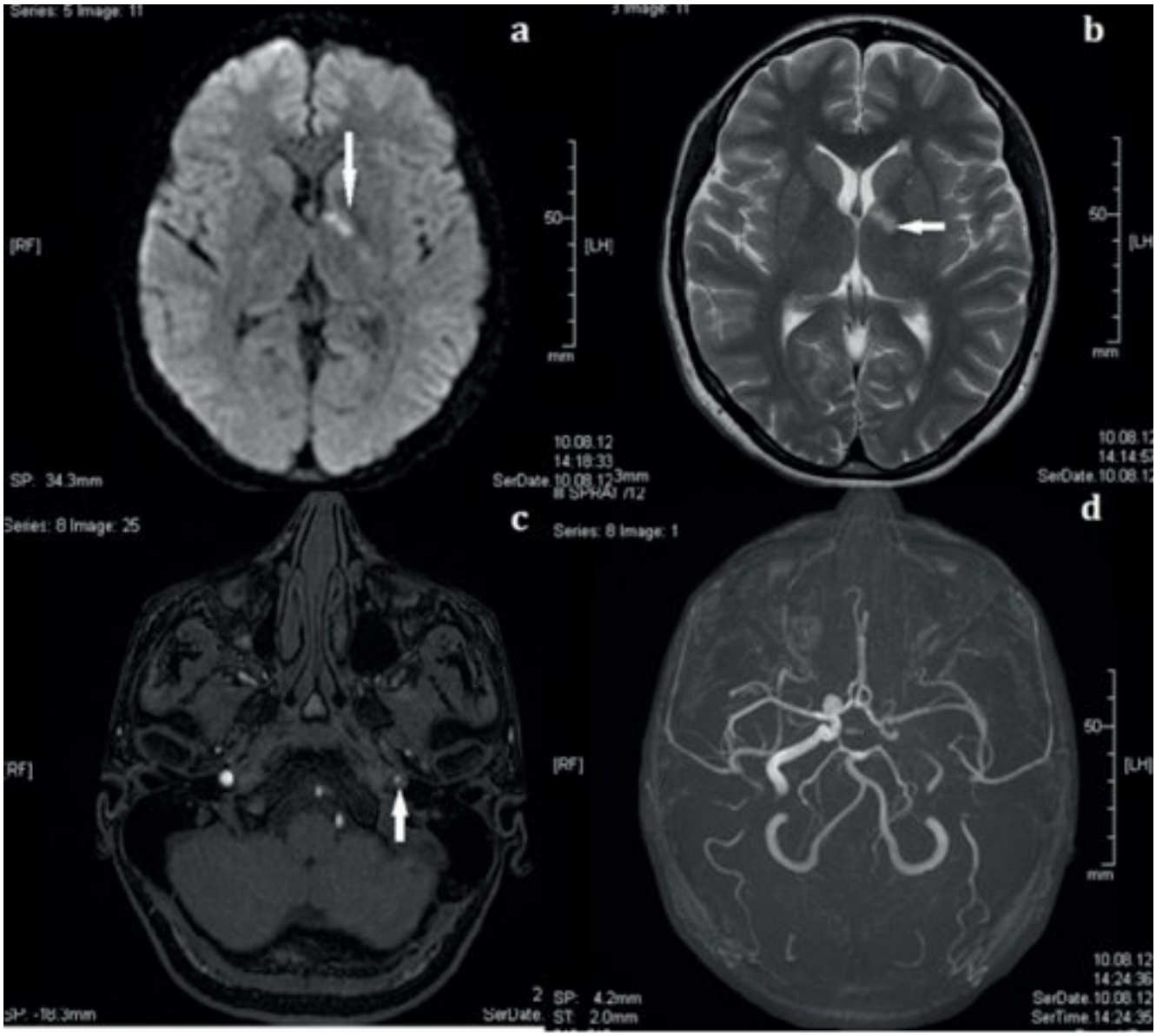

Fig 3. Control magnetic resonance imaging and angiography images four days after DSA

the right side. The fourth TIA was longer and lasted 30 minutes. Digital subtraction angiography (DSA) studies were performed using a digital angiography system (Innova 3100 Single Plane; GE Milwaukee). DSA using the left ICA approach showed an occlusion of the supraclinoid segment of the left ICA, with a visible collateral flow from the external carotid artery (Fig. 2a, c). DSA using an approach from the right ICA revealed good collateral flow through AcomA to the left ACA, middle cerebral artery (MCA), and terminal part of the left ICA over a length of 5 mm after the origin of ophthalmic artery (Fig. 2b). The carotid duplex showed a high resistance "stump" waveform, with end-diastolic absent flow in the left ICA (Fig. 2d). Four days after DSA, the MRI showed a new acute ischemic lesion in the left basal ganglia (Fig. 3a, b). The MRA showed a slow blood flow in the intracranial petrous segment of the left ICA and an occlusion in the supraclinoid segment, with a distal propagation of arterial wall dissection (Fig. 3c, d).

The patient completely recovered and was dismissed with a modified Rankin score of 0. After discharge, she took oral anticoagulant (OAC) medication and statin drug. We followed her for two years and monitored the rate of recanalisation by duplex ultrasound and with MRA. One month after occlusion, a duplex scan showed recanalisation with a homogenous smooth surface and thrombosis on the false lumen, with 50% diameter stenosis. Upon examination thirty days later, recanalisation of the left extracranial segment of ICA was complete, but the transcranial Doppler ultrasound (TCD) showed probable high-grade stenosis of the left terminal-ICA or ACA. The MRI performed 3 months after the onset of disease showed recanalisation with residual 40% tubular stenosis of the supraclinoid segment of the left ICA over the length of 6 mm.

Due to an inability to achieve the therapeutic levels of INR, the OAC was terminated, and double antiplatelet therapy was introduced. Another MRI performed 7 months later showed recanalisation in the ACA and residual 20% stenosis in the supraclinoid segment of the left ICA over a length of 4 mm.

DISCUSSION

Postpartal CCAD is a rare postpartum complication that accounts for 6% of spontaneous CCAD in women under 50 years of age, as previously described in case reports and a small number of case studies (3). We reviewed pertinent research articles concerning CCAD within the last fifteen years and found that CCAD occurred within the first six weeks after childbirth (4-15) (Table 1). Dissection is much more common in extracranial segments of the carotid than it is in vertebral arteries. The most preferred site for intracranial carotid artery dissection is the supraclinoid segment beyond the origin of the ophthalmic artery, with or without extension to the MCA or ACA (3, 16). It was observed that CCAD occurred more commonly in older child-bearing females and had a mean age of onset of 33,7 years (5-15) (Table 1). The current view is that CCAD is related to the combination of various intrinsic and extrinsic risk factors. The most important intrinsic factors are generalised arteriopathy that can result in weakness of the arterial wall, vascular anomaly, or genetic predisposition. Extrinsic factors include traumatic lesions of the neck, recent viral infection, and other factors (17) (Table 1). Hormonal changes during pregnancy can result in an increase in intravascular volume and cardiac output and, thus, an impaired arterial distensibility and compliance of large arteries. Karkkainen et al showed that carotid artery elasticity decreased towards the end of pregnancy and was not correlated with either hyperlipidaemia or the diameter of the vessel (18). Furthermore, oestrogens have favourable effects on the vascular endothelium and vascular smooth muscle cells, with an increase in arterial stiffness observed during the postpartal period (19). The CCA tunica media is thinner than the intima layer and has a higher intima/media ratio during pregnancy. This ratio is dependent on the age, level of oestradiol, blood pressure and BMI of the expecting mother (20). The thinner media can be a predilection site for traumatic lesion. The exceptions are intracranial arteries with very thin tunica adventitia and no external elastic lamina (27).

The association between migraine and CCAD is still unclear. A recent meta-analysis reported that migraine doubles the risk of CAD (22). Tzourio et al. found a highly significant correlation between migraine and serum elastase activity, which is involved in matrix degradation (23). Importantly, postpartal CCAD is associated with a reversible cerebral vasoconstriction syndrome, reversible posterior leukoencephalopathy syndrome, and subarachnoidal haemorrhage without signs of an intracranial extension of CCAD (4). A history of migraine has been frequently reported in patients with reversible cerebral vasoconstriction syndrome during pregnancy and is most likely a result of transient cerebral *dysregulation* (25). In patients with postpartal CCAD, labour had been performed most commonly via the vaginal route. Only in two cases could the effect of positive intrathoracal pressure (Valsalva manoeuvre) during labour be dismissed because the childbirth was performed by the planned caesarean's section. In the other two cases, the labour was performed by caesarean's section after an unsuccessful vaginal delivery (5-15).

During the Valsalva manoeuvre, the cerebral blood flow velocity can increase up to 50% (25). This can have an effect on the arterial intima layer. Pain is a main neurological symptom of CCAD and is often very intense at the time of dissection. It is usually located at the retro-orbital, frontal, and/or temporal cranial regions. Unilateral headache and contralateral weakness are the most common neurological symptoms at the onset of disease. Generally, neurological symptoms appear within the first 24 hours but may occur later (3, 24). The most common clinical presentations of CCAD are stroke or TIA, and intracranial haemorrhage is less common (4). Haemorrhagic stroke due to an arterial dissection is more common at the intracranial segments of the carotid arteries.

The clinical course of CCAD is unpredictable. There can occasionally be an extension of arterial wall dissection along either the cranial or caudal segments, as observed in our patient. The clinical outcome of cerebral artery dis-

Table 1. The main characteristics of CCAD

% of CCAD in general population	Time of CCAD occurance	Age of CCAD occurance (mean)	Enveloped CCA segment	Factors for CCAD development	Clinical presentation	Clinical course of CCAD
Six (6)	first six weeks after childbirth	33.7 years	supraclinoid segment (beyond the origin of the ophthalmic artery)	a) Intrinsic: generalized arteriopathy, vascular anomaly, genetic predisposition. b) Extrinsic: traumatic lesions of the neck, recent viral infection	1. stroke, 2. TIA 3. intracranial hemorrhage	1. spontaneous recanalization, 2. further progression of CAD to the intra and extracranial segments

section may be spontaneous recanalisation. Not rarely, a further progression of CAD to the intra and extracranial segments during first months from onset has been observed (12) (Table 1). The clinical outcome of the arterial dissection is dependent not only on the degree of recanalisation but also on the preserved ability to supply collateral flow through the circle of Willis. Determining these haemodynamic factors during follow-up is necessary via neurosonological or angiographic means. In our patient, the collateral flow was obtained mainly through the AcomA and remained observable even after recovery due to ACA trifurcation. Thus, it is important to access the flow direction in the A1 segment of ACA to avoid the misinterpretation of measurable collateral arterial circulation. The activation of two or more collateral flows is shown to reduce the neurological deficit after stroke due to CCAD and is an important sign for good outcome in these patients (26).

CONCLUSION

Intense voluntary effort by the mother to deliver the baby during the second stage of labour, along with haemodynamic and hormonal changes of the vessel wall, particularly in patients with a migraine, may cause postpartal CCAD in otherwise healthy women. Postpartal CCAD can have an unpredictable course due to an extension of intramural arterial wall haematoma in either the distal or proximal direction. Good clinical outcome is largely dependent on preserved collateral blood flow. Noninvasive Doppler sonography provides early recognition of CCAD and its subsequent resolution. Regular monitoring of the collateral flow is necessary to access the course of disease and its outcome.

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