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INNATE LYMPHOID CELLS: ROLES IN TUMOUR GENESIS AND PROGRESSION

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UROĐENE LIMFOIDNE ĆELIJE- ULOGA U GENEZI I RASTU TUMORA

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ABSTRACT

Innate lymphoid cells (ILCs) represent the most recently identified members of the innate immune system. These cells play important roles in inflammation, tissue remodelling and metabolic disease. ILCs can be subdivided into three major groups according to their cytokine production. The role of ILCs in tumourigenesis and tumour progression is not completely clarified. In this review, we discuss whether and how ILCs are involved in tumour genesis, growth and metastasis.

Keywords: *Innate lymphoid cells, tumour, progression, antitumor immunity*

SAŽETAK

Urođene limfoidne ćelije (engl. Innate lymphoid cells- ILCs) predstavljaju populaciju nedavno opisanih ćelija urođene imunosti. Ove ćelije igraju značajnu ulogu u zapaljenju, obnavljanju tkiva i metaboličkim poremećajima. U zavisnosti od produkcije citokina, ILCs se mogu podeliti u tri glavne subpopulacije. Uloga ILCs u tumorogenezi i progresiji bolesti nije u potpunosti razjašnjena. U ovom preglednom članku, mi razmatramo da li i kako ILCs utiču na genezu, rast i metastaziranje tumora.

Ključne reči: *Urođene limfoidne ćelije, tumor, progresija, antitumorska imunost*

INNATE LYMPHOID CELLS

The innate immune response is important in combating various microbes during the early phases of infection. Innate lymphoid cells (ILCs) represent the most recently identified constituents of the innate immune system by playing a role in inflammation, tissue remodelling and metabolic disease (1). ILCs lack the known immune cell lineage markers. Unlike T and B lymphocytes, ILCs do not have antigen receptors and memory functions (2). ILCs are localized in intestinal and lung mucosae as well as the skin and are capable of rapidly switching on responses to pathogens, even upon first exposure (1). These cells can be subdivided into three major groups according to their cytokine production (Fig 1; 3-4). The ILC2 group represents the innate equivalent of Th2 cells. This group only includes ILC2 cells (e.g., nuocytes, natural helper cells, innate helper cells, and multipotent progenitor cells) that secrete IL-5 and IL-13 in response to IL-25, IL-33 and thymic stromal lymphopoietin (TSLP), and they mediate innate responses during helminth infections and allergies (1-2). The ILC1 group is composed of ILC1 cells and natural killer (NK) cells. They represent the innate equivalent of adaptive Th1 and cytotoxic T cells, respectively. While NK cells play well-known roles in antiviral and antitumour immunity, several additional ILC1s have recently been identified that produce IFN-γ. The ILC3 group includes ILC3 cells and lymphoid tissue inducer (LTi) cells (1, 5). These cells mainly secrete IL-17 and IL-22 in response to IL-23 and IL-1b, and they represent the innate equivalents of Th17 and Th22 cells, respectively. The development and differentiation as well as effector functions of the ILCs are dependent upon the transcription factor, GATA3 (1, 6).

The role of ILCs in inflammatory immune responses, tissue remodelling and metabolic disease are well documented (Fig 1; 1, 2, 7). Recent studies described the involvement of ILCs in tumour growth and progression (8-11). In this paper, we summarize the role of ILCs in tumour genesis and anti-tumour immunity modulation.

THE HISTORY OF INNATE LYMPHOID CELLS

Natural killer (NK) cells were the first discovered ILCs. Five years ago, a new type of innate lymphoid cells was described in fat-associated lymphoid clusters (FALCs). These cells did

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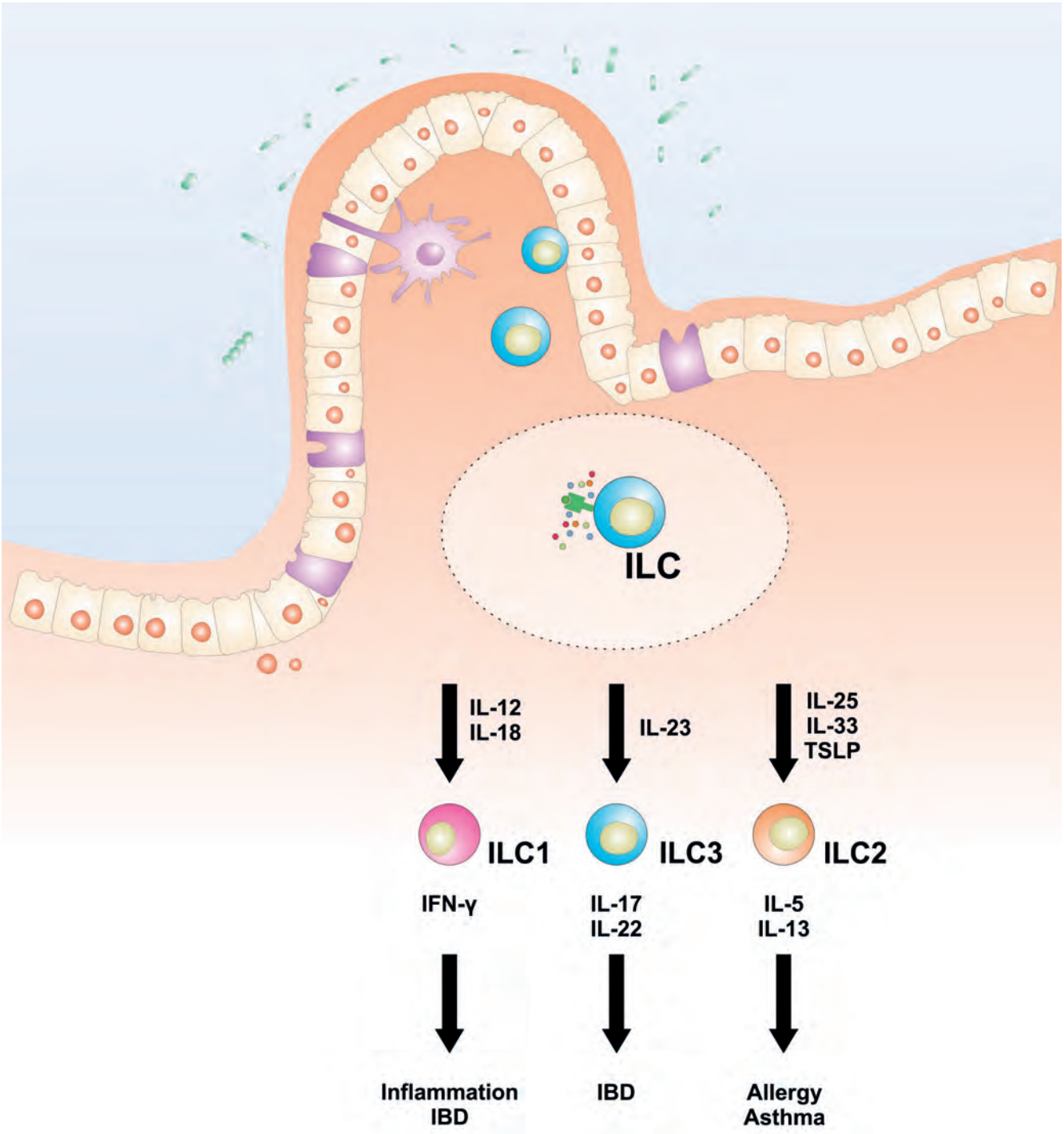

Figure 1. Innate lymphoid cell classifications. The classification of innate lymphoid cells (ILCs) is based on functional criteria. ILC1s are defined by their capability to produce interferon-γ (IFNγ). ILC2s produce type 2 cytokines, interleukin-5 (IL-5) and IL-13. ILC3s are capable of producing the type 17 cytokines IL-17 and IL-22. Different subsets of ILCs cells play various roles in disease immuno-pathogenesis and subsequent tissue destruction and systemic manifestations.

not express lineage (Lin) markers but expressed c-Kit, Sca-1 (also known as Ly6a) and ST2 (12). The authors named them natural helper cells (NHCs). NHCs were shown to proliferate in response to IL-2 and to produce large amounts of type 2 cytokines such as IL-5 and IL-13 in response to IL-33 stimulation and also to require IL-7 for survival (12). NHCs regulate B-cell antibody production and self-renewal of B1 cells. The authors concluded that the described FALC Lin^- c-Kit^+ $Sca\text{-}1^+$ cells (NHCs) acted like Th2-type innate lymphocytes (12). Novel innate lymphocytes, termed multipotent progenitor (MMP) cells, were discovered at a same time (13). IL-25 (also known as IL-17E) promotes the accumulation of Lin^- $Sca\text{-}1^+$ c-Kit^{int} MMP cells in gut-associated lymphoid tissue with subsequent type 2 cytokine production (13). In the same year, this second group of investigators identified a population of lineage negative IL-13 producing cells (14). They named them "nuocytes" due to their IL-13 production (nu is the 13^{th} letter of the Greek alphabet). These cells did not express Lin markers but expressed ICOS, ST2, IL17BR and IL17Rα (14). Nuocytes play a critical role in inducing helminth expulsion. These cells expand rapidly in response to IL-25 and IL-33 (type 2 inducing cytokines) and in response to infection of the helminth, *Nippostronglylus brasiliensis*. Nuocytes secrete high IL-5 and IL-13 levels and present highly specialized type 2 regulatory cells (14). Three independent research groups defined innate lymphoid cells, termed multipotent progenitor (MMP) cells, natural helper cells and nuocytes in the gut-associated mucosa tissues, in the same year (12-14). Whether these cell types exist in organs other than the digestive system was unknown. At that time, it was already known that intranasal administration of the type 2-inducing cytokines, IL-25 and IL-33, induced eosinophilia in the lungs of mice lacking T and B lymphocytes, indicating that another cell population was capable of facilitating localized inflammation (15). Chang et al. reported the presence of Lin^- c-Kit^+ $Sca\text{-}1^+$ $ST2^+$ cells in the lungs and their contribution to the development of airway hyper-reactivity via IL-33 signalling (16). In this study, H3N1 influenza infection induced airway hyper-reactivity, which is a cardinal feature of asthma, through the accumulation and activation of innate lymphoid cells. These cells produced large amounts of IL-5 and IL-13, which facilitate the genesis and progression of airway hyper-reactivity and intensive mucus secretion in the airways (16). Another research group described innate lymphocytes, termed lung natural helper (LNH) cells, as a T cell-independent source of type 2 cytokines (17). The cells were defined as Lin^- $Sca\text{-}1^+$ c-$Kit^{+/lo}$ $CD25^+$ $CD127^+$ cells. After stimulation with IL-33 and IL-2, IL-7 or TSLP, the LNH cells produced IL-5 and IL-13 (17). Innate lymphoid cells in the lungs were capable of rapidly responding to lung epithelium-derived cytokines. Further, they can be divided into two distinct populations: NK cells and cytokine producing ILCs (17). Barthemes et al. described the IL-33 responsive ILCs that reside in the lungs (18). These lung lymphoid cells were Lin^- c-Kit^- $Sca\text{-}1^+$ $CD44^+$ $CD25^+$ $Thy1.2^+$ $IL\text{-}7R\alpha^+$ $ICOS^+$ and produced large quantities of IL-5 and IL-13 after exposure to the fungal allergen, *Alternaria alternata* (18). The authors suggested that allergic airway inflammation can develop independently of adaptive immunity, and lung Lin^- $CD44^{hi}$ $CD25^+$ cells were sufficient to induce airway inflammation (18). An important pathogenic role of novel ILCs was confirmed by the identification and expansion of these cells in various allergy and parasitic inflammation models (18-21). ILCs are important producers of IL-9 during airway inflammation (14, 22). It has now been shown that Lin^- $ST2^+$ cells are the main source of IL-9. IL-33 was shown to induce the accumulation of ILCs that produce IL-9 (22).

Additionally, ILCs play an important role in tissue remodelling. It was shown that, during influenza virus-induced airway inflammation, ILCs produce the epidermal growth factor-related cytokine, amphiregulin, which further regulates epithelial cell repair (23).

Common traits for all types of the described innate lymphoid cells are that they are systemically dispersed and expanded in response to IL-33 and/or IL-25, *in vivo*, express similar surface markers and produce large quantities of IL-5 and IL-13 after activation (24). This finding indicates that these populations might be closely related. All of these cells have been classified into the ILC2 group.

ILC1s are defined by the production of IFNγ and the inability to produce type 2/17 cytokines (1, 2). The prototypical member of this group is the NK cell. First discovered in 1975, natural killer (NK) cells were the first discovered ILCs (25). Unlike T and B lymphocytes, these innate lymphocytes do not express antigen receptors but rapidly exhibit cytotoxic activities against virus-infected and tumour cells and produce various cytokines (1, 2). The origins and roles of NK cells in anti-viral and anti-tumour immunity are well established and they will not be discussed here. NK cells not only display cytotoxic activity, but they are also able to produce large quantities of IFN-γ following activation. Recent studies have identified another ILC1 subset that produces IFN-γ (2, 26, 27). These cells do not secrete type 2/17 cyto-

Table 1. Innate lymphoid cells in tumour genesis and progression

ILC2s	Eosinophil accumulation and antitumour immunity via IL-5 (11)
	MDSCs and Treg accumulation and immunosuppression via IL-13 (8)
	Carcinogenesis via IL-13 (51,52)
ILC3s ($IL\text{-}17^+$)	Facilitate tumourigenesis, angiogenesis and metastasis (55)
ILC3s ($IL\text{-}22^+$)	Stimulate pro- and anti-tumour mechanisms (59)
ILC1s (NK)	Antitumour cytotoxicity and facilitate potent antitumour immunity (62-66)

kines and represented a population that is distinct from NK cells (2, 26, 27). The development of NK cells is dependent upon T-bet expression, and they occur independently of GATA3, while the development of ILC1s is dependent upon both GATA3 and T-bet expression (4). ILC1 cells express high T-bet levels and low RORγt levels (27). These $CD117^-$ cells express IL-7Ra and CXCR3 and secrete IFN-γ, TNF-α, CCL3, and CCL4 in response to activation via IL-12, IL-15 and IL-18, and they play role in immune defence to Salmonella spp. infections and in Crohn's disease immunopathogenesis (1, 2, 27-29).

A recent study described various ILC3 subsets in intestinal tissues (1, 5, 30-33). The ILC3 group includes ILC3 cells and lymphoid tissue inducer (LTi) cells (1, 5). One of the ILC3 subsets that was described in the tonsils, Peyer's patches, appendix and colon lamina propria consists of cells that expresses the NK cell receptor, NKp44/NKp46, and CCR6 and secretes IL-22 but not IL-17 upon IL-23 stimulation (30-32). Another ILC3 subset was discovered in disease affected tissue of Crohn's disease patients and secretes IL-17 and IFN-γ (33). Foetal mesenteric lymph nodes harbour ILC3s that secrete IL-17 and IL-22 (34). LTi cells, which are mediators of innate immunity, can be activated via an IL-23-dependent manner and represent the dominant source of IL-22 during early infections (36). ILC3s mainly secrete IL-17 and IL-22 in response to IL-23 and IL-1b, and represent the innate equivalents of Th17 and Th22 cells (30-35). IL-22 triggers antimicrobial peptide release and promotes epithelial cell proliferation, thereby preserving epithelial barrier integrity (1, 2, 5, 30-35). IL-17 elicits neutrophil recruitment. A recent study found that ILC3s, in particular LTi cells, express MHC class II molecules and present antigen to $CD4^+$ T cells, indicating an impact of ILC3s on adaptive immune responses (37). However, interactions with T lymphocytes did not lead to activation and subsequent proliferation, but these interactions led to tolerance of presented antigens (37). Cella et al. revealed that $NKp44^+$ ILC3s produce BAFF (B-cell activating factor) and promote B-cell activation and survival within mucosal tissues (30, 32).

THE ROLE IF ILCS IN TUMOUR GROWTH AND PROGRESSION

The role of ILC2s in tumour growth and progression was established in a few tumour models (Table 1). A recent report showed that IL-33 mediated an increased level of IL-5-producing ILCs that further regulated the antitumour activity of eosinophils in a lung tumour metastasis model (11). In this study, the authors used a lung metastatic melanoma cell line and observed an accumulation of innate IL-5-producing cells that regulated eosinophil influx into the lung. The innate IL-5-producing cells were similar to the natural helper cells, nuocytes and innate type 2 helper cells, and the majority of which were lineage negative and expressed Sca-1, Thy1.2, CD25, CD27, CD44, CD69, IL-7Ra and ST2, which is a subunit of IL-33R (11). Further, they showed responsiveness of ILCs to IL-25 and IL-33, indicating that these IL-5-producing cells belong to the ILC2 class of cells. After induction of a lung metastatic melanoma model, the authors found that exogenous IL-25 and IL-33 mediated infiltration of eosinophils into the lung via IL-5. Specifically, IL-25 and IL-33 induced accumulation of the innate IL-5-producing ILC2s, which in turn recruited eosinophils into the lung. Antitumour immunity is mediated by the innate and adaptive immune system (38, 39, 40, 41, 42). Polarization of antitumour immune response influences tumour growth in a dual manner. Polarization towards the type 1 response preferentially activates cellular immunity (by producing IFN-γ and IL-2), while the type 2 immune response suppresses cellular immunity by eliciting humoral immunity (via IL-4, IL-5 IL-10, and IL-13 production) (40, 43- 45). The type 1-mediated antitumour immune response is followed by potent stimulation of T-cell cytotoxic activity (39, 40, 41, 46). On the contrary, type 2 polarization results in the production of growth factors and cytokines that support tumour growth and metastasis (43). Although type 2 cytokines downregulate antitumour immunity, they can promote the recruitment of tumouricidal eosinophils and macrophages into the tumour microenvironment (40, 41, 43, 44). In the present study, Ikutani et al. concluded that ILC2s facilitate the accumulation of tumouricidal eosinophils in the lung and may play an important role in tumour surveillance (11). However, eosinophils possess both pro- and anti-tumour activities that are dependent on the tumour microenvironment and type (47, 48). The role of ILC2s in tumour growth needs to be further clarified.

Our initial work on this subject was based on a highly malignant and poorly immunogenic murine tumour model, which shares many characteristics with naturally occurring human breast cancer (8). In this study, we aimed to investigate the effects of exogenously administered IL-33 on tumour appearance and progression and on the mechanisms of anti-tumour immunity. We found that IL-33 enhanced mammary carcinoma growth and lung and liver metastasis by facilitating the expansion of immune suppressor cells within the tumours and by diminishing innate anti-tumour immunity (8). IL-33 administration expanded IL-13 producing innate lymphoid cells within the mammary tumour. These tumour infiltrating lymphoid cells were $CD45^+$ Lin^- $Sca\text{-}1^+$ $CD44^+$ $CD25^+$ $ST2^+$ and produced IL-5 and IL-13 in response to IL-33 and were IL-4, IL-10 and IFN-γ negative, indicating that they had an ILC2 phenotype (8). IL-33 increased the frequencies of IL-5 and IL-13 expressing ILCs and circulating levels of IL-13 in tumour-bearing hosts (8). ILC2s have been shown to facilitate Type 2 immune responses while preventing Type 1-mediated immunity (11, 12, 14, 17, 49); however, their roles in cancer progression are not well defined. We assumed that ILCs directly affect myeloid-derived suppressor cell (MDSC) activity via IL-13. MDSCs are usually recruited to tumour sites from peripheral lymphoid organs where they promote the $CD4^+Foxp3^+$ Treg generation (IJC 33) and exert immunosuppressive effects via TGF-b production. It is well known that MDSCs require the presence of IL-13 for

their activity, e.g., arginase and nitric oxide synthase II expression (50). In line with our conclusion, we also demonstrated IL-33 mediated an increase of $CD11b^+$ $CD11c^-$ $Gr\text{-}1^+$ MDSCs within mammary tumours (8). These MDSCs expressed IL-13α1 receptors and produced TGF-β (8). Our findings revealed a novel role for IL-33 in the mechanisms of breast cancer immune escape via the ILC2s/IL-13/MDSCs/ TGF-β/Tregs axis (8).

In another study, intra-biliary injection of IL-33 into mice with active Akt and Hippo pathways facilitated cholangiocarcinoma development, indicating a role of IL-33 in cholangiocyte proliferation, biliary repair, and carcinogenesis via the ILC2s/IL-13 axis (51, 52).

The role of ILC3s in tumour growth and progression was also described (Table 1). $ROR\gamma t^+$ ILC3s are a main source of IL-17 and IL-22 (second to Th17 cells in terms of production) (53). Studies have shown that $ROR\gamma t^+$ ILC3s accumulate in the intestine of inflammatory bowel disease (IBD) patients and are a crucial IBD pathogenic factor (33, 53). In chronic gastrointestinal infection or acute stimulation with chemical carcinogens, IL-17-producing $IL\text{-}23R^+$ ILC3s induce gut tumourigenesis through the IL-23/IL-17 signalling pathway, which promotes angiogenesis and tumour metastasis (54, 55). Some experiments suggested that IL-22 producing ILC3s promote inflammation in active intestinal diseases (33, 56-58). IL-22 may promote pro- and anti-tumour mechanisms depending on the tissue microenvironment and tumour characteristics. Thus, in pathogen-induced cancers, IL-22 inhibits tumour growth by promoting the elimination of viral or bacterial infections and termination of inflammation. In contrast, IL-22 may facilitates angiogenesis and promotes cancer growth (59). Studies have shown that IL-22 together with other factors contributes to tumour formation (60, 61).

It has been known for decades that NK cells provide protection against viruses and tumour cells. The role of NK cells in immune surveillance is well established (62-66). NK cell activity is variable during tumour progression and is related to clinical stages and disease outcome (65-70). During the cytotoxic killing of tumour cells, NK cells and $CD8^+$ T cells rapidly release granules that contain perforin and granzymes into immunological synapses, thereby inducing target cell death (71). NK cell activity is the major mechanism of innate immunity against tumours (42). NK cells lyse tumour cells without prior sensitization and represent the first line of defence against tumours and cancer metastasis (42).

Recent studies have achieved significant progress towards defining subpopulations of ILCs. ILC1s, ILC2s and ILC3s are now emerging as important cell populations that regulate tissue homeostasis and inflammation. Several studies have described the effects of ILCs on the genesis, growth and progression of tumours and the modulation of antitumour immune responses. However, much of the current knowledge regarding ILCs is based on experimental models and still requires confirmation in humans. Extensive clinical investigations are still needed to clarify the intrinsic roles of ILCs in response to tumours.

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CONFLICT OF INTEREST

The authors declare no financial or commercial conflicts of interest.

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CYP3A5 POLYMORPHISM IN SERBIAN PAEDIATRIC EPILEPTIC PATIENTS ON CARBAMAZEPINE TREATMENT

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POLIMORFIZAM CYP3A5 KOD DECE SA EPILEPSIJOM LEČENE KARBAMAZEPINOM U SRBIJI

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ABSTRACT

*Carbamazepine exhibits significant inter-individual variability in its efficacy and safety, which leads to unpredictable therapy outcomes for the majority of patients. Although its complex biotransformation depends on CYP3A5 activity, evidence of association between carbamazepine treatment outcomes and CYP3A5 functional variations remains inconclusive. The aim of the present study was to investigate the distribution of two of the functionally important CYP3A5 variants *2 and *3 as well as their effects on carbamazepine dose requirements, plasma concentrations and clearance in a Serbian population. The study involved 40 paediatric epileptic patients on steady-state carbamazepine treatment. Genotyping was conducted using the PCR-RFLP method, and carbamazepine plasma concentrations were determined using the HPLC method. CYP3A5*2 and *3 polymorphisms were found at frequencies of 0.0% and 97.5%, respectively, which corresponds well to previously published data for Caucasians. No differences in CYP3A5*3 allele frequencies were detected among epileptic patients in comparison to healthy volunteers within similar ethnic populations (p>0.08), indicating that CYP3A5 polymorphism does not represent a risk factor for epilepsy development. There was an observed tendency towards lower dosage requirements (mean±SD: 15.06±4.45 mg/kg vs. 18.74±5.55 mg/kg; p=0.26), higher plasma concentrations (mean±SD: 0.45±0.13 mg/kg vs. 0.38±0.03 mg/kg; p=0.47) and lower clearance (mean±SD: 0.14±0.05 mg/kg vs. 0.15±0.01 mg/kg; p=0.79) of carbamazepine in homozygous carriers of CYP3A5*3/*3 compared to heterozygous CYP3A5*1A/*3 Serbians. Because these genotype groups did not differ significantly in terms of their carbamazepine pharmacokinetics parameters, the proposed effects of CYP3A5*3 on carbamazepine metabolism could not be confirmed.*

Key words: *CYP3A5 polymorphism, Serbian, epilepsy, carbamazepine*

SAŽETAK

*Karbamazepin odlikuje značajna inter-individualna varijabilnost u efikasnosti i bezbednosti, zbog koje je ishod terapije kod većine pacijenata neizvestan. Iako njegova složena biotransformacija zavisi od aktivnosti CYP3A5 enzima, definitivni dokazi o povezanosti ishoda lečenja karbamazepinom i funkcionalnih varijacija CYP3A5 gena još uvek ne postoje. Cilj ove studije bio je da ispita distribuciju dve funkcionalno značajne varijacije CYP3A5 gena *2 i *3, kao i njihov uticaj na potrebnu dozu, plazma koncentraciju i klirens karbamazepina, u srpskoj populaciji. Studija je uključila 40 pedijatrijskih pacijenata sa epilepsijom lečenih karbamazepinom, nakon postignutog ravnotežnog stanja. Genotipizacija je sprovedena PCR-RFLP, a plazma koncentracija karbamazepina izmerena HPLC metodom. Učestalost CYP3A5*2 i *3 polimorfizama bila je 0.0% i 97.5%, što odgovara prethodno publikovanim podacima za belu populaciju. Nije bilo razlike u učestalosti CYP3A5*3 alela kod pacijenata sa epilepsijom u poređenju sa zdravim ispitanicima iz istih populacija (p>0.08), što ukazuje da CYP3A5 polimorfizam nije faktor rizika za razvoj epilepsije. Uočena je tendencija ka nižim potrebnim dozama (mean±SD: 15.06±4.45 mg/kg naspram 18.74±5.55 mg/kg; p=0.26), višim plazma koncentracijama (mean±SD: 0.45±0.13 mg/kg vs. 0.38±0.03 mg/kg; p=0.47) i nižem klirensu (mean±SD: 0.14±0.05 mg/kg vs. 0.15±0.01 mg/kg; p=0.79) karbamazepina kod homozigotnih CYP3A5*3/*3 u poređenju sa heterozigotnim CYP3A5*1A/*3 genotipovima kod Srba. Obzirom da nije bilo značajne razlike u farmakokinetičkim parametrima karbamazepina među različitim grupama genotipova, predloženi efekat CYP3A5*3 na metabolizam karbamazepina nije mogao biti potvrđen.*

Ključne reči: *CYP3A5 polimorfizam, srpski, epilepsija, karbamazepin*

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ABBREVIATIONS

CYP – Cytochrome P450;
CYP3A5 – Cytochrome P450 3A5;
EDTA - Ethylene diaminetetracetic acid;
PCR - Polymerase chain reaction;
PCR-RFLP - Polymerase chain reaction - restriction fragment length polymorphism;
HPLC - High-performance liquid chromatography

INTRODUCTION

Epilepsy is a common, widely distributed, neurological disorder, known for its frequent resistance to and serious adverse reactions during treatment (1, 2). Since the introduction of potassium chloride in the 19th century, numerous anticonvulsant drugs have been used in therapy for epilepsy (3). Unfortunately, all of these anticonvulsants have exhibited significant inter-individual variability in their efficacy and safety, which leads to an unpredictable therapy outcome in the majority of patients (4, 5). This can be a consequence of many different factors, including variations in the genes coding for drug metabolizing enzymes, transporters and receptors (6). Nevertheless, the pharmacogenetics of epilepsy are still largely unknown (6).

Carbamazepine is a well-known anticonvulsant, frequently used as the first line therapy in several forms of both adult and childhood epilepsy (7, 8). It undergoes a complex biotransformation that involves several drug metabolizing enzymes, with major routes depending primarily upon CYP2C8 and members of the CYP3A family CYP3A4 and CYP3A5 (7, 9). In contrast with CYP3A4, which contributes to variable drug responses through inducibility rather than polymorphism, CYP3A5 exhibits high genetic variability dependent upon ethnicity, as well as strong associations between its genetic background and drug metabolizing activity (10, 11). The clinical significance of *CYP3A5* polymorphism has been reported for several CYP3A5 substrates, including verapamil, tacrolimus and saquinavir (12-14). However, due to conflicting reports, the evidence of association between carbamazepine treatment outcomes and *CYP3A5* functional variations remains inconclusive. As an example, although *CYP3A5* genotype affected serum concentration of carbamazepine in Koreans (15), Chinese (16), and Japanese (17), as well as drug half-life in African-Americans (18), no influence of carbamazepine pharmacokinetics parameters were observed in Caucasian epileptic patients (18). The apparent underlying cause of this discrepancy might be interethnic variability, which represents a multidimensional determinant that comprises both genetic heritage and environment (19, 20). Thus, additional studies on other ethnic populations could contribute to a better understanding of inter-individual differences in carbamazepine response. To our knowledge, no similar investigation has been conducted in Serbian epileptic patients to date.

With an aim to explore the potential role of *CYP3A5* genetic polymorphisms in carbamazepine metabolism, we investigated the distribution of two of the functionally important *CYP3A5* variants and their effects on drug dosage requirements, plasma concentrations and clearance in Serbian epileptic patients undergoing carbamazepine treatment. As the relative risk of adverse drug reactions in children is known to be several-folds higher than in adults (21), only a paediatric population was included in the study.

MATERIALS AND METHODS

Study subjects

The study involved 40 Serbian epileptic patients on steady-state carbamazepine treatment (Table 1). The subjects were recruited from the paediatric department of the Clinical Centre, Kragujevac, Serbia. To be enrolled in the study, all patients had to meet the following inclusion criteria: 1) age between 2 and 20 years, 2) diagnosed partial or generalized tonic-clonic seizures, 3) ongoing carbamazepine treatment, and 4) Serbian origin. The exclusion criteria were as follows: 1) presence of known contraindications for carbamazepine; 2) use of grapefruit juice; 3) presence of atrioventricular block, suppression of bone marrow or porphyria; 4) diagnosed absence or myoclonic epilepsy; 5) presence of increased intraocular pressure; and 6) pregnancy or breastfeeding. Of the 40 enrolled patients, four were co-treated with valproate, whereas others were on carbamazepine monotherapy. Written informed consent was obtained from all patients and their parents, and the study was approved by the ethics committee at the Clinical Centre, Kragujevac, Serbia. The study was conducted in accordance with the Declaration of Helsinki and its subsequent revisions.

Table 1: Patient characteristics

Number of patients	40
Gender (male/female)	24/16
Total body weight (kg)	mean ± SD: 40.38±12.82; range: 17-65
Age (years)	mean ± SD: 10.58±2.88; range: 4-20
Epilepsy (idiopathic/symptomatic)	34/6

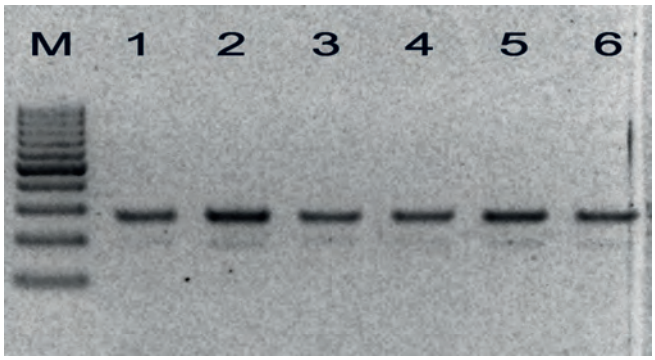

Figure 1. PCR-RFLP analysis of *CYP3A5*2* allele.
Lane M: 100 bp DNA ladder; lanes 1-6: 27289C/C genotype

Genotyping and drug analysis

DNA was extracted from whole-blood samples with EDTA using the PurelinkTM genomic DNA kit (Invitrogen, Carlsbad, CA). DNA concentration was measured using a Qubit® 2.0 Fluorometer and Qubit™ dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA).

*CYP3A5*2* (27289C>A, rs28365083) was genotyped using the PCR-RFLP method described by van Schaik et al. (22), with minor modifications. In brief, a 269-bp-long *CYP3A5* region of interest was amplified in 20 µl PCR of reaction mixture, consisting of ~20 ng of DNA, 0.2 µM dNTP Mix (Thermo Scientific, Waltham, MA), 1.7 mM $MgCl_2$, 0.2 µl of primers 5'-CTGTTTCTTTCCTTCCAGGC-3' and 5'-CTCCATTTCCCTGGAGACTTG-3' (Invitrogen, Carlsbad, CA) and 0.5 U DreamTaqDNA Polymerase (Thermo Scientific, Waltham, MA), in 1XPCR buffer (Qiagen, Hilden, Germany). The amplification was conducted under the following conditions: initial denaturation at 94°C for 7 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 70°C for 1 min; and final extension at 72°C for 7 min. PCR products were subjected to the restriction enzyme FastDigest® Tsp509I (Thermo Scientific, Waltham, MA), which cuts only variant-type alleles to fragments of 182 bp and 87 bp (Fig. 1).

Genotyping for *CYP3A5*3* (6986A>G, rs776746) was performed according to King et al. (23). Namely, PCR yielded 196-bp-long amplicons in a 15 µl mixture containing ~20 ng of DNA, 1XPCR buffer (Qiagen, Hilden, Germany), 0.2 µM dNTP Mix (Thermo Scientific, Waltham, MA), 2.5 mM MgCl2, 0.2 µl of primers 5'-CTGTTTCTTTCCTTCCAGGC-3' and 5'-CTCCATTTCCCTGGAGACTTG-3' (Invitrogen, Carlsbad, CA) and 0.5 U DreamTaqDNA Polymerase (Thermo Scientific, Waltham, MA). The conditions of the PCR reaction were as follows: initial denaturation at 94°C for 2 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 61°C for 1 min, extension at 70°C for 1 min; final extension at 72°C for 7 min. Restriction digestion at 37°C with FastDigest® RsaI enzyme (Thermo Scientific, Waltham, MA) resulted in cutting wild type alleles to 102-bp, 94-bp, 74-bp and 20-bp fragments, and variant type alleles to 102-bp, 74-bp and 20-bp fragments (Fig. 2).

Both PCR reactions were conducted in Techne Genius PCR Thermal Cyclers (Techne, Cambridge, UK). The PCR products and restriction fragments were detected by gel electrophoresis on a 1.2% or 2.4% agarose gel stained with Sybr® safe DNA gel stain (Invitrogen, Carlsbad, CA).

Carbamazepine plasma concentrations at steady-state were determined by high-performance liquid chromatography (HPLC) according to Jankovic et al. (8), using a Chrompack ISOS/GRAS pump (Chrompack, Middelburg, The Netherlands), UV-VIS Chrompack detector (Chrompack) and Spectra Physics 4600 Data Jet integrator (Spectra Physics, San Jose, CA, USA). Samples were prepared by liquid–liquid extraction with diethyl ether. LiCrospher analytical columns (4.6 x 250 mm, 5 µm) with methanol:water:glacial acetic acid (55:44:1) and a 1 ml/min flow rate were used for molecules separation.

Statistical analysis

Genotype data were presented as haplotype and genotype frequencies, and the 95% confidence interval calculations were calculated according to the modified Wald method. Chi-squared tests were used to compare the observed and expected allele frequencies (Hardy- Weinberg equilibrium) as well as the values obtained from previously reported allele frequencies from other populations. The effects of genotype on carbamazepine dosage requirements, plasma concentrations and clearance were examined by Student's t-tests for independent groups. Carbamazepine clearance was estimated based on the assumed 12-h half-life of carbamazepine in children (24). Statistical analyses were performed with Statistica, version 7.1 (StatSoft, Tulsa, OK, USA). $P<0.05$ was considered statistically significant.

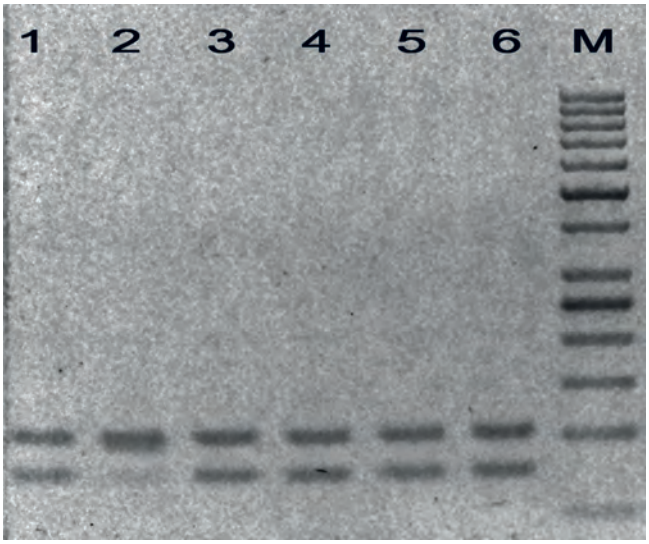

Figure 2. PCR-RFLP analysis of *CYP3A5*3* allele.
Lanes 1, 3-6: 6986G/G genotype; lane 2: 6986A/G genotype; lane M: 50 bp DNA ladder

Table 2. Nucleotide change, haplotype and genotype frequencies of *CYP3A5* in Serbian paediatric epileptic patients on carbamazepine treatment

		Observed frequency	95% Confidence interval
Nucleotide change			
	27289C>A	0.000 (0/80)	0.000, 0.056
	6986A>G	0.975 (78/80)	0.907, 0.998
Haplotype			
	*CYP3A5*1A*	0.025 (2/80)	0.000, 0.093
	*CYP3A5*2*	0.000 (0/80)	0.000, 0.056
	*CYP3A5*3*	0.975 (78/80)	0.907. 0.998
Genotype			
	*CYP3A5*1A/*3*	0.050 (2/40)	0.893, 0.989
	*CYP3A5*3/*3*	0.950 (38/40)	0.893, 0.989

Table 3. *CYP3A5*2* and *CYP3A5*3* distribution in Caucasian populations

Population	CYP3A5 *2 allele frequency	CYP3A5 *3 allele frequency	Reference
Serbian	0.00 (0/80)	0.98 (78/80)	present study
Bosnian		0.93 (259/278)	(48)
Macedonian		0.92 (320/348)	(49)
Greek		0.94 (532/566)	(35)
Italian		0.93 (93/100)	(50)
Polish		0.94 (376/400)	(51)
British		0.94 (188/200)	(23)
Dutch	0.01 (10/1000)	0.92 (920/1000)	(22)
	0.01 (2/200)	0.94 (188/200)	(52)
Finish		0.92 (826/898)	(36)
Russian		0.94 (368/392)	(53)
Australian		0.95 (110/116)	(54)

RESULTS

Genotyping for *CYP3A5*2* and *CYP3A5*3* was performed on 40 Serbian paediatric epileptic patients on carbamazepine treatment, and the nucleotide change, haplotype and genotype distributions are presented in Table 2. All *CYP3A5* genotype frequencies were in accordance with Hardy-Weinberg equilibrium ($\chi 2<0.026$, p=0.05). *CYP3A5*2* was not found. All valproate users were homozygous carriers of *CYP3A5* wild type alleles 27289C and 6986A.

Comparisons with the *CYP3A5* variant allele frequencies observed earlier in other Caucasian populations (Table 3) revealed no significant differences between our results and previously published data in terms of both *CYP3A5*3* ($\chi^2<3.20$, df=1, p>0.07) and *CYP3A5*2* ($\chi 2=0.81$, df=1, p=0.37). Similarly, no differences in *CYP3A5*3* allele frequencies were observed among epileptic patients in comparison to healthy volunteers within different ethnic populations (Table 4, $\chi 2<3.00$, df=1, p>0.08).

CYP3A5 genotype groups did not differ significantly in terms of carbamazepine dosage requirements (p=0.26), dose-normalized plasma carbamazepine concentrations (p=0.47) or carbamazepine clearance (p=0.79). However, there was a tendency towards observed lower dosage requirements (mean ± SD: 15.06 ± 4.45 mg/kg vs. 18.74 ± 5.55 mg/kg), higher plasma concentrations (mean ± SD: 0.45 ± 0.13 mg/kg vs. 0.38 ± 0.03 mg/kg) and lower clearance (mean ± SD: 0.14 ± 0.05 mg/kg vs. 0.15 ± 0.01 mg/kg) in homozygous carriers of *CYP3A5*3/*3* compared to heterozygous *CYP3A5*1A/*3*.

DISCUSSION

In the present study, we investigated the distribution of *CYP3A5*2* and *CYP3A5*3* variants as well as their effects on carbamazepine metabolism in Serbian paediatric epileptic patients. To the best of our knowledge, this is the first study of *CYP3A5* genetic polymorphism in relation to carbamazepine in Serbs. Our results indicate similar frequencies of *CYP3A5* alleles in our Serbian population to those found in other Caucasians and in epileptic patients compared to healthy populations of the same ethnic background. Based on our findings, the proposed effects of *CYP3A5*3* on carbamazepine metabolism in Serbian epileptic patients seem to be possible, but due to the extremely high frequency of *CYP3A5*3* and the small sample size, the effects could not be confirmed.

CYP3A5 is located on chromosome 7q21-q22.1, in a 231-kb cluster with other five members of the CYP3A subfamily:

Table 4. *CYP3A5*3* allelic distribution in epileptic patients compared to healthy volunteers within different populations

Population	Epileptic patients	Healthy volunteers	Reference
Serbian	0.98 (78/80)		present study
		0.92 (252/274)	(55)
		0.94 (113/120)	(56)
Polish	0.88 (130/148)		(47)
		0.85 (120/142)	(47)
Korean	0.77 (54/70)		(15)
		0.70 (70/100)	(30)
Chinese	0.79 (133/168)		(16)
		0.78 (471/604)	(33)
		0.76 (164/216)	(57)
		0.73 (658/902)	(58)
Japanese	0.73 (210/288)		(17)
		0.76 (284/374)	(34)
African–American	0.36 (22/60)		(18)
		0.30 (53/178)	(31)
		0.27 (80/292)	(32)

three genes (*CYP3A4, CYP3A7* and *CYP3A43*) and two pseudogenes (*CYP3A5P1* and *CYP3A5P2*) (25, 26). The gene is highly polymorphic, with more than 25 alleles (http://www.cypalleles.ki.se/cyp3a5.htm) identified to date. It has been shown that the expression of the enzyme is possible only in carriers of at least one wild type *CYP3A5*1A* allele and that the most of the variant alleles are functionally defective (27, 28). The first genetic variant, *CYP3A5*2*, represents a non-synonymous substitution in exon 11 (27289C>A) that leads to an amino acid change at residue 398 (T398N), causing decreased stability and reduced hepatic content of the CYP3A5 protein (27). *CYP3A5*2* is found to be rare in all populations, ranging from 0% in Asians and Blacks (29, 30) to 1% in Caucasians (22). On the other hand, the most frequent and functionally important *CYP3A5* variation, *CYP3A5*3*, is intronic, and consists of a 6986A>G substitution that generates a cryptic splice site and exon 3B, introducing a stop codon and premature termination of a protein translation (28). *CYP3A5*3* represents the most common cause of CYP3A5 loss of expression, found in approximately 30% of African–Americans (31, 32), 75% Asians (33, 34) and more than 90% of Caucasians (22, 35, 36). In the present study, we did not observe carriers of CYP3A5*2 among Serbs, whereas all of the participants were carriers of at least one *3 allele. The results obtained correspond well to the previously published data for Caucasians.

The CYP3A5 enzyme is primarily extrahepatic (10), suggesting that it might play a role in other biological processes in addition to metabolism (28). It has been observed that its level and activity correlates with the risk of developing several diseases, including hypertension (31), acute lymphoblastic leukaemia (37), chronic myeloid leukaemia (38), or breast cancer (39). Epilepsy has a strong hereditary background that involves mutations in multiple genes (40, 41), thus genetic variability in metabolism might potentially contribute to the aetiology of this disease as well. However, based on our results and previously published data, frequency distributions of the most important CYP3A5 variant do not differ between epileptic and non-epileptic subjects within the same populations. Therefore, it is highly unlikely that *CYP3A5* polymorphism represents a risk factor for epilepsy development.

It is well known that metabolism of many drugs, including carbamazepine, largely depends on CYP3A activity (7, 9). Although CYP3A4 plays the leading role, CYP3A5 could contribute substantially, depending on its expression (11). Yet, previous investigations dealing with the influence of *CYP3A5* genotype on drug disposition seem to be conflicting, reporting significant effects in some (12-14, 42, 43) but not all (44-46) CYP3A substrates. Studies on carbamazepine also yielded contradictory results, most probably due to the different ethnic origins of the participants. Namely, the effects of *CYP3A5* genotype on carbamazepine serum concentrations or half-life were observed in Asians and Blacks (15-18) but not in a Caucasian population (18). Similarly, no association between *CYP3A5* polymorphism and carbamazepine resistance was detected in Caucasians (47). In the present study, comparisons between carriers and non-carriers of the non-functional *CYP3A5*3* allele in terms of carbamazepine-pharmacokinetics parameters did not show significant differences. However, a tendency towards lower dosage requirements, higher plasma concentrations and lower clearance of carbamazepine was observed in subjects having the non-functional *CYP3A5* genotype. The lack of statistical significance could be explained by the extremely high frequency of *CYP3A5*3/*3* carriers (95%) but also by the small sample size, caused by a low number of available subjects that met the inclusion criteria for the study. Additional investigations would be necessary to determine the importance of *CYP3A5* genotyping in Caucasian epileptic patients undergoing carbamazepine treatment.

In conclusion, the frequency distribution of *CYP3A5*2* and *CYP3A5*3* alleles in Serbian epileptic patients corresponds well to previously published data for Caucasians. *CYP3A5* polymorphism does not seem to represent a risk factor for epilepsy development. The proposed effects of *CYP3A5*3* on carbamazepine metabolism, although possible, could not be confirmed.

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THE EFFECTS OF TWO FITNESS PROGRAMS WITH DIFFERENT METABOLIC DEMANDS ON OXIDATIVE STRESS IN THE BLOOD OF YOUNG FEMALES

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EFEKTI DVA FITNES PROGRAMA SA RAZLIČITIM METABOLIČKIM ZAHTEVIMA NA OKSIDATIVNI STRES U KRVI MLADIH DEVOJAKA

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ABSTRACT

The aim of the present study was to assess the effects of two metabolically different exercise programs on the redox state of women who were physically inactive before the beginning of the study. For this purpose, participants (women 25±5 years old) chose one of two popular fitness programs, Pilates or Tae Bo, and attended it 3 times a week for 12 weeks. At the beginning and end of the study, body composition analysis and venous blood sampling were performed. The levels of superoxide anion radical, hydrogen peroxide, nitric oxide and lipid peroxidation were measured in plasma, and the levels of reduced glutathione and the activity of superoxide dismutase and catalase were measured in erythrocytes. Only the Tae Bo program induced changes (positive) in body composition, whereas both exercise programs induced slight oxidative stress in exercisers. In the Tae Bo group, the levels of hydrogen peroxide were significantly increased, whereas the levels of reduced glutathione were decreased after three months of training. In the Pilates group, hydrogen peroxide and catalase activity were increased, and nitrites decreased. However, at the end of the study, those two groups had no significantly different values for any pro/antioxidant compared with the subjects who served as controls. This finding suggests that moderate physical activity, such as recreational fitness programs, may induce the increased production of reactive oxygen species but do not lead to a serious disturbance of the redox homeostasis of exercisers.

Keywords: *oxidative stress, redox balance, fitness, tae bo, pilates, women*

SAŽETAK

Cilj ove studije je da ispita efekte dva programa vežbanja sa različitim metaboličkim zahtevima na redoks status žena koje su inicijalno bile fizički neaktivne. Žene starosti 25±5 godina samostalno su odabrale jedan od dva ponuđena fitnes programa, Pilates ili Tae bo, i pohađale ga 3 puta nedeljno tokom 12 nedelja. Na početku, i na kraju studije, ispitanicama je procenjen telesni sastav i uzeti uzorci venske krvi. Nivoi superoksid anjon radikala, vodonik peroksida, azot monoksida i lipidne peroksidacije mereni su u plazmi, dok su nivoi redukovanog glutationa, i aktivnost superoksid dismutaze i katalaze mereni u eritrocitima. Samo Tae bo program je doveo do pozitivnih promena telesne kompozicije, dok su oba programa vežbanja dovela do narušavanja redoks homeostaze ispitanica. U Tae bo grupi nivoi vodonik peroksida su bili povećani, a nivoi redukovanog glutationa sniženi nakon 3 meseca treninga. U Pilates grupi, nivoi vodonik peroksida i aktivnost katalaze su bili povećani, a nivoi nitrita smanjeni. Ipak, na kraju studije nije bilo značajnih razlika u nivoima pro-antioksidanata između ove dve grupe vežbačica i osoba koje su činile kontrolnu grupu. Ovi rezultati upućuju na zaključak da umerena fizička aktivnost, kao rekreacioni fitnes programi, može dovesti do povećane produkcije reaktivnih kiseoničnih vrsta, ali ne dovodi do ozbiljnog narušavanja redoks homeostaze vežbača.

Ključne reči: *oksidativni stres, redoks ravnoteža, fitnes, tae bo, pilates, žene*

ABBREVIATIONS

ADS - antioxidative defense system;
CAT - catalase;
GSH - reduced glutathione;
RBCs - red blood cells;
RONS - reactive oxygen and nitrogen species;
SOD - superoxide dismutase;
TBARS - thiobarbituric acid reactive substances

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INTRODUCTION

Hypokinesia represents one of the major risk factors for numerous physical disorders, including cardiovascular diseases, obesity, diabetes, and osteoporosis (1). Recreational physical activities, such as different fitness programs, function in both the prevention and treatment of these abnormalities. Accumulating data indicate that exercise with moderate intensity has systemic and complex health-promoting effects, which undoubtedly involve the regulation of redox homeostasis and signalling (2). The relationship between exercise and oxidative stress has been intensively investigated for decades (3, 4); however, more data are required to address this association and its dependence on various relevant factors (5). For example, few studies have investigated the redox state of females. The reason for the disproportionate number of investigations on exercise-induced oxidative stress in male and female populations likely lies in the complexity of the examination and interpretation of the redox state of females, which occur due to hormonal differences between the sexes and their influence on results. It is believed that women are less susceptible to oxidative stress since because oestrogen is a potent antioxidant (6); however, one study showed that the phase of the menstrual cycle (i.e., estradiol concentration) exerts a minimal influence on the exercise-induced redox changes in young women (7).

In recent decades, a number of popular programmed group exercises for women have emerged, such as Pilates and Tae Bo. Those two fitness programs differ significantly in their metabolic and motoric demands. Tae Bo is high-intensity aerobic training that uses different movements from martial arts, dance and aerobics that are combined in choreography with fast music. In contrast, Pilates is a specific form of training that is based on breathing and uses untypical initial positions and exercises to develop muscular strength and flexibility (8). Keeping in mind the deficiency of data on exercise-induced oxidative stress in females as well as the popularity of the above-mentioned fitness programs, the aim of our research was to assess the changes in the redox state of young, previously sedentary females after three months of programmed exercise. The secondary aim of the research was to compare the effects of two metabolically different exercise programs because different mechanisms and the quantity of reactive oxygen species production may be expected from a highly aerobically demanding and static, strength-oriented physical activity.

MATERIAL AND METHODS

Subjects

The sample consisted of 59 sedentary women (25±5 years old). The control group consisted of 10 sedentary women, and there were two experimental groups: the Pilates (n=19) and Tae Bo (n=20) groups. The Pilates and Tae Bo groups took part in the experiment at will and were obligated to attend these programmed activities in addition to maintaining their usual everyday activities and nutrition. All participants were healthy, did not use medications or supplements before the beginning of the study, and were non-smokers. The study was performed in accordance with the Declaration of Helsinki and was approved by the ethical committee of The Faculty of Medical Sciences, University of Kragujevac.

Protocol

Venous blood samples were taken from all participants before the beginning of the study and 3 months later. The characteristics of the two fitness programs are presented in Table 1.

The intensity of both programs was monitored by the Polar Team² System (Polar Electro Oy, Finland) for heart rate monitoring, which was worn by every second exerciser. Warming up in Pilates and Tae Bo consisted of exercises that included large muscle groups and lasted approximately 10 minutes. The main part lasted 45 minutes and consisted of specific exercises depending on the program (Pilates or Tae Bo). Relaxing and stretching included exercises that provided body cool down and relaxation and lasted approximately 5 minutes.

Body composition was measured using the *Tanita BC-418* apparatus for bioelectrical impedance analysis.

Table 1. Characteristics of Pilates and Tae Bo programs.

Characteristic	Pilates	Tae Bo
Duration of class	60 minutes	60 minutes
Duration of program	3 months	3 months
Number of classes per week	3 classes per week	3 classes per week
Intensity	50-65 % HRmax	60-85 % HRmax
Structure of class	1) Warm up 2) Main portionRelaxation (breathing exercises)	1) Warm up 2) Main portionRelaxation and stretching
Musical tempo of main portion of class	100-110 bpm	130-150 bpm
Equipment	Resistance bands	Weights

Biochemical assays

Blood samples were taken from an antecubital vein into a Vacutainer test tube containing sodium citrate anticoagulant. Blood samples were analyzed immediately. Blood was centrifuged to separate plasma and red blood cells (RBCs). Biochemical parameters were measured spectrophotometrically.

Superoxide anion radical determination

The level of superoxide anion radical (O_2^-) was measured using nitro blue tetrazolium reaction in TRIS-buffer combined with plasma samples and read at 530 nm (9). The levels of O_2^- are presented in nmol/ml of plasma.

Hydrogen peroxide determination

The protocol for measuring hydrogen peroxide (H_2O_2) is based on the oxidation of phenol red in the presence of horseradish peroxidase (10). A 200 µl sample with 800 µl phenol red solution) and 10 µl horseradish peroxidase were combined (1:20). The level of H_2O_2 in plasma was measured at 610 nm. The levels of H_2O_2 are presented in nmol/ml of plasma.

Nitric oxide determination

Nitric oxide (NO) decomposes rapidly to form stable metabolite nitrite/nitrate products. Nitrite (NO_2^-) was determined as an index of nitric oxide production with Griess reagent (11). Approximately 0.1 ml 3N perchloride acid, 0.4 ml 20 mM ethylenediaminetetraacetic acid and 0.2 ml plasma were put on ice for 15 min and were then centrifuged for 15 min at 6000 rpm. After pouring off the supernatant, 220 µl K_2CO_3 was added. Nitrites were measured at 550 nm. Distilled water was used as a blank probe. The levels of NO_2^- are presented in nmol/ml of plasma.

Index of lipid peroxidation (thiobarbituric acid reactive substances, TBARS)

The degree of lipid peroxidation in plasma was estimated by measuring the thiobarbituric acid reactive substances (TBARS) using 1 % thiobarbituric acid in 0.05 NaOH, whch were incubated with plasma at 100 °C for 15 min and read at 530 nm. Distilled water was used as a blank probe. Thiobarbituric acid extract was obtained by combining 0.8 ml plasma and 0.4 ml trichloroacetic acid; the samples were then put on ice for 10 minutes and centrifuged for 15 min at 6000 rpm. This method was described previously (12). The levels of TBARS are presented in µmol/ml of plasma.

Determination of antioxidant enzymes

Isolated RBCs were washed three times with 3 volumes of ice-cold 0.9 mmol/l NaCl and hemolysates containing approximately 50 g Hb/l (prepared according to McCord and Fridovich (13)) were used for the determination of catalase (CAT) activity. CAT activity was determined according to Beutler (14). Lysates were diluted with distilled water (1:7 v/v) and treated with chloroform-ethanol (0.6:1 v/v) to remove haemoglobin (15). Then, 50 µl catalase buffer, 100 µl sample and 1 ml 10 mM H_2O_2 were added to the samples. Detection was performed at 360 nm. Distilled water was used as a blank probe. Superoxide dismutase (SOD) activity was determined using the epinephrine method of Misra and Fridovich (16). Approximately 100 µl lysate and 1 ml carbonate buffer were mixed, and 100 µl of epinephrine was added. Detection was performed at 470 nm. The activities of SOD and CAT in red blood cells (RBCs) are presented in units per gram of haemoglobin x 10^3 (U/g Hb x 10^3)

Determination of glutathione

The level of reduced glutathione (GSH) was determined based on GSH oxidation with 5.5- dithio-bis-6.2-nitrobenzoic acid using the Beutler method (17); the concentration is expressed as nanomoles per millilitre of RBCs.

Statistics

The distribution of the data was checked with the Shapiro-Wilk test, and depending on its result, the appropriate parametric or nonparametric test was used. The differences between the values of means from two related samples (before and after the exercise period) were assessed by a paired t-test or Wilcoxon's test. The difference among three unrelated samples (between groups on initial and on final examination) was assessed by ANOVA or the Kruskal Wallis test, followed by the T test or Mann-Whitney U test. The alpha level for significance was set to $P < 0.05$.

Table 2. Anthropometric characteristics of the investigated group (*P<0.05 when compared to the initial value).

Characteristic	Beginning of the study (X±SD)	End of the study (X±SD)
Height (cm)		
Controls	171.50±4.08	
Pilates	169.15±6.36	
Tae Bo	168.70±6.20	
Weight (kg)		
Controls	61.85±7.29	62.16±7.84
Pilates	63.14±7.93	62.49±6.98
Tae Bo	67.35±12.80	65.23±12.04*
Body mass index (kg/m²)		
Controls	21.36±2.21	21.47±2.30
Pilates	22.17±3.28	21.89±3.03
Tae Bo	23.64±4.04	23.03±3.79*
Fat (%)		
Controls	19.91±5.11	20.20±5.35*
Pilates	21.69±7.59	21.30±6.80
Tae Bo	25.23±9.40	24.02±8.88*
Muscle (%)		
Controls	13.76±0.97	13.84±1.07
Pilates	13.71±1.37	13.67±1.18
Tae Bo	12.98±1.62	13.19±1.55*

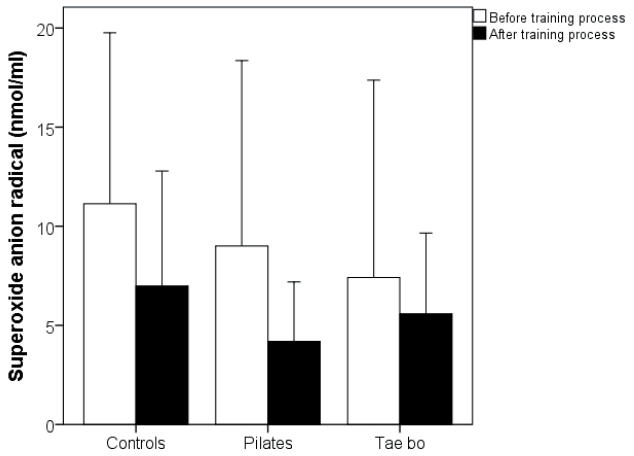

Figure 1. Levels of superoxide anion radical (X±SD) in the investigated groups before and after the training period.

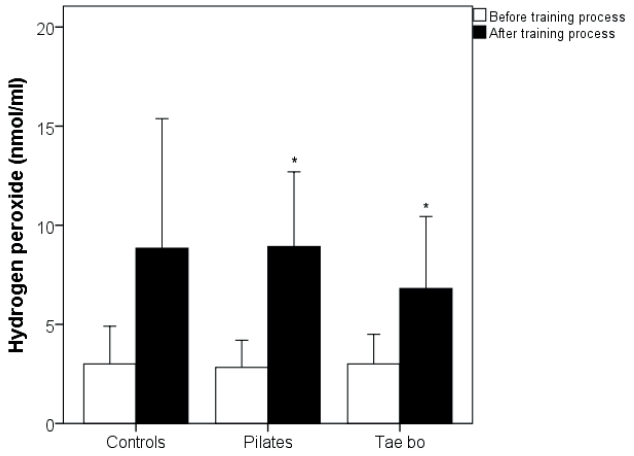

Figure 2. Levels of hydrogen peroxide (X±SD) in the investigated groups before and after the training period (*P<0.05 compared with the beginning of the study).

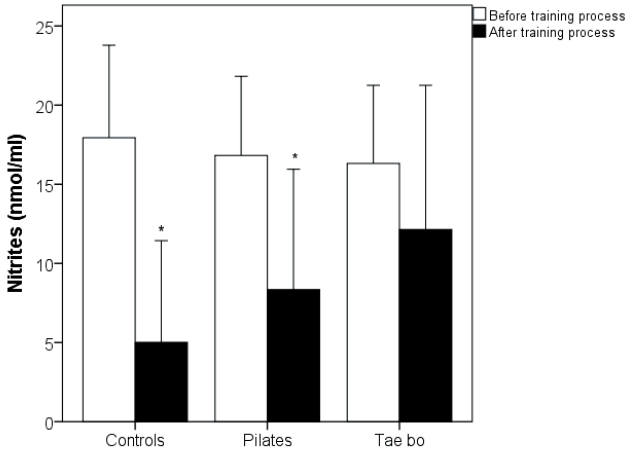

Figure 3. Levels of nitrites (X±SD) in the investigated groups before and after the training period (*P<0.05 compared with the beginning of the study).

RESULTS

The anthropometric characteristics of the investigated groups at the beginning and at the end of the study are presented in Table 2. After three months of training, all parameters of body composition changed significantly in subjects who practiced Tae Bo (body weight: P=0.002, % fat: P=0.005, % muscle: P=0.008, BMI: P=0.002), and the body fat increased in the control group (P=0.038). However, there was no significant difference between groups in the investigated anthropometric parameters at either the beginning or the end of the study.

Changes in levels of pro/antioxidants in plasma and red blood cells of subjects are presented in Figures 1 to 7. The levels of NO_2^- were significantly lower in the Control (P=0.043) and Pilates (P=0.007) groups compared with the levels measured at the beginning of the study. The Pilates group also had higher levels of H_2O_2 (P=0.001) and CAT activity (P=0.011) at the end of the study. In the Tae Bo group, H_2O_2 increased (P=0.002), but GSH decreased (P=0.006) after three months of training.

The differences between groups in the levels of investigated redox parameters are also presented in Figures 1 to 7. At the beginning of the study, subjects from the Control group had significantly lower SOD activity compared with both the Tae Bo (P=0.040) and Pilates groups (P=0.037). At the end of the study, the groups did not significantly differ in any redox parameter.

DISCUSSION

The aim of the present study was to assess the effects of two metabolically different exercise programs on the redox state of women who were physically inactive before the beginning of the study. For this purpose, participants chose one of two popular fitness programs, Pilates or Tae Bo, and attended it three times a week for 12 weeks. At the beginning and end of the study, body composition analysis and blood sampling were performed. Only the Tae Bo program induced (positive) changes in body composition, although both exercise programs disturbed the redox homeostasis of subjects.

There is a common acceptance that Pilates and other forms of low-intensity exercises, when they are performed slowly and with proper breathing, will do far more to improve health status than will a vigorous cardiovascular or strength workout (18). Those types of exercise may be classified as parasympathetic exercise: they do not raise the heart rate and breathing rate significantly, but they significantly reduce stress levels (18). In contrast, intensive aerobic exercise, such as Tae Bo, is characterized by increased oxygen consumption and a possible disturbance of intracellular pro/antioxidant homeostasis. In general, the body has adequate antioxidant reserves to cope with the production of reactive oxygen species (ROS) under physiological conditions and perhaps during low- to moderate-

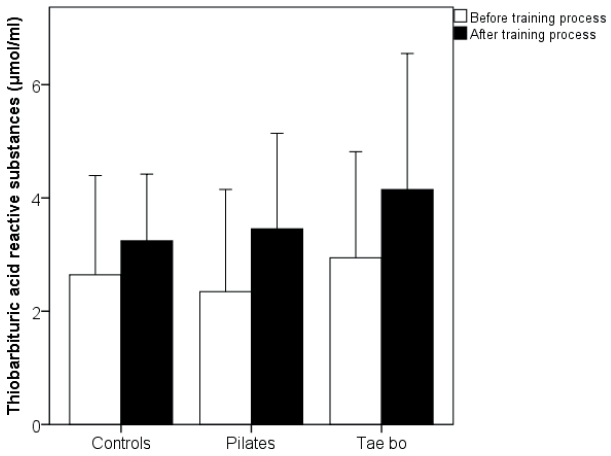

Figure 4. Levels of thiobarbituric acid reactive substances (X±SD) in the investigated groups before and after the training period.

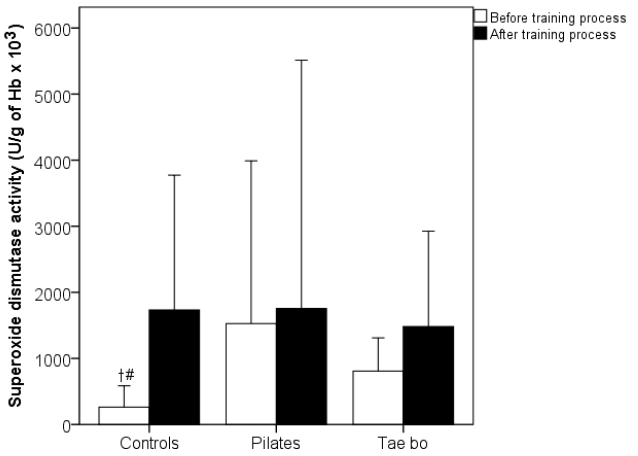

Figure 5. Superoxide dismutase activity (X±SD) in the investigated groups before and after the training period (†P<0.05 compared with the Pilates group at the beginning of the study; #P<0.05 compared with the Tae Bo group at the beginning of the study).

intensity exercise, but when ROS production is excessive, as occurs during intensive physical efforts, an imbalance between prooxidants and antioxidants in favour of the prooxidants may occur and may lead to a disruption of redox signalling and control and/or molecular damage (19). The effect of exercise on the redox state of an individual depends on many factors, such as the type of training, training load, and individual reaction of an athlete depending on age, gender, and the coexisting factors of risk and physical condition (20). In our study, subjects from both the Pilates and Tae Bo groups had increased levels of H_2O_2 compared with the levels measured before engagement in exercise training, although the levels of this prooxidant all other measured redox parameters were not significantly different among the investigated groups (Control, Pilates and Tae Bo) at the end of the study. In addition to the increase in H_2O_2 levels, at the end of the study, the Pilates group had increased CAT activity and decreased levels of NO_2^- (as a marker of NO) in blood, whereas the Tae Bo group experienced a decrease in the levels of GSH. In the Control group, a decrease in the NO_2^- levels was observed at the end of the study. Those results are consistent with the results of the only similar previously published study (18). In that study, Radovanovic and colleagues reported increased values of the activity of malondialdehyde, protein carbonyls and total sulphydryls in both Pilates- and Tae Bo-trained subjects, but there were no significant differences between the groups (18). After 12 weeks of training, the CAT activity increased in both groups, but the increase was significant only in the Pilates group, whereas Tae Bo subjects had significantly increased total serum antioxidant activity (18).

The results that we obtained suggest that both of the exercise programs induced changes in the redox state of exercisers in the direction of oxidative stress. At the end of the study, the Tae Bo subjects had significantly higher levels of H_2O_2 and lower levels of GSH, which is an element of the endogenous antioxidant system that is directly involved in H_2O_2 elimination. In contrast, in the Pilates group, the

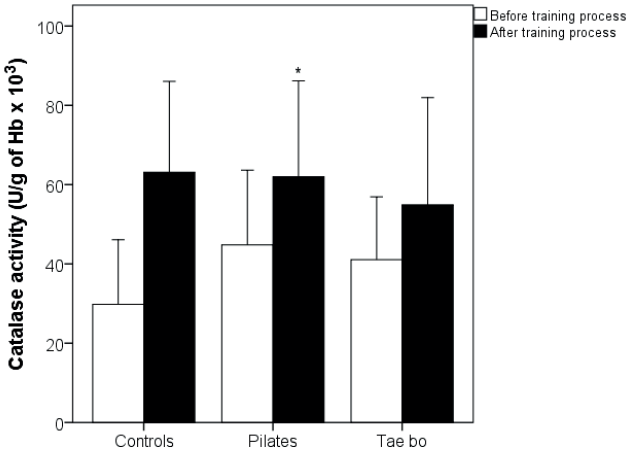

Figure 6. Catalase activity (X±SD) in the investigated groups before and after the training period (*P<0.05 compared with the beginning of the study).

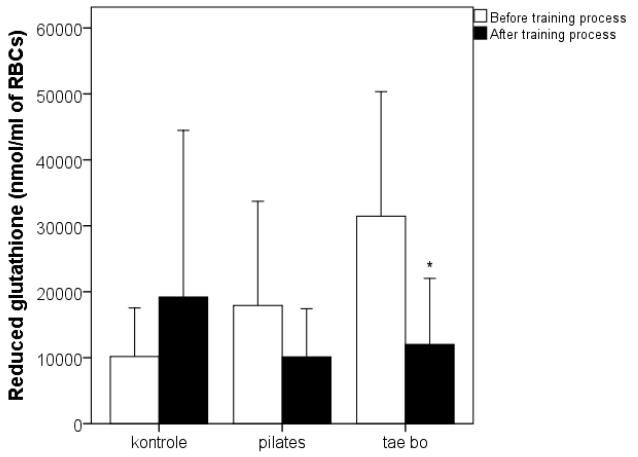

Figure 7. Levels of reduced glutathione (X±SD) in the investigated groups before and after the training period (*P<0.05 compared with the beginning of the study).

increased H_2O_2 levels were followed by increased CAT activity, with no significant change in the levels of GSH. GSH both directly and indirectly (as a cofactor for glutathione peroxidase - GPx) eliminates free radicals (21). CAT and GPx are both engaged in H_2O_2 elimination, but their affinity for it is different and dose-dependent. The affinity of GPx for H_2O_2 is higher at low H_2O_2 levels, whereas CAT affinity increases with an increase in the H_2O_2 levels. Although the difference was not found to be statistically significant, the Pilates group had higher levels of H_2O_2 than did the Tae Bo group, which increased the CAT activation to higher (significant) levels. Interestingly, no group had increased levels of lipid peroxidation (measured through TBARS), which would be expected, especially in the Tae Bo group, whose levels of GSH were exhausted. It was previously shown that GSH blood levels are a determinant of plasma TBARS at rest (22). Subjects with a favourable blood glutathione redox status at rest maintain a more favourable redox status in response to exercise-induced oxidative stress, and vice versa (22). Furthermore, the levels of NO_2^- were also found to be decreased in the Control and Pilates groups, though that was not the case in subjects who practiced Tae Bo. The absence of change in the Tae Bo group may be explained by the effect of increased shear stress on endothelium, characteristic for high-intensity aerobic exercise. Numerous previous studies have shown that regular endurance activity increases the bioavailibity of NO (23-25) and that physically active people have greater basal NO production compared with a sedentary population (26, 27). Finally, the observed differences between the groups in the levels of SOD at the beginning of the study may be explained by the more active lifestyle of subjects who chose to be engaged in training, and they likely do not represent the control group. The absence of significant changes in SOD activity due to the three-month training program is surprising because the majority of previously published studies showed that it is the enzyme that is most susceptible to change due to acute or chronic exercise (28).

CONCLUSION

Although both exercise programs induced an increase in the production of reactive oxygen species, the fact that exercisers did not have significantly different values for any pro/antioxidant relative to controls suggests that moderate physical activity, such as participation in recreational fitness programs, does not lead to a serious disturbance of the redox homeostasis of exercisers. Furthermore, as we previously reported (29), both Pilates and Tae Bo induced significant improvements of the motoric status of women, especially balance and leg and abdominal strength. However, because only Tae Bo induced significant changes in the body composition of exercisers, this high-intensity aerobic activity should be the activity of choice for those who wish to lose and control their weight.

ACKNOWLEDGEMENTS

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PROGNOSTIC VALUE OF NORMAL MYOCARDIAL PERFUSION IMAGING IN ASYMPTOMATIC DIABETIC PATIENTS WITH MODERATE AND HIGH CALCIUM SCORES

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PROGNOSTIČKI ZNAČAJ RADIO-TOMOGRAFSKOG ISPITIVANJA PERFUZIJE MIOKARDA KOD PACIJENATA SA ASIMPTOMATSKIM DIJABETISOM I UMERENIM DO VISOKIM VREDNOSTIMA KALCIJUM SKORA

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ABSTRACT

The purpose of this study was to evaluate the intermediate prognostic value of normal myocardial perfusion imaging (MPI) in asymptomatic diabetic patients with intermediate and high coronary artery calcium (CAC) scores. Methods: A total of 115 asymptomatic diabetic patients with no known coronary artery disease (CAD) underwent MPI after multi-slice computed tomography CAC assessment for the detection of suspected CAD. The study included 75 patients with normal MPI results. A 17-segment model for myocardial perfusion and function analysis was used. Patients were divided into three groups: I gr-20 patients with a diabetes duration between 1-5 years; II gr-24 patients with a diabetes duration 5-10 years; and III gr-31 patients with a diabetes duration >10 years. End points (cardiac death, non-fatal myocardial infarction, heart failure, new angina, revascularization) were assessed at 6, 12 and 24 months.

All patients had normal resting left ventricular function and normal myocardial perfusion scans. Forty patients had moderate coronary artery calcification with an average CAC of 290+/-95. Thirty-five patients had severe coronary calcification with an average CAC of 568+/-67. A correlation was found between diabetes duration and CAC severity (r-0,62 for diabetes duration over 10 years). Three cardiac events were identified at 24--month follow-up (new angina with percutaneous coronary intervention-PCI). Hard events (cardiac death, nonfatal myocardial infarction) were observed at a rate of 0% in group I, 4.2% in group II and 6.4% in group III. The overall event rate at 24--month follow-up was 4.0%.

MPI and CAC are valuable techniques for the preclinical assessment of CAD in asymptomatic diabetic patients. This could guide decision-making to result in optimal treatment and prognosis. Even diabetic patients with normal MPI are at increased intermediate risk for CV events.

Key words: *myocardial imaging, coronary calcium score, coronary artery disease, prognosis*

SAŽETAK

Cilj ovog istrazivanja je da utvrdi prognostički značaj radio-tomografskog ispitivanja perfuzije miokarda (eng. MPI) kod pacijenata sa asimptomatskim dijabetisom i umerenim do visokim vrednostima kalcijum skora.

115 asimptomatskih dijabetičnih pacijenata je bilo podvrgnuto radio-tomografskom ispitivanju perfuzije miokarda nakon tomografskog ispitivanja koronarnog kalcijuma skora (eng. CAC) radi dijagnoze koronarne arterijske bolesti (KAB). Studija je obuhvatila 75 pacijenata sa normalnim rezultatima MPI-ja. Korišćen je 17-osegmentni model MPI-ja. Pacijenti su bili podeljeni u tri grupe: I grupa – 20 pacijenata sa istorijom dijabetesa od 1-5 godina, II grupa – 24 pacijenta sa istorijom dijabetesa od 5-10 godina, III - 31 pacijenat sa istorijom dijabetesa od >10 godina. Krajnje tačke (srčani zastoj, ne-fatalni infarkt miokarda, srčana insufucijencija, nova epizoda angine, revaskularizacija) su ispitivane u šestom, dvanaestom i dvadesetčetvrtom mesecu.

Svi pacijenti su imali normalne vrednosti funkcije leve komore u mirovanju i normalne miokardne perfuzione skenere. 40 pacijenata je imalo srednje vrednosti CAC-a koje su iznosile 290 +/- 95. Tridesetpet pacijenata je imalo visoke vrednosti CAC-a, u proseku 568 +/- 67. Pronadjena je korelacija izmedju dužine trajanja dijabetesa i težine CAC-a (r-0,62 za trajanje dijabetesa >10 godina). Tri srčane smetnje su identifikovane tokom 24-voromesečnog perioda praćenja (nova epizoda angine, sa perkutanom koronarnom intervencijom - PCI). Teži srčani poremećaji (srčani zastoj, ne-fatalni infarkt miokarda) su zabeleženi u grupi I sa stopom od 0%, u grupi II sa stopom od 4.2% i u grupi III sa stopom od 6.4%. Ukupna stopa srčanih poremećaja tokom 24-voromesečnog perioda praćenja je iznosila 4.0%.

MPI i CAC su korisne tehnike za preklinička ispitivanja KAB kod pacijenata sa asimptomatskim dijabetesom. To može olakšati donošenje odluka o izboru adekvatne terapije i imati prognostičku vrednost. Čak i dijabetični pacijenti sa normalnim rezultatima MPI-ja imaju povećan rizik za nastanak neželjenog kardiovaskularnog dogadjaja.

Ključne reči: *radio-tomografija miokarda, koronarni kalcijum skor, koronarna arterijska bolest, prognoza*

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ABBREVIATIONS

MPI - myocardial perfusion imaging
CAC - coronary calcium score
CAD - coronary artery disease
PCI - percutaneous coronary intervention
SPECT - single photon emission computed tomography
LVEF - left ventricular ejection fraction
BMI - body mass index
CV - cardiovascular

INTRODUCTION

Coronary artery disease (CAD) is a leading cause of morbidity and mortality and is associated with high medical costs in the management of high-risk patients. Because of the lack of sufficient CAD screening, asymptomatic patients' first clinical presentation is often in the form of acute myocardial infarction or sudden cardiac death. On-going debate exists over the best diagnostic and therapeutic approaches for asymptomatic diabetic patients at high risk for CAD. There is also controversy regarding whether non-invasive imaging-based risk stratification improves patient management and prognosis when compared with traditional risk factor-based stratification. Strategies that can identify asymptomatic patients who are at high risk of future cardiac events may favourably affect CAD risk and outcomes in this population. MPI with gated SPECT is a well-established test for the diagnostic and prognostic evaluation of patients with known or suspected CAD (1, 2). A normal myocardial perfusion study is associated with low cardiac risk, while increasing coronary calcium scores are associated with increased risk. There has been increased interest in the use of coronary artery calcium (CAC) imaging to diagnose early subclinical atherosclerosis and to improve risk stratification in asymptomatic individuals (3–5). Our study aimed to evaluate the intermediate prognostic value of normal myocardial scintigraphic results with moderate and high calcium scores in diabetic patients without known coronary artery disease.

MATERIALS AND METHODS

Study Population

Seventy-five consecutive asymptomatic diabetic patients (41 male, 34 female; age range 63 ± 15 years) with high CV risk based on the European Society of Cardiology SCORE risk stratification system (>5% risk for fatal cardiovascular event in the next 10 years) and without previously known or established CAD were included in the study. All patients completed the World Health Organization (WHO) Rose Angina Questionnaire for confirmation of their asymptomatic status.

Physical examination, including blood pressure, weight, height, waist circumference, and body mass index (BMI), was performed on all patients; we also performed risk factor analysis and documented the duration of diabetes. Full blood laboratory analyses, including lipid status, fasting glucose levels, HbA1C in diabetic patients, and creatinine levels, were performed on all patients. Urine testing for evidence of albuminuria (30-300 mg/l) was conducted on patients with diabetes and hypertension. Patients were divided into three groups: I gr- 20 patients with a diabetes duration between 1-5 years; II gr- 24 patients with a diabetes duration 5-10 y; and III gr- 31 patients with a diabetes duration >10 y. The clinical and laboratory characteristics of the patients are presented in Tables 1 and 2.

Exclusion criteria were the following: typical stable angina pectoris, previously known or established CAD (history of myocardial infarction, acute coronary syndromes, previous percutaneous intervention or coronary artery bypass surgery), LVEF <50% at rest, severe valvular disease, atrial fibrillation, left bundle branch block, presence of pacemaker, and severe chronic pulmonary disease.

Patients were treated with optimal medical therapy and risk factor control based on the latest European Society of Cardiology guidelines on cardiovascular disease prevention in diabetes and heart disease.

All patients signed a written informed consent form before study enrolment. The study was approved by all authors and the local ethical committee.

Study protocol

Patients who met the inclusion criteria underwent ECG and resting transthoracic 2D echocardiography for the assessment of left ventricular systolic and diastolic function. All patients had preserved resting systolic LV function and left ventricular ejection fraction (LVEF >55%) and then underwent myocardial perfusion Single Photon Emission Computed Tomography (SPECT) imaging for the detection of silent myocardial ischaemia or silent myocardial infarction. The coronary artery calcium (CAC) score was calculated, and the results were evaluated using Agatston units.

Myocardial perfusion SPECT imaging (MPI)

MPI SPECT imaging was performed using a one-day rest-stress protocol with a Tc-99 m sestamibi radiotracer, using 15 mCI for the rest and 25 mCI for the stress study.

We used a single-head gamma camera with a commercially available quantitative gated and perfusion SPECT software package (4DM-SPECT). Patients were instructed to abstain from caffeine-containing beverages for at least 12 h, nitrates for 24 h, and beta-blockers for 48 h before the study. All patients were subjected to a pharmacological stress with Dipiridamole. We used a 17-segment model for a quantitative bulls-eye analysis of regional myocardial perfusion and function. Myocardial perfusion was assessed by a 5-point scoring system (0-normal radiotracer uptake, 1-mild, 2-moderate; 3-severe hypo perfusion; 4-absent uptake). Semi -quantitative analyses of regional perfusion at rest and stress were performed using the summed stress score (SSS), summed rest score (SRS) and summed differential score (SDS) to assess the presence and extent of myocardial ischaemia. Scan abnormalities were defined as follows for SSS: SSS <4, normal perfusion; 4-8, mild; 9-13, moderate; and >13, severely abnormal perfusion scan. Abnormalities for SDS were broken downcategorized as follows: SDS <6, mild (<10% of LV); SDS 7-10, moderate (10-15% of LV); and SDS >10, severe ischemiaischaemia (>15% of LV). In addition, LV volumes, LVEF at rest and stress, presence of transit ischaemic LV dilation (TID), visualization of the right ventricle and lung uptake were also analysed. Regional wall motion was assessed by a 6-point scoring system at rest and stress (0-normal wall motion, 1-mild, 2- moderate; 3-severe hypokinesia, 4- akinesia, 5- dyskinesia) using a wall motion score index.

Coronary Artery Calcium (CAC) Score Imaging

For CAC imaging, a non-enhanced retrospectively ECG-gated scan was obtained with a 128-slice CT scanner (Siemens Somatom Definition 128). The estimated effective radiation dose for this protocol was below 1 mSv. Patients with a heart rate greater than 65 beats per minute received metoprolol 5–10 mg IV before the CT scan. Image reconstruction was performed at 55% of the R-R interval. The total calcium burden in the coronary arteries was measured according to the scoring algorithm of Agatston. On the basis of the total Agatston score, only patients with CAC scores 101–400 (moderate CAC) and 401–1,000 (severe CAC) were included in the study and were referred for MPI. The total CAC score and the CAC score in the each coronary artery was evaluated.

Medical therapy and lifestyle advice

Medical therapy was reviewed, and all patients were put on optimal medical treatment with lifestyle advice based on the latest ESC guidelines for CV prevention and the management of stable CAD.

Statistical analysis

We used the SPSS statistical package (version 18.0). Categorical values were expressed in percentages, while continuous variables were expressed as the mean value ± SD. We used the Pearson method for correlation assessment. Multivariable regression analysis was built to identify factors independently associated with cardiovascular events. Statistical significance was defined at $p<0.05$ for all statistical tests.

RESULTS

The prevalence of metabolic risk factors, laboratory findings and medical therapy are presented in Tables 1 and 2. All patients had, on average, 2 risk factors.

The risk factor distribution among the groups was as follows: Calcium Score of 100-400 (n=40): hypertension (n=32, 80, 1%), hypercholesterolemia (n=18, 44.5%), smoking n=12, 30.0%), obesity (n=3, 7.5%), family history of heart disease (n=13, 32.5%), average HbA1C 7, 6%; Calcium Score >400 (n=35): hypertension (n=27, 77, 0%), hypercholesterolemia (n=37, 61.6%), smoking (n=13, 37.1%), obesity (n=4, 11, 4%), family history of heart disease (n=7, 20.0%), average HbA1C 7, 9+/-1, 1%.

Table 1. Clinical characteristics of the study population

Variables	Patients n=75
Age	65+/-9
Gender	45 m; 30 f
Hypertension	52 (69.5%)
Dislipidaemia	45 (59, 8%)
Obesity	7 (9.2%)
Peripheral artery disease	6 (8.0%)
Smoking	26 (34.6%)
Family history	20 (26.6%)
HbA1C % in diabetic patients	7.9+/-1.1
Ejection fraction (%)	58+/-6%
Microalbuminuria	33 (44.0%)
Mean risk factors per patient	2+/-1

Table 2. Therapeutic characteristics of the study population

Variables	Values
ACE/ARB	75 (82.6%)
Ca antagonist	47 (62.6%)
Thiazide diuretic	45 (60.0%)
Beta blocker	6 (8.0%)
Statins	69 (92.0%)
Oral therapy for diabetes	49/75 (65.3%)
Insulin therapy	26/75 (34.6%)

ACE-angiotensin receptor inhibitors, ARB-Angiotensin receptor inhibitors

Myocardial SPECT findings

A total of 1275 segments were analysed. All patients had normal resting left ventricular function with EF >50% assessed by Gated SPECT. All patients had normal MPI findings, with SDS 2+/1. Five patients had transient ischaemic dilation all of them had hypertension. All patients had normal wall motion.

Coronary Artery Calcium Score findings

Forty patients had moderate calcification of the coronary arteries with average CAC 290+/-95. Thirty-five patients had severe coronary calcification with average CAC 568+/-67. A correlation was seen observed between diabetes duration and CAC severity (r-0,62 for diabetes duration over 10 years). No patients had extensive CAC >1000. Calcium was present in the left main artery in 8 patients (10%), in the left anterior descending coronary artery (LAD) in 22 patients (29%), in the left circumflex artery (LCX) in 13 patients (17%), and in the right coronary artery (RCA) in 16 patients (21%). The average calcium score in the LAD (289+/-72) was significantly higher than in the LCX (115 +/-56) or in the RCA (192+/-68).

Cardiac events

Only 3 cardiac events were noted at the 24-month follow up in the II and III groups (new angina with percutaneous coronary (PCI) revascularization). The rate of hard events (cardiac death or nonfatal myocardial infarction STEMI/NSTEMI) was 0% in gr I, 4.2% in gr II and 6.4% in gr III. The overall rate of CV events at the 24-month follow up was 4.0%. Two patients required PCI to the RCA and one patient underwent PCI to the LAD.

DISCUSSION

Patients with diabetes mellitus are a special population atwith increased CV risk that haves been the focus of a robust amount of prognostic literature. The prevalence of diabetes is increasing dramatically. Globally, it is estimated that 382 million people suffer from diabetes with a prevalence of 8.3%; this number is expected exceed 380 million within the next 20 years. European guidelines on CV prevention and the treatment of stable CAD and American guidelines on screening for CAD in asymptomatic adults suggest that the imaging of atherosclerosis and functional imaging of CAD can be applied in this population (4, 9). On-going debate continues regarding the best screening approach in asymptomatic diabetic patients, as well as the period of usefulness for normal MPI scan results in these patients. Screening for silent CAD in high-risk patients aimsis performed to detect the disease in an early stage and initiate appropriate treatment, mindful of the fact that, in up to 60% of males and 42% of females, the initial presentation of CAD is acute myocardial infarction; in approximately 40% of patients, the initial presentation of CAD is sudden cardiac death (1, 2). Consequently, there is a strong argument for using atherosclerosis and myocardial ischaemia imaging to evaluate the risk of subsequent CAD and guide treatment decisions (3).

Diabetes is considered to be a CAD equivalent, and diabetic patients are considered to be at high -risk of CAD (5). The European and American guidelines recommend screening of asymptomatic high-risk patients; these include diabetic patients with evidence of peripheral or carotid occlusive arterial disease, microvascular disease (proliferative retinopathy, nephropathy), or at least two cardiovascular risk factors (diabetic dyslipidaemia, hypertension, smoking, family history of premature CAD) (4, 9). Risk stratification by normal stress MPS may identify patients with and without CAD who do not require further intervention. It has also been demonstrated that the incremental prognostic value of MPS is greater in diabetic patients than in those without diabetes (6-10). Although the presence of a normal scan should indicate a good prognosis, a normal MPS in diabetic patients may be less encouraging than in non-diabetic subjects. Giri et al. showed that diabetic men have a 13.8% risk of death or MI at 3 years (11). Despite the prognostic benefit of SPECT MPI, the rate of cardiac events is unacceptably high in diabetic patients with normal myocardial perfusion (12, 13). Our study results found hard events (cardiac death, nonfatal myocardial infarction STEMI/NSTEMI) to occur at 0% in gr I, 4.2% in gr II and 6.4% in Gr III. The overall rate of CV events at the 24-month follow-up was 4.0%. This high rate is likely secondary to both false-negative studies for significant CAD by SPECT MPI and to the increased prevalence of mild stenosis with a higher risk of plaque ruptureThis , indicating that non-invasive techniques, such as PET MPI, should be improved to more accurately diagnose CAD and that more aggressive medical therapy should be pursued (13).

Several studies have shown that the duration of the low-risk status after a normal stress MPS depends on several factors that may influence the natural progression of CAD (14). Kang and colleagues (11) reported a 1.9% annual event rate in 440 patients with diabetes mellitus who had normal results on dual isotope SPECT examinations (15). These studies have shown that abnormal stress perfusion study results enable the identification of subjects with diabetes mellitus who are at high risk. However, normal scan results in patients with diabetes mellitus have not been as accurate in identifying subjects who are at low risk, such as patients without diabetes mellitus. Negative stress MPS reliably predicts an excellent outcome, as less than 1% of patients with a normal study will experience hard cardiac events such as cardiac death or nonfatal myocardial infarction. The results of these studies also suggest that a normal stress imaging study predicts a relatively good short-term prognosis, but the predictive value of the test declines steadily after 2 years. However, it appears that the expected event rate is driven not only by MPS findings but also

by the underlying risk factors and comorbidity burden as well as the extent of atherosclerosis and rest and stress left ventricular function. The diagnostic and prognostic value of MPI SPECT is well established, although there are less data for asymptomatic high-risk patients. A meta-analysis involving patients with normal MPI SPECT demonstrated that the annual rate of cardiac death or non-fatal myocardial infarction is much smaller in non-diabetic patients (0.6%) than in diabetic populations, in whom published rates have ranged from 1.6 to 3.3% (16, 17). In particular, combining perfusion and functional data, patients with normal perfusion and LV function had a higher annualized event rate compared with those with discordant perfusion and LV function. The highest probability of cardiac death or nonfatal myocardial infarction and the major risk acceleration was observed in patients with diabetes and abnormal post-stress LVEF.

The decision to undertake coronary artery calcium screening should be based on clinical judgement, and the test should be performed only if the results have the potential to change patient management. If coronary calcium testing is performed, it appears reasonable to proceed with further testing in diabetic patients with calcium scores >400, considering factors such as age and renal function (18). In higher risk groups, a higher prevalence of CAC has been shown to impart a high short-term risk of CV events. In an 8-year follow up study of 716 asymptomatic diabetic patients, it was shown that those with higher CAC scores (>400) had a significantly higher prevalence of annual cardiac events compared with those with lower scores (5.6% versus 0.7%, P<0.01) (18). There are fewer studies assessing the value of normal SPECT scans in patients with moderate and high CAC values.

Our study results confirm the literature data on the increased intermediate risk for CV events in diabetic patients, even with normal myocardial SPECT scan results. The difficulty in identifying diabetic patients at low risk for CV events on the basis of negative cardiac imaging stress test results has major clinical implications. In our study, all patients with CV events had evidence of transient ischaemic left ventricular dilatation, reduced stress LV function and a diabetes duration of over 10 years. From a therapeutic standpoint, the threshold for proceeding to angiography should be lower in diabetic patients. Additionally, measures of risk factor control should be no less aggressive in patients with normal versus abnormal noninvasive imaging results. Recently, Simonsen and colleagues evaluated long-term temporal risk variations in patients with suspected or known CAD and suggested a warranty period of 5 years following a normal MPS. However, these authors did not stratify according to LVEF (20). Our results indicate that an increased CAC score is not always related to haemodynamically significant CAD. It is well known that most unstable plaques causing acute coronary events are angiographically non-significant. Combining anatomic with functional data provides complementary information due to the evaluation of different pathophysiologic aspects of CAD. However, the presence of atherosclerosis does not necessarily result in perfusion abnormalities, nor does a normal perfusion SPECT finding exclude obstructive CAD. Berman and colleagues found that patients with normal SPECT results frequently have extensive atherosclerosis on the basis of CAC criteria, which is perceived as subclinical CAD (21). Even in patients with normal MPI results, a high CAC score is a marker of increased long-term risk. Several studies indicate that patients' knowledge of increased CAC scores improved their compliance with medical therapy and led to intensified medical treatment (19-21).

CONCLUSION

Coronary calcium score and SPECT MPI are valuable methods for the preclinical assessment of atherosclerosis and haemodynamically-ally significant CAD. CAC imaging is useful for identifying patients with extensive atherosclerosis, without haemodynamically significant CAD, and who may be referred for risk factor modification and aggressive medical treatment. Diabetic patients, even those with normal MPI, have an increased intermediate risk for CV events. Among patients with a normal study, the calcium score may represent an additional risk factor for future cardiac events.

CONFLICTS OF INTEREST: none declared.

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EFFICACY AND SAFETY OF IVUS-GUIDED PERCUTANEOUS CORONARY INTERVENTIONS

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EFIKASNOST I BEZBEDNOST IVUS VOĐENIH PERKUTANIH KORONARNIH INTERVENCIJA

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ABSTRACT

The inclusion of IVUS-guided PCI has yet to become a routine approach in invasive cardiology due to the relatively high cost of the procedure, equivocal positive results in important studies and the steep learning curve. As an additional diagnostic tool, IVUS seems to be irreplaceable in stent apposition research, edge dissections and the determination of plaque composition.

To examine the efficacy and safety of IVUS-guided PCI vs. angiographically guided PCI in a centre without prior experience.

A total of 35 patients were examined using IVUS prior to stent placement, and the gathered data were used to determine adequate implanted stent size and length. The acquired parameters were as follows: percentage of stenosis, MLD, CSA and distal reference diameter. After PCI, IVUS studies were repeated and data on the CSA, residual stenosis and MLD were acquired. Additionally, stent size and length, deployment pressure and the use of additional post-dilatations were recorded. There were 35 patients with similar demographic data in the control group who underwent treatment of the same vessel segment with the same stent type. Additional data regarding acute complications (dissections, slow flow during or after the procedure, IM) were obtained from the existing database and complications after six months, such as the need for TVR, MACE and death, were also obtained.

Significant differences were observed in terms of stent size and length, residual stenosis, post-dilatation and MLD in favour of the IVUS group; in addition, TVR was 36% lower in the IVUS group with no significant difference in MACE and death between the groups.

After introduction of the method, IVUS-guided PCI exhibited reasonable safety and efficacy compared to conventional angiographically guided PCI at centres without prior experience.

SAŽETAK

Uvođenje IVUS vođenih PCI još uvek ne predstavlja rutinsku metodu u invazivnoj kardiologiji zbog relativno visoke cene procedure, dvosmislenih pozitivnih rezultata u važnim studijama i visokoj krivi učenja. Kao pomoćno dijagnostičko sredstvo IVUS se pokazao nezamenjivim u ispitivanju apozicije stenta, pojavi ivičnih disekcija kao i u ispitivanju sastava plaka.

Ispitati efikasnost i bezbednost IVUS vođenih PCI naspram angiografski vođenih PCI u centru bez prethodnog iskustva.

Ukupno 35 pacijenata je ispitivano IVUS pre postavljanja stenta, i dobijeni podaci su korišćeni pri odabiru veličine i dužine implantiranog stenta. Korišćeni podaci su bili procenat stenoze, MLD, površina preseka krvnog suda (CSA) i distalni referentni dijametar. Nakon PCI, IVUS ispitivanja su ponovljena i dobijeni su podaci o CSA, rezidualnoj stenozi i MLD. Takođe, notirani su veličina i dužina stenta, raspoređivanje pritiska i korišćenje dodatnih post-dilatacija. U kontrolnoj grupi je bilo 35 pacijenata sličnih demografskih podataka, tretirani istim koronarnim segmentom i korišćenim istim tipom stenta. Dodatni podaci o akutnim komplikacijama (disekcija, spor protok tokom i posle procedure, IM) su dobijeni iz postojeće baze podataka i posle šest meseci poput potrebe za TVR, MACE i smrtni ishod.

Uočena je statstički značajna razlika u veličini i dužini stenta, rezidualnoj stenozi, post-dilataciji i MLD u korist IVUS grupe, takođe TVR je bio 36% niži u IVUS grupi sa beznačajnim razlikama u MACE i smrtnom ishodu.

Po uvođenju metode, IVUS vođene PCI su se pokazale sigurnim i efikasnim u odnosu na konvencionalne angiografski vođene PCI u centru bez ranijeg iskustva.

DE GRUYTER OPEN

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ABBREVIATIONS

IVUS – intravascular ultrasound
PCI – percutaneous coronary intervention
MLD – minimal lumen diameter
CSA – cross sectional area
TVR – target vessel revascularization
MACE – major cardiac adverse event

INTRODUCTION

Intravascular ultrasound (IVUS) is based on applying high frequency ultrasound for the detection and visualization of intracoronary structures and for localization and for determining the size and significance of intravascular atherosclerosis plaques. Different structure detection methods are based on different echogenic characteristics of the vessel parts. Due to excellent image quality and spatial resolution, IVUS represents the complementary method of choice for ordinary angiographic diagnostic approaches during the examination of lumen size and vessel size, plaque load and plaque composition as well as remodelling of the artery. IVUS enables the identification of lesions for which revascularization does not need to be performed. The treatment procedure during PCI can include IVUS, and an appropriately placed stent can be assessed (1).

The major limitation of coronary angiography is that the anatomy cannot be strictly defined due to the two-dimensional projections of a three-dimensional coronary lumen. Additionally, diffuse disease distant from the lesion, lesion abbreviation, angulations, calcifications, plaque eccentricity, vessel overlap, and turbulent contrast may complicate the determination of the lesion size. More accurate lumen and vessel dimensions are obtained using IVUS, so as reproduced data.

Intermediary lesions are still a therapeutic dilemma in interventional cardiology, and there is significant inconsistency in defining the significance of these lesions between different operators and after repeat evaluations by the same operators (2). IVUS is a well-established method in interventional cardiology, and its introduction led to significant advances in the efficacy, procedure technique and adjunct medication protocols with PCI (3, 4, 5). IVUS contributed to a better understanding of the possibilities and limitations of angiographic examination (6, 7).

Currently, the question is whether the routine use of IVUS is medically and financially effective. The use of high pressure during stent implementation directly derived from IVUS studies significantly reduced the need for repeat interventions, and it was proved to be a safe and efficient method. Previous studies proved that angiographically guided procedures are not inferior to IVUS-guided procedures regarding repeat revascularization, myocardium infarcts and mortality, although MLD and the residual stenosis percentage were better with IVUS-guided procedures (8, 9, 10).

MATERIALS AND METHODS.

Both observational and retrospective research were performed. A total of 70 patients were tested in the two groups. In one tested group, 35 patients underwent diagnostic IVUS with elective PCI. Angiographic data gathered before the IVUS procedure were used to classify the lesions according to weight (11), stenosis level, minimal stenosis diameter and DRD vessel size (DRD). Patients with three-vessel disease and disease of the left main coronary artery were not included in this study. The monitored data included stenosis percentage, MLD, cross section area (CSA), and size of the distal reference diameter. After stent implementation, IVUS was repeated and the CSA as well as the residual stenosis percentage and MLD were measured. The size of the stent, implanted pressure, the use of the NC balloon (size and post-dilatation pressure) were entered into the database (register). The patients in the control group underwent treatment of the same segment and implantation with the same stent type; in addition, values derived from the angiographic examination of MLD, stenosis percentage, and the size of the distal reference diameter measured by QCA. Data on the type, size and length of the stent, NC balloon use (with size and pressure of the balloon inflation) were also obtained from the database. Data on dissection and spasm were also obtained from the patient database.

The patients were questioned by telephone or the database was accessed for the following reasons: there were repeat revascularizations on an implanted stent over 6 months (TVR - target vessel revascularisation); composite MACE (major adverse cardiac event) with bleeding that required treatment; acute complications during PCI, including spasm lasting more than 5 minutes, vessel dissection and permanent lateral branch block; and acute coronary changes (defined by an increase of TnI>0.16 and EKG changes, death outcome related to cardiac function occurring between a month and six months after the intervention).

Data were collected retrospectively and measurements were performed by an independent examiner using producer equipment software: QCA – Siemens Medical and iLAB - Boston Scientific. All procedures were performed by two operators who did not have significant prior experience with diagnostic IVUS.

There were no differences between the two groups regarding the mean age, percentage of diabetes, percentage

of DES or lesion type in the vessels. The vessel size defined by IVUS was 15% larger than the angiographically estimated size, and there were differences in the measurements between the two methods within the IVUS group (3, 35±0, 5 vs. 2, 95±0, 7 measured by QCA, p<0, 05); in addition, during angiographic estimation of the vessel size, significant differences were not observed (2, 95±0, 7 vs. 2, 89±0, 6, p=0, 11). The TVR number was smaller than in the IVUS group, and there were more patients who underwent PCI in the IVUS group. NC balloon use was two-fold higher in the IVUS tested group.

Statistical data were processed using the IBM Statistics – SPSS 19 program package; the T-test was used for independent samples for values with a normal distribution, whereas for other values, the Mann-Whitney test was used.

Due to the small number of patients, MACE varied from case to case and occurred in patients with many restenosis episodes and on implanted DES (death outcome with 24 hours due to thrombosis on POBA with cutting balloon) in the IVUS group as well as dissection distal to the implanted stent; in the control group, the lateral branch was blocked and dissection and acute bleeding at the puncture location occurred (3 patients in both cases).

RESULTS.

Table (1, 2, 3)

The following observations were based on the results:

1. Differences in the estimation of vessel size between groups, such as lesion length (figures 1, 2, and 3)
2. Considerably greater use of NC balloons for post-dilatation of implanted stents, which can be explained by the advanced quality of IVUS, which can be used to assess inadequate stent expansion, as well as the inflation intensity due to operator knowledge of the actual size of the vessel (figures 4, 5)
3. Reduced need for repeat revascularization after IVUS-guided procedures with a similar complication percentage for both procedures.

DISCUSSION

The results of significant studies indicate that an experienced operator is required for routine guided procedures for the interpretation of the data gathered with IVUS; these results were found in a few studies that involved the use of IVUS at a small centre and without significant prior experience. Earlier data do not support the routine use of IVUS (8, 9, 10, 11, 12), although we obtained results in favour of a decrease in TVR without a significant difference. Documentary data indicate that the differences are related to acquisition in cases involving small and long lesions, such as lesions on venous grafts for which appropriate angiographically obtained data on the lesion type and length

Table 1. Patients characteristics and coronary lesion

Mean age	60,3	61	NS
Diabetes	23	24	NS
DES (%)	21%	23%	NS
% of treated vessels	**IVUS**	**Control**	**P**
One	75%	67%	NS
Two	25%	33%	NS
Previous PCI	15%	12%	NS
Previous CABG	0%	0%	/
Blood vessel %	**IVUS**	**Control**	**P**
LAD	45	46	NS
LCx	25	36	NS
RCA	33	18	NS
Lesion type %	**IVUS**	**Control**	**P**
Type A	15	13	NS
Type B1	25	22	NS
Type B2	52	60	NS
Type C	8	5	NS
1 vessel	77	68,5	NS
2 vessel	23	31,5	NS
Previous PCI	17	11,5	NS

Table 2. Parameters of procedure

Measurement	IVUS	Control	P
MLD	1,1±0,04	1,3±0,2	NS
MLD (post)	3,5±0,5	3,1±0,4 (QCA)	P=0,002
Distal reference diameter	3,38±0,5	2,89	P=0,042
Stent length	18,5±2,2	16,9±1,7	P=0,002
Inflation pressure	15,5±1,9	13,9±1,8	P=0,001
Stent size	3,44±0,6	3,03±0,5	P=0,004
postdilatation (%)	45,7	22,9	P<0,0001
Residual stenosis %	5,7%	14,28%	P=0,23
Postdilatation pressure	22,7±2,2	20,3±1,9	P<0,001

Table 3. Coronary events

Clinical data	IVUS	Control	P
TVR (%)	6,7%	10,6%	P=0,039
MACE	5,7%	5,7%	NS
Death	/	/	NS
Slow/no reflow	/	2,8%	NS
Dissection	2,8%	/	NS

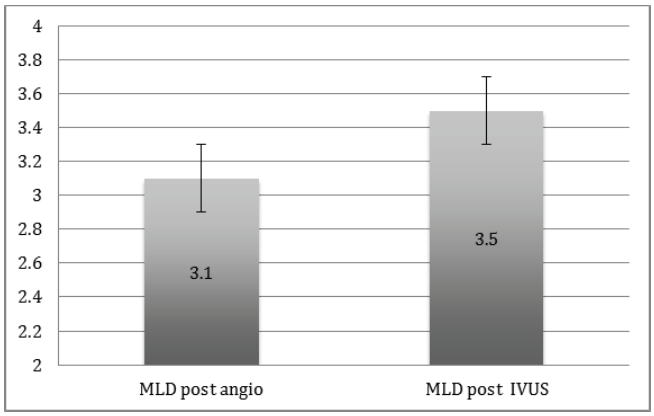

Figure 1. MLD post

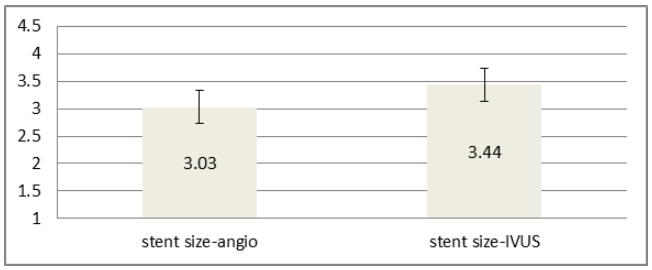

Figure 2. Stent lenght

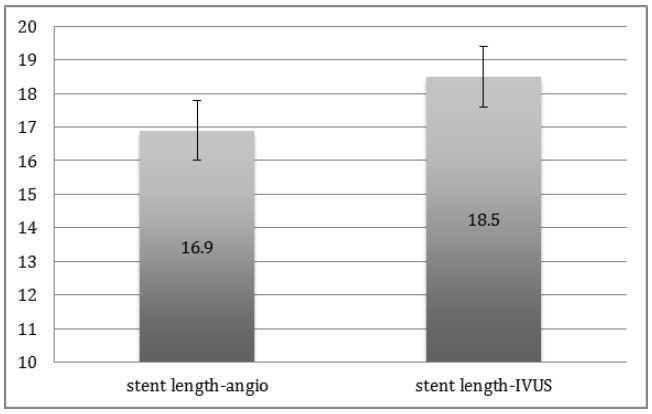

Figure 3. Stent lenght

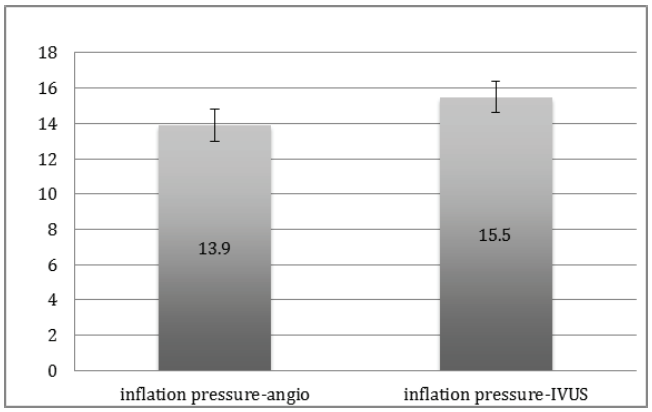

Figure 4. Inflation pressure

cannot be obtained; these lesions are often treated using inadequate stents (which are smaller but not sufficiently long), resulting in the need for additional treatment or repeat interventions. Negative results indicate that stent premeasurements and vessel injury, such as shear stress decreases, can additionally accelerate the restenosis process as shown in porcine models (13). Based on a significant increase in DES use in clinical practice, the dependency of stent strut apposition to the vessel is noted and decreases the acute complications of percutaneous interventions (14); in addition, low lumen size estimates, existing diseased segments proximally and distally from the placed stents and existing but unobservable angiographic dissections are predictors of early PCI complications (15). On the other hand, IVUS did not show a significant influence on stent restenosis in patients treated with DES (15).

IVUS remains a useful instrument for LM interventions, and many studies have demonstrated the benefit from the precise determination of stent size and post-implant results (16, 17, 18, 19). Our cumulative data without determining the subgroups regarding stent size and type do not prove that routine IVUS produces better overall results regarding composite MACE but significantly decreased the need for additional interventions. Data also proved that IVUS-guided procedures can be performed at centres without significant IVUS experience. Further research and more IVUS-guided procedures would indicate whether this PCI method has clinical benefits at small-to-medium-sized centres. We emphasize the need for IVUS-guided interventions at centres without cardiosurgical support where acute complications can significantly endanger the clinical status of patients and the procedure effects. Furthermore, because of the emerging trend of introducing early treatment of acute coronary syndrome by percutaneous interventions that are accompanied by a significantly higher early and late mortality and morbidity than that of elective PCI, the need arises to re-examine the origin of unstable plaques and therapeutic treatment options (17).

Conclusions. After introduction of the method, IVUS-guided PCI proved to be of reasonable safety and efficacy compared to conventional angiographic guided PCI at centres without prior experience.

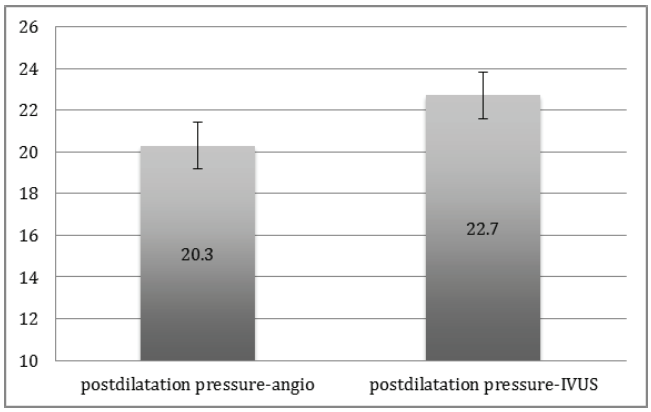

Figure 5. Postdilatation pressure

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RISK FACTORS FOR DEVELOPMENT OF ACUTE NECROTIZING PANCREATITIS

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FAKTORI RIZIKA ZA RAZVOJ AKUTNOG NEKROTIZUJUĆEG PANKREATITISA

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ABSTRACT

Acute necrotizing pancreatitis (ANP) is a severe form of acute pancreatitis that is associated with high morbidity and mortality. Thus, an adequate initial treatment of patients who present with acute pancreatitis (AP) based on correct interpretation of early detected laboratory and clinical abnormalities may have a significant positive impact on the disease course.

The aim of the study was to determine patient- and initial treatment- related risk factors for the development of acute necrotizing pancreatitis.

For the purpose of this study a case-control design was chosen, including adult patients treated for AP in the surgical Intensive Care Unit (sICU) of Clinical Center of Kragujevac, from January 2006 to January 2011. The cases (n=63) were patients who developed ANP, while the controls (n=63) were patients with AP without the presence of pancreatic necrosis. The controls were randomly selected from a study sample after matching with the cases by age and sex.

Significant association with the development of ANP was found for the presence of comorbidity (adjusted OR 6.614 95%CI 1.185-36.963), and the use of somatostatin (adjusted OR 7.460, 95%CI 1.162-47.833) and furosemide (adjusted OR 2710.57, 95%CI 1.996- 56.035) started immediately upon admission to the sICU.

This study suggests that comorbidities, particularly the presence of serious cardio-vascular disease, can increase the risk for development of acute necrotizing pancreatitis. The probability for the development of ANP could be reduced by the avoidance of the initial use of loop diuretics and somatostatin.

Key words: *acute necrotizing pancreatitis, risk factors, comorbidity, furosemide, somatostatin*

SAŽETAK

Akutni nekrotizujući pankreatitis (ANP) je teška forma akutnog pankreatitisa koja je praćena visokim morbiditetom i mortalitetom. Adekvatno inicijalno lečenje pacijenata sa kliničkom slikom akutnog pankreatitisa, koje se zasniva na tačnoj interpentaciji rano dijagnostikovanih labarotorijskih i kliničkih abdormalnosti, može imati značajan pozitivan uticaj na tok bolesti.

Cilj ove studije je bio da odredi faktore rizika koji su povezani sa stanjem pacijenta i inicijalnim lečenjem na razvoj ANP.

Izabrana je „case-control" studija koja je uključila odrasle osobe koji su lečeni od akutnog pankreatitisa u hirurškoj jedinici intenzivnog lečenja Kliničkog centra Kragujevac, u periodu od januara 2006. do januara 2011. godine. Slučajevi (n=63) su pacijenti koji su razvili ANP, dok su kontrole (n=63) sa akutnim pankreatitisom bez razvoja nekroze pankreasa. Kontrole su slučajno izabrane iz studijskog uzorka nakon slaganja u polu i starosti sa slučajevima.

Značajna povezanost sa razvojem ANP je nađena za prisustvo komorbiditeta (adjusted OR 6.614 95%CI 1.185-36.963), inicijalno lečenje somatostatinom (adjusted OR 7.460, 95%CI 1.162-47.833) i furosemidom (adjusted OR 2710.57, 95%CI 1.996- 56.035) .

Rezultati ove studije ukazuju da komorbiiditet, naročito prisustvo kardiovaskularnih bolesti može povećati rizik za razvoj ANP. Verovatnoća za razvoj ANP mogla bi se smanjiti izbegavanjem inicijalne upotrebe somatostatina i diuretika Henleove petlje.

Ključne reči: *akutni nekrotizujući pankreatitis, faktori rizika, komorbiditet, furosemid, somatostatin.*

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ABBREVIATIONS

ANP- Acute necrotizing pancreatitis
AP- Acute pancreatitis
sICU- Surgical Intensive Care Unit
CCK- Clinical Center of Kragujevac
CRP- C-reactive protein
PaO_2 -Arterial oxygen tension
IL- Interleukin
TPN-Total parenteral nutrition
TNF-α- Tumour necrosis factor alpha
HES- Hydroxyethyl starch
OR- Odds Ratio
CI- Confidence interval
Df- Degree of freedom

INTRODUCTION

Acute necrotizing pancreatitis (ANP) is an inflammatory response to functional and / or structural damage to the acini of the pancreas. It is a severe disease and is frequently associated with unfavourable outcomes. The necrosis involves pancreatic parenchyma, peripancreatic tissue or both. Approximately 5-10% of patients with acute pancreatitis develop the necrotizing form of the disease, with mortality ranging from 8% to 30%. In fact, the development of pancreatic necrosis is the most important prognostic factor that imposes a high risk of secondary infection, multiple organ failure and fatal outcome.(1.-4.)

The association with the development of acute necrotizing pancreatitis and consequent increased mortality rate has been well established for numerous factors, including older age, obesity, signs of systemic inflammatory response syndrome, increased levels of creatinine and blood urea nitrogen, hyperglycaemia, low serum calcium level, hypoalbuminaemia, high C-reactive protein level, raised lactate dehydrogenase level, and pleural effusion at admission to the hospital. (5.-7.) On the other hand, there are factors whose role in the development of the necrotizing form of AP is still controversial. These include: initial use of different modalities of enteral or parenteral nutrition, need for early and aggressive fluid replacement, the type of optimal resuscitative fluid (particularly use of various colloid solutions), and the impact of comorbidity that can be defined as the presence of one or more additional serious diseases co-occurring in patients with acute pancreatitis.(7.-8.)

The aim of this study was to investigate the association of patient- and initial treatment - related factors in patients admitted to the sICU for acute pancreatitis with the development of the necrotizing form of disease, as well as to determine their potential additive effects on the occurrence of the observed outcome.

MATERIALS AND METHODS

We have conducted a retrospective, observational, case-control study of 126 adult patients treated for AP at the surgical Intensive Care Unit (sICU) of Clinical Center of Kragujevac (CCK), Serbia, during the five-year period between January 2006 and January 2011. The main criterion for admission to sICU was haemodynamic instability that required continuous monitoring. All data were collected through review of the patients' files. The diagnosis of AP was established by the presence of abdominal pain consistent with the disease and serum amylase and / or lipase greater than three times the upper limit of normal.(1.) The presence of necrosis of the pancreatic parenchyma or the peripancreatic tissues was diagnosed by abdominal contrast-enhanced computed tomography (CECT) and is defined by the presence of nonperfusion of the pancreatic parenchyma and/or presence of local inflammatory changes with associated heterogeneous collection of both solid and liquid components.(2.) CT was performed within at least three days of admission to the sICU, and a single radiologist retrospectively interpreted the CT scans. The study protocol was approved by the Ethics Committee of CCK.

The group of cases (n=63) consisted of patients who developed ANP, and the controls (n=63) were patients with AP without the presence of pancreatic necrosis. The controls were randomly selected from a study sample after matching the cases by age and sex.

Exclusion criteria were: patients under 18 years of age, patients who developed acute pancreatitis after an operation, pregnant women, patients who were referred from the other hospitals to the sICU of the Clinical Center of Kragujevac after more than 2 days from the disease onset, and patients with incomplete data in their medical records.

The variables analysed as potential risk factors for the development of necrotizing AP included:

a) Comorbidity (including myocardial infarction, congestive heart failure, coronary artery disease, hypertensive heart disease, moderate or severe stage of chronic kidney failure, liver cirrhosis, peripheral vascular disease, cerebrovascular disease, chronic lung disease, moderate or severe liver disease, diabetes, and malignancy).
b) The levels of blood urea nitrogen, serum creatinine, blood glucose, arterial oxygen tension (PaO_2), partial pressure of carbon dioxide (pCO_2), C-reactive protein (CRP), serum protein, serum albumin, leukocyte count, heart rate, and the amount of intravenous solutions. These continuous variables were measured in the first 2 days of after admission to the sICU.
c) The presence of pleural effusion on first day of admission of a patient to the sICU, diagnosed by chest X ray.

d) Type of intravenous solution for fluid resuscitation. Colloids that were used for resuscitation were gelatin and/or hydroxyethyl starch.
e) Use of albumin 20% solution.
f) Type of nutritional support started within the first 3 days of admission to the sICU (Nutrison® solution for enteral nutritional support was used early if that could be tolerated; if not, Nutriflex® solution was used for parenteral nutrition). This variable was divided into four categories:
   1. No need for the nutrition support because regular oral feeding was resumed
   2. Total enteral nutrition,
   3. Total parenteral nutrition, and
   Combined enteral and parenteral nutrition.
g) Initial use of opioid analgesics (tramadol), somatostatin analogue octreotide, parenteral calcium preparations, and loop diuretics (furosemide).
h) Multiple Organ Dysfunction Score (MOD score) in the first 24 hours of admission to the sICU.

The differences between cases and controls in the observed numeric (continuous) variables were assessed by Student T-test for two independent samples or a Mann-Whitney U test (after estimation of data distribution using Kolmogorov-Smirnov test for normality), while for categorical variables, Chi-squared test was used. The differences were considered significant if probability of null hypothesis was less than 0.05. To estimate the association of potential risk factors and the development of necrotizing AP, crude and adjusted odds ratios (OR) with 95% confidence intervals (95% CI) were estimated using logistic regression analysis.

RESULTS

Baseline characteristics of the patients with and without necrotizing pancreatitis, and differences between them, are shown in Table 1. The differences between the cases and controls were significant in terms of comorbidity, presence of cardio-vascular disease, the values of the laboratory tests (CRP, blood glucose, urea, creatinine, serum protein and albumin), presence of pleural effusion and parameters of initial treatment, such as use of 20% albumin, use of opioid analgesics, use of furosemide, use of parenteral calcium preparations, type of nutritional support and type of intravenous solution used for fluid resuscitation. There were not significant differences for leukocyte count, pCO_2 value, heart rate or amount of intravenous solutions.

The results of both univariate and multivariate logistic regression analysis (Cox & Snell R square 0.450, Nagelkerke R square 0.600, Hosmer-Lemeshow Chi square 7.639, df=7, p = 0.392, overall model accuracy of 84.8%) presented in Table 2 suggest that the presence of comorbidity, as well as initial use of somatostatin and furosemide, are significantly associated with the development of necrotizing AP. For certain variables, univariate regression models indicated significant influence on the development of acute necrotizing pancreatitis (see crude ORs in the Table 2). The variables contributing to development of necrotizing AP were as follows: level of C-reactive protein, presence of pleural effusion at admission to sICU, type of solution used for intravenous fluid replacement, use of opioid analgesics and nutritional support in initial treatment. The factor that was associated with protection from the development of acute necrotizing pancreatitis was the level of serum albumin at admission to the sICU. After adjustment all of these effects were lost (see adjusted OR in Table 2).

The interactions between factors that are likely to have potential additive effects on the risk of development of necrotizing AP were also examined. The analysis showed strong synergistic effects for the use of furosemide and somatostatin in initial treatment and the presence of comorbidity (Table 3).

DISCUSSION

Acute pancreatitis (AP) is an acute inflammation of the pancreas and adjacent tissue that can potentially be life threatening. There are two main clinical courses of the AP: the first is self-limited, mild form of disease, which is experienced by the majority of patients (approximately 80-85% of all cases of AP). The second form is severe pancreatitis with development of tissue necrosis and is associated with high morbidity and mortality (approximately 15–20% of patients suffer from severe, necrotizing pancreatitis). (9) Necrosis of the pancreas is a consequence of the inflammatory process and hypoperfusion due to loss of intravascular volume (marked as haemoconcentration or increasing haematocrit values). Pancreatic necrosis is an ideal place for the development of infection, which occurs in 40-70% of all patients with necrotizing pancreatitis and is the primary cause of death in the second or late phases of the disease. For these reasons, tracking a patient's progress early in the disease course and initial aggressive management have significant clinical importance. (10)

Our study showed that the presence of certain patient features (such as the presence of comorbidity), pathological laboratory findings and initial treatment were associated with development of ANP.

It ishas been shown that patients with important related comorbidities, especially with chronic cardiovascular disease, often develop necrotizing pancreatitis. This can be explained by the fact that in patients with cardiovascular disease, there is impaired perfusion of all tissues including the pancreas, which leads to the development of necrotic pancreatitis. However, in a study by Uomo et al., related comorbidity was not associated with the development of necrotizing pancreatitis. (11)

CRP is an acute phase reactant secreted by hepatocytes after stimulation by cytokines IL-1 and IL-6, and its level is increased in many inflammatory conditions. The values of CRP peak 72 hours from the onset of pain in AP,

Table 1. Baseline characteristics of the study patients

Variable	Acute pancreatitis with necrosis (cases)	Acute pancreatitis without necrosis (controls)	Test value and significance of null hypothesis	Crude odds ratios with confidence intervals (1.96*SE)
Comorbidity	*Without chronic diseases* 16 (12.7%) *With any chronic diseases* 47 (37.3%)	*Without chronic diseases* 29 (23%) *With any chronic diseases* 34 (27%)	x^2=5.842 p=0.016	2,506 (1.180, 5.321)
Chronic cardio-vascular disorder	Without chronic cardio-vascular disorder 26 (20,6%) With presence of chronic cardio-vascular disorder 36 (29,4%)	Without chronic cardio-vascular disorder 38 (30,2%) With presence of chronic cardio-vascular disorder 25 (19,8%)	χ^2 =6,623 p=0,036	2,285 (1.169, 4.466)
C-reactive protein (CRP) level at admission to sICU	186.47±16.32	116.78±15.89	T=-3,508 p=0,003	1.007 (1.002, 1.012)
Blood glucose level at admission to sICU	9.72±0.65	7.77±0,36	U=1462.5 Z=-2.547 p=0,011	1.140 (1.023, 1.271)
The level of blood urea nitrogen at admission to sICU	10.00±0.85	6.84±0.55	U=1343 Z=-3.013 p=0.003	1.127 (1.034,1.229)
Serum creatinine level at admission in sICU	195.82±30.171	89.90±4.33	U=1304.5 Z=-3.203 P=0.001	1.013 (1,004, 1.022)
The level of the total serum protein at admission to sICU	53.65±1.38	59.13±1.20	T=2.926 p=0,004	0.931 (0.885,0.981)
The level of the serum albumin at admission to sICU	28.41±1.15	34.07±1,12	U=559 Z=-3.874 p=0,000	0.909 (0.856, 0.965)
The presence of pleural effusion at admission	With pleural effusion 37 (29,4%) Without pleural effusion 26 (20,6%)	With pleural effusion 18 (14.3%) Without pleural effusion 45 (35,7%)	χ^2 =11,648 p=0,001	3,558 (1.697, 7.471)
Type of intravenous solution used for fluid resuscitation in initial treatment	only crystalloids 29 (23,0%) crystalloids plus colloids 34 (27,0%)	only crystalloids 45 (35,7%) crystalloids plus colloids 18 (14,3%)	χ^2 =8,383 p=0,004	2.931 (1.402, 6.129)
Type of nutritional support in initial treatment	no need for the nutritional support 18 (14,3%) total enteral nutrition 4 (3,2%) total parenteral nutrition 18 (14,3%) combined enteral and parenteral nutrition 23 (18,3%)	no need for the nutritional support 29 (23%) total enteral nutrition 3 (2,4%) total parenteral nutrition 25 (19,8%) combined enteral and parenteral nutrition 6 (4,8%)	χ^2 =13,822 p=0,003	1.535 (1.133, 2.080)
Use of albumin 20% solution in initial treatment	Yes 22 (17,5%) No 41 (32,5%)	Yes 4 (3,2%) No 59 (46,(%)	χ^2 =15,702 p=0,000	0,126 (0.041, 0.394)
Use of opioid analgesics in initial treatment	Yes, tramadol 31 (24,6%) No 32 (25,4%)	Yes, tramadol 14 (13,5%) No 46 (2635%)	χ^2 =6,596 p=0,017	2,621 (1.246, 5.516)
Use of somatostatin analogue octreotide in initial treatment	Yes 33 (26,2%) No 30 (23,8%)	Yes 17 (13,5%) No 46 (36,5%)	χ^2 =8,488 p=0,004	2,976 (1.414, 6.265)
Use of calcium in initial treatment	Yes 32 (25,4%) No 31 (24,6%)	Yes 14 (22,2%) No 49 (38,9%)	χ^2 =11,093 p=0,001	0,277 (0.128, 0.599)
Use of furosemide in initial treatment	Yes 36 (28,6%) No 27 (21,4%)	Yes 13 (10,3%) No 50 (39,7%)	χ^2 =17,666 p=0,000	5,128 (2.332, 11.280)

Table 2. Crude and adjusted odds ratios of the investigated factors potentially associated with death in patients with severe acute necrotizing pancreatitis

Risk factors	Crude OR (95% CI)	Adjusted OR (95%CI)
Comorbidity	2.506 (1.180, 5.321) *	6.614 (1.185, 36.963)*
C-reactive protein (CRP) value at admission to the sICU	1.007 (1.002, 1.012)*	1.003 (0.994, 1.112)
The value of the serum albumin at admission to the sICU	0.909 (0.856, 0.965)*	1.057 (0.941, 1.188)
The presence of pleural effusion at admission	3.558 (1.697, 7.471)*	3.399 (0.581, 19.904)
Type of solution used for intravenous fluid replacement	2.931(1.402, 6.129)*	3.041 (0.551, 16.780)
Use of furosemide in initial treatment	5.128 (2.332,11.280)*	10.57 (1.996, 56.035)*
Use of opioids analgesics (tramadol vs. no opioids analgesic was performed) in initial treatment	2,621 (1.246, 5.516)*	1.338 (0.231, 7.762)
Use of Somatostatin/Octreotide in initial treatment	2,976 (1.414, 6.265)*	7.460 (1.162, 47.833)*
Nutritional Support in initial treatment	1.535(1.133, 2.080)*	0.708 (0.292, 1.715)
Multiple Organ Dysfunction Score within the first 24 h of admission to the sICU	1.219 (0.967, 1.535)	1.009 (0.568, 1.791)

* Statistically significant association (OR)

Table 3. Interactions between the risk factors influencing development of ANP

Risk factors	Crude OR (95% CI)	Adjusted OR (95% CI)
Only comorbidity	2.506 (1.180, 5.321) *	6.614 (1.185, 36.963) *
Only use of furosemide in initial treatment	5.128 (2.332,11.280) *	10.57 (1.996, 56.035) *
Only use of somatostatin in initial treatment	2,976 (1.414, 6.265) *	7.460 (1.162, 47.833) *
Both use of furosemide and comorbidity	4.818 (2.086; 11.131) *	16.755 (2.680; 104.771)*
Both use of somatostatin and comorbidity	3.220 (1.342; 7.726) *	6.614 (1.059; 35.868) *

* Statistically significant association (OR)

and increased CRP 48 hours after admission to hospital is good predictor for development of the severe form of disease. (12) There are many studies that examined the predictive value of CRP for the development of acute necrotizing pancreatitis, and thus far, CRP has proven to be the best predictive marker. Barauskas et al. showed that patients with a CRP level below 110 mg/l have lower risk of developing necrotizing pancreatitis. (13) Cardoso et al. showed that a cut-off level of CRP that may indicate the development of necrotizing pancreatitis varied between 170 and 190 mg/l. (12) However, multiple cut-off levels have been described, and a CRP level of 150 mg/L is currently widely used as the cut-off level for development of pancreatic necrosis. (14) Our study showed that an increased level of CRP in the first 48 hours from the onset of disease is correlated with the development of pancreatic necrosis; the average CRP level of patients with pancreatic necrosis was 186 mg/l.

In severe forms of AP, pathological changes in the lung are common and are manifested as functional changes that are related to a disturbance in gas exchange, leading to the development of hypoxia and morphological changes that are manifested as pleural effusion and/or pulmonary infiltrates. For a long time, these changes have been considered significant predictive parameters, and they have been included among the Ranson, Glasgow, and APACHE II criteria. (15) Our study showed that presence of pleural effusion 24 hours after the admission was associated with the development of necrotizing pancreatitis, which is in agreement with findings by Talamini et al. (16)

Haemoconcentration in the early course of the disease leads to stasis, thrombosis and eventually pancreatic necrosis. To prevent it, aggressive fluid resuscitation is recommended early in the course of pancreatitis, and the quantity of liquid necessary for resuscitation is 2-4 times higher (60-160 mL/kg body weight) than the needs of a healthy person.(17) Two types of fluids can be used for resuscitation, which are colloid fluids with large molecules (hetastarch, dextran 40, and albumin) and crystalloid fluids with added electrolytes (normal saline, Ringer's, and lactated Ringer's solution). Fluid resuscitation begins with intravenous crystalloid solutions, such as Ringer's lactate solution. It has been reported that lactated Ringer's solution reduced the inflammatory response in patients with acute pancreatitis in comparison to normal saline.[18] On the other hand, the combination of crystalloids and colloids (hydroxyethyl starch - HES) is also effective, increasing colloid osmotic pressure and diminishing the loss of fluid in the third space, as well as modulating the inflammatory reaction through inhibition of nuclear factor-κB activation and neutrophil adhesion and migration.(19) Our study showed that fluid resuscitation in patients with ANP was carried out with combination crystalloids plus colloids because of a high degree of haemoconcentration and a need for increased colloid osmotic pressure and decreased fluid leakage.

Acute pancreatitis is characterized by an increased metabolism and thus increasing nutritional needs. The concept of gut rest (prohibiting enteral intake) has not proved effective in the treatment of acute pancreatitis; therefore, total parenteral nutrition-TPN was initially given priority with the aim to achieve adequate nutritional needs and to prevent secretion of exocrine pancreas. However, use of TPN can increase disease severity, incidence of septic complication and hyperglycaemia. Early enteral nutrition through naso-jejunal tube maintains gut integrity, reduces translocation of bacteria from the gut, down-regulates the systemic immune response, and has many other beneficial effects, including increased production of anti-inflammatory cytokines by intestinal mucosa, especially IL-10, and increase of gastrointestinal motility.(20.-21.) Our study showed that patients with necrotizing pancreatitis received a combination of enteral and parenteral feeding due to increased metabolic needs.

Many studies have reported that hypoalbuminaemia in the early stage can lead to development of pancreatic necrosis.(22), Additionally, a lower level of plasma albumin in early stages is associated with high incidence rate of infection and variation of albumin levels is a significant risk factor for poor prognosis of patients with severe acute pancreatitis.(23) We confirmed this, because hypoalbuminaemia in the early stage of disease was associated with development of necrotizing pancreatitis, and thus, albumin solution was administered to our patients in order correct hypoalbuminaemia and to replace lost volume.

Somatostatin is a 14 amino acid peptide that acts as an inhibitor of growth hormone, as well as gastric, pancreatic, intestinal secretion, gastrointestinal motility and blood flow in splanchnic area. Octreotide is synthetic analogue of somatostatin that has a much longer half-life and causes less glucose intolerance than the native hormone.(24) The initial use of somatostatin and octreotide was justified by the traditional concept of resting the pancreas in AP. The data for use of somatostatin are controversial. Some studies reported beneficial effects of somatostatin in AP with fewer surgical complications, decrease in systemic inflammatory response (by decrease IL-6 and TNF-α level) and improvement in kidney function. (25.) Di Francesko et al. showed that administration of somatostatin induces excitation of the Sphincter of Oddi and pancreatic outflow obstruction.(26.) We show here that use of somatostatin in the early stage of disease was associated with the development of necrotizing pancreatitis.

Aggressive fluid resuscitation is very important in preventing the development of pancreatic necrosis and is performed until an adequate urinary volume is achieved. Use of diuretics before an adequate fluid resuscitation is achieved can aggravate the disease.(17) We confirmed this because patients with early use of diuretics such as furosemide more often developed necrotizing pancreatitis.

This study does have the following limitation. The influence of certain potentially confounding variables on the development of necrotic pancreatitis could not be estimated due to incomplete data records (e.g., body mass index, aetiology of acute pancreatitis, use of antibiotics in initial treatment, and type of cytokines response).

In conclusion, this study suggests that the presence of comorbidities, particularly of chronic cardiovascular diseases, is significantly associated with development of ANP. Certain laboratory findings such as high levels of CRP, low values of albumin and presence of pleural effusion could indicate which patients will develop ANP. The probability for the development of ANP could be reduced by the avoidance of the initial use of loop diuretics and somatostatin.

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COMPETING INTERESTS

The authors declare that have no conflict of interest in this study.

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DISCOID LATERAL MENISCUS INCIDENCE DURING KNEE ARTHROSCOPY

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DISKOIDNI LATERALNI MENISKUS - INCIDENCA KOD ARTROSKOPIJE KOLENA

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ABSTRACT

Discoid meniscus is a morphological anomaly of the lateral meniscus that appears in 1–5% of meniscectomies. A precise diagnosis remains difficult to make, and many dilemmas hinder effective treatment.

To evaluate the incidence of discoid meniscus in patients who underwent knee arthroscopy, as well as the discoid meniscus type, follow-up problems, combined knee lesions, and postoperative results.

This retrospective study included 1357 patients who received knee arthroscopy during the period between January 2007 and December 2013. We analysed the discoid meniscus incidence, sex distribution, type distribution (Monllau classification), noted preoperative symptomatology, rupture incidence and type (O'Connor classification), anomaly presence and other intra-articular lesion correlations. The IKDC score was used to evaluate the operative treatment results.

The DLM incidence was 1.03%. The most common type of discoid meniscus was complete 5 (35.71 %). The dominant symptom was pain, which was reported by 12 (85.71%) patients. Eleven (78.57 %) patients exhibited ruptures, and the most common type was horizontal, which was reported in 4 (36.36%) cases. The number of ruptures was significantly higher in patients older than 18 (9 patients; 90%), compared to those younger than 18 (2 patients; 40%). Operative treatment resulted in an improvement for all patients in terms of subjective symptom reduction.

The incidence of DLM in our study was 1.03%, and the dominant symptom was pain in the knee (85.71%). The most common lesion of the meniscus was a horizontal split, primarily in patients older than 18 years. Patients also presented with joint intra-articular lesions; the most common type was ACL rupture. In all patients, an improved postoperative IKDC score was reported.

Key words: *discoid meniscus, knee arthroscopy, saucerization*

SAŽETAK

Diskoidni meniskus je morfološka anomalija lateralnog menisusa koja se srece u 1 – 5% meniscektomija. I dalje postoje poteškoće u preciznoj i tačnoj dijagnostici kao i dileme o najboljem načinu rešavanja

Pokazati učestalost diskoidnih meniskusa kod pacijenata kojima je rađena artroskopija kolena, tipove diskoidnog meniskusa, prateće tegobe, udružene lezije kolena, postoperativni rezultat.

Retrospektivna studija je obuvatila 1357 pacijenata kojima je u periodu od januara 2007. godine do decembra 2013. godine rađena artroskopija kolena. Analizirana je incidenca diskoidnog meniskusa, raspodela prema polu, tipu (Monllau klasifikacija), notirana je preoperativna simptomatologija, incidenca i tip raskida (O'Conor klasifikacija), korelacija prisustva anomalije i drugih intraartikularnih lezija. IKDC skor je korišćen radi procene rezultata operativnog lečenja.

Incidenca javljanja diskoidnog meiskusa bila je 1.03%. Kod 5 (35.71 %) pacijenta je bio kompletni tip diskoidnog meniskusa. Dominantni simptom bio je bol - 12 (85.71%) pacijenata. Raskid je postojao kod 11 (78.57 %) pacijenata i to najčešće horizontalni 4 (36.36%) slučaja. Značajno više raskida kod starijih od 18 godina - 9 (90%) u odnosu na mlađe od 18 goidna - 2 (40%). Operativno lečenje dovelo je do poboljšanja kod svih pacijenta u smislu subjektivnog smanjenja tegoba.

Incidenca javljanja DLM-a u našoj studiji je 1, 03% od čega je 35.71% kompletan tip DLM. Dominantan simptom je bio bol u kolenu (85, 71%). Najčešća lezija meniskusa je bio horizontalni rascep i to prevashodno kod pacijenata starijih od 18 godina. Više je bilo pacijenata sa uduženom intraartikularnom lezijom, najčešća je bila ruptura LCA. Kod svih pacijenata došlo je do popravljanja IKDC skora postoperativno.

Ključne reči: *diskoidni meniskus, artroskopija kolena, saucerizacija*

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ABBREVIATIONS

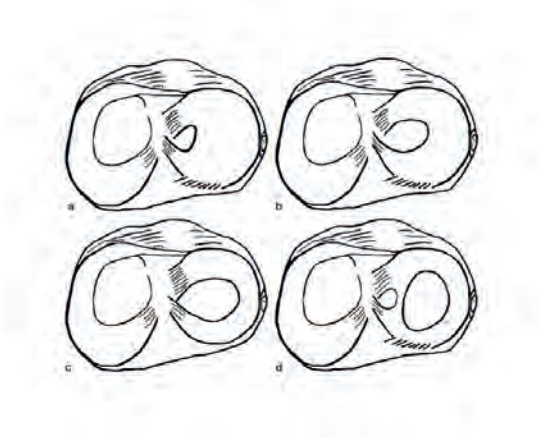
DLM - Discoid lateral meniscus
IKDC - International Knee Documentation Committee
LCA (ACL) - Ligamnetum cruciatum anterior
SPSS - Statistical Package for the Social Sciences
MRI - Magnetic resonance imaging

INTRODUCTION

Discoid lateral meniscus (DLM) represents the most common structural and morphological anomaly of the knee meniscus.(1, 2) The first DLM description was reported by Young in 1889. (3) Thus far, research has indicated the existence of clear racial differences in the incidence of this morphological anomaly, with the Asian population exhibiting the highest incidence (from 9.1% to 16.6%), while the European population demonstrates a somewhat lower incidence of DLM (from 0.4% to 5%). (4, 5, 6) In 1979, Watanabe et al. established a classification system in which DLM was divided into three groups: complete (a), incomplete (b) and Wrisberg type (c), and, in 1998, Monllau et al. suggested adding "ring-shaped" lateral meniscus (d) as the fourth group in this classification. (7, 8) The most common lesions are complete and incomplete, which are usually asymptomatic and represent incidental findings unless combined with meniscus lesions.

The imprecise symptomatology and common asymptomatic presentation of DLM renders this meniscus anomaly difficult to diagnose. Common symptoms that occur in DLM cases include pain, popping and movement limitation related to knee extension. Ultrasonographic imaging of the menisci may demonstrate a wide and irregularly shaped lateral discoid meniscus in type 1 and 2 discoid menisci with an overall accuracy of more than 70%. (9, 11). Magnetic resonance imaging (MRI) is widely used to diagnose musculoskeletal pathologies affecting the knee. The most accurate criterion for the diagnosis of discoid meniscus using MRI is the ratio of the minimal meniscal width to maximal tibial width. This ratio provided a sensitivity and specificity of 95% and 97%, respectively, even when torn menisci were present. (10, 17)

Discoid lateral meniscus is a common incidental finding during arthroscopy of the knee. In 1957, Kaplan recommended the total meniscectomy of the discoid meniscus (31). Long-term follow-up points to a greater prevalence of osteoarthritis changes after total meniscectomy, and many authors have since recommended partial arthroscopic meniscectomy. (13, 14, 15, 34, 35) Rosenberg et al. (1987) described a case in which a Wrisberg ligament type of DLM was reattached peripherally after central partial meniscectomy (32). The currently used treatment method consists of f arthroscopically assisted partial meniscectomy. DLM remodelling, including processing of all unstable and separated meniscus parts (saucerization), must be performed. (16, 17, 18) The treatment of asymptomatic DLM by partial meniscectomy and saucerization is not recommended because the knees of such patients are considered to be biomechanically adapted to DLM; therefore, the disturbance of such biomechanics by partial meniscectomy would favours the more rapid occurrence of degenerative knee changes. (19, 38)



Monllau classification of DLM [8]

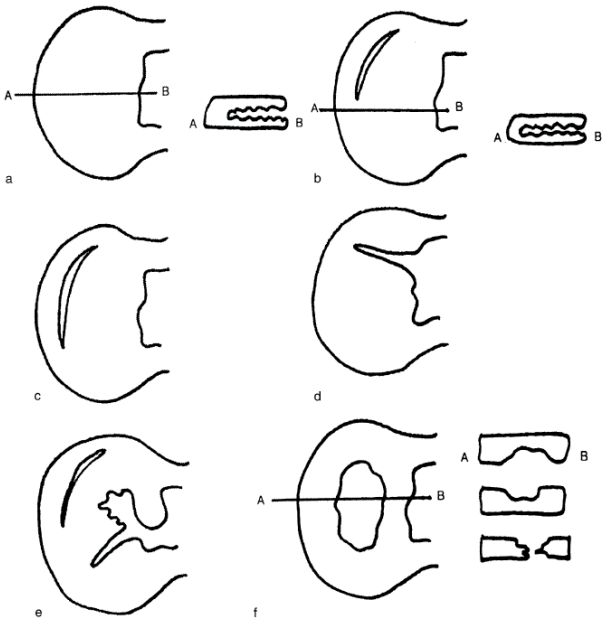
AIM

Because doubts remain regarding the diagnosis and treatment of these rare anatomical anomalies, the aim of this paper was demonstrate the frequency of DLM in our patient population, diagnosed types of DLM, associated symptoms, associated intra-articular knee lesions, and results of partial meniscectomy.

MATERIAL AND METHODS

This retrospective study was conducted in IOS "Banjica" from January 2007 to December 2013, and we analysed 1357 patients who underwent arthroscopic knee surgery. Symptoms such as joint pain, popping, and movement limitations and McMurray test results were noted preoperatively in medical documents.

Indications for arthroscopic surgery were defined after presenting each patient to the Department Consulting Body and were based on subjective problems, clinical results and additional diagnostic procedures. Standard knee plain roentgenograms were performed in all cases, and MRI was performed for 8 patients.



O'Connor's classification of DLM ruptures: a) simple horizontal, b) combined horizontal, c) longitudinal rupture, d) transverse rupture, e) multiple rupture, f) central rupture [**17**]

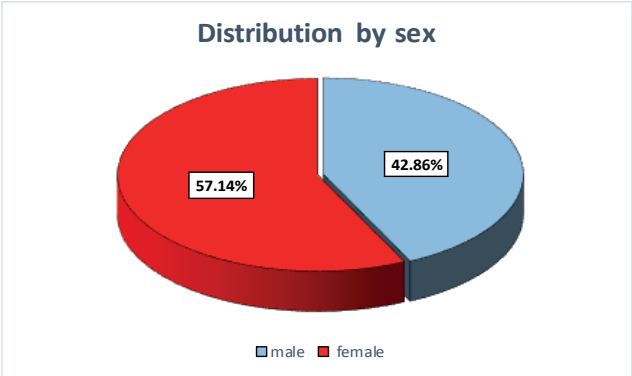
The demographic data were obtained from the institute's medical documents. The discoid lateral meniscus type, as well as lesion type (if any was observed), was noted postoperatively in medical documents. All patients with diagnosed DLM received saucerization, i.e., partial meniscectomy.

We analysed the DLM incidence, DLM distribution according to Monllau's classification (8), patient sex, preoperative symptoms, and type of DLM rupture according to O'Connor classification (17), as well as the correlation between the presence of DLM and other intra-articular lesions.

Patients were included in the study according to the order in which they were admitted to the hospital and underwent surgery. The criterion for inclusion was the intraoperative finding of DLM. All patients were regularly invited to check-ups by the end of rehabilitation. The data were statistically analysed in SPSS software, which was used for descriptive statistics. The obtained results are shown as graphs.

RESULTS

Out of 1357 total arthroscopies performed in IOS "Banjica" during the period from January 2007 to December 2013, DLM was diagnosed in 14 (1.03 %) patients. On average, the patients were 23 years old (from 10 to 43). Six patients were male (42.86%), and 8 were female (57.14%). (Graph 1)



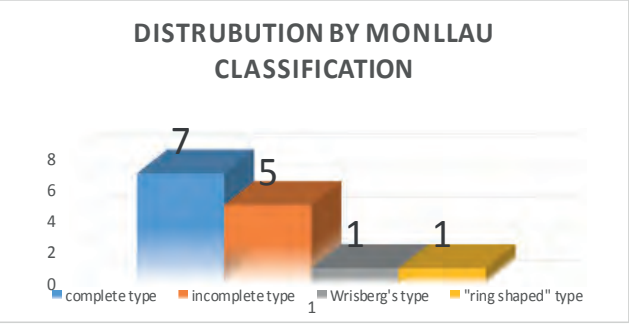
Graph 1. Patient distribution by sex

According to Monllau's classification (8), out of 14 diagnosed DLMs, 7 cases (50%) were complete, 5 (35.71 %) incomplete, 1 (7.14%) Wrisberg type and 1 (7.14%) "ring-shaped" type. In 8 (57.14 %) cases, DLM was diagnosed in the right knee, while DLM was present in 6 (42.86%) left knee cases. (Graph 2)

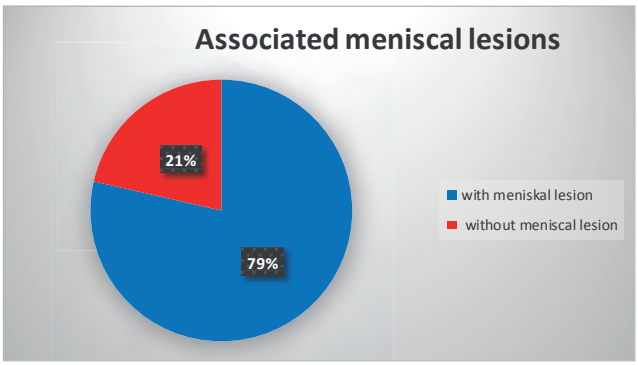
The anamnestic data on knee trauma was positive for 8 (57.14%) patients. Pain was the dominant symptom that was present in 12 patients (85.71%), and these individuals reported palpation painful sensitivity in the projection of the lateral joint gap. Popping and occasional knee movement limitations were present in 9 (64.28%) patients. On average, 4 months passed between the onset of symptoms to arthroscopy.

Out of 14 diagnosed DLMs, 11 (78.57 %) cases were combined with meniscus lesions. Using O'Connor's classification, we diagnosed 4 (36.36%) horizontal fissures, 3 (27.27%) vertical fissures, 2 (18.18 %) radial fissures, 1 (9.09%) combined rupture and 1 (9.09%) multiple rupture. In patients over the age of 18, combined DLM lesions were diagnosed more often (9 patients; 90%) than in patients younger than 18 years of age (2 patients; 40%). *This difference demonstrated strong statistical significance ($p < 0.0005$).* (Graph 3 & Graph 4)

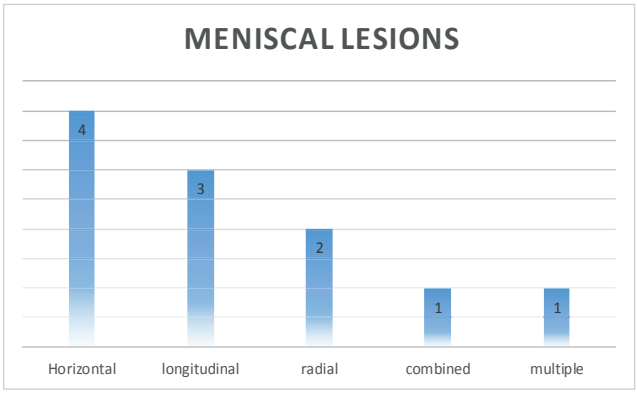
In 5 (35.71%) cases, another combined intra-articular lesion besides DLM was found: in 3 (21.42%) cases, lesions of the anterior cruciate knee ligament were observed; 1 (7.14 %) patient was diagnosed with chondral lesion and 1 (7.14%) individual exhibited both damage of the anterior cruciate ligament and chondral lesion. (Graph 5 & Graph 6)



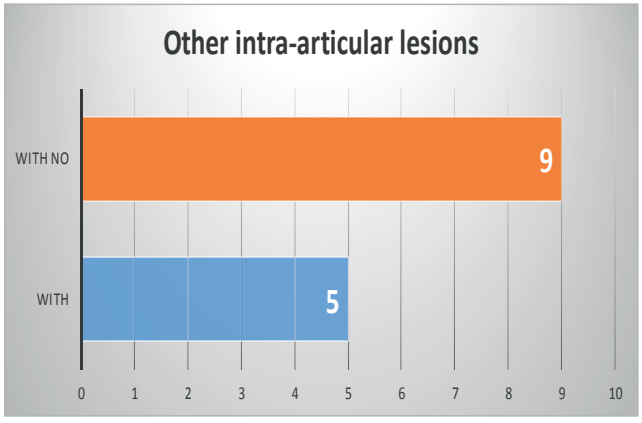
Graph 2. Patient distribution by Monllau classification



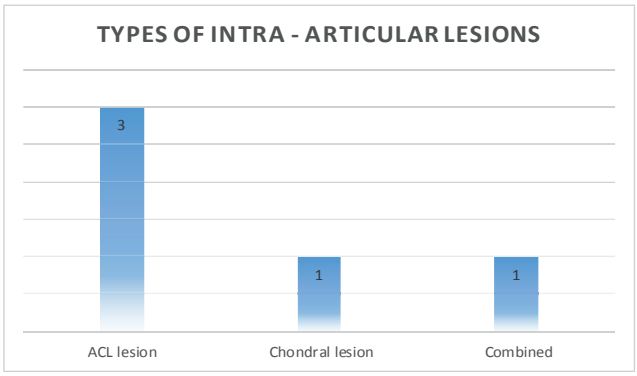
Graph 3. Incidence of associated meniscal lesions



Graph 4. Type of meniscal lesion



Graph 5. Incidence of other intra-articular lesions



Graph 6. Type of intra-articular lesion

On average, the obtained results were collected over 6 months of monitoring (from 3 to 9 months). All patients recovered successfully after rehabilitation, and they continued their normal life activities. The average preoperative IKDC score (**20**) of all patients was 70; postoperatively, the average patient IKDC score improved to 90. No complications were observed during intervention and the immediate postoperative course, and no other delayed complications were reported.

DISCUSSION

The cause of discoid menisci has been a matter of considerable discussion. In 1948, Smillie (21) postulated that the discoid shape is a normal stage in the developing embryo and that failure of absorption of the central portion will persist during the foetal state. Kaplan (22) claimed that insufficient posterior meniscal attachment to the tibia causes increased meniscal excursion during flexion and extension and that repetitive microtrauma subsequent to this increased mobility produces morphological changes. Complete and incomplete types of DLM could result from the cessation of absorption during foetal life, while the Wrisberg type is mainly hypermobile and lacks the posterior attachment to the tibial plateau.

The incidence rate of 1.03% recorded in our study falls in the incidence range 0.4–5% reported by arthroscopic studies in the Caucasian population. (6, 23, 24) Much higher incidence rates have been reported among Indian (5.8%), Korean (9.1–10.5%) and Japanese (16.6%) patients (25, 26, 4, 5). The reasons for this prevalence distribution are unknown.

No statistically significant differences were observed in terms of the sex of individuals diagnosed with DLM (8:6 in favour of females). In a Swedish report by Abertsson et al., of 29 patients with DLM, 11 were male and 18 female.(23) In another study performed in India by Sriptahi et al., 41 male and 46 female patients (out of 87 patients) with DLM were studied. (9) Thus, these studies also failed to find a significant difference in the male-to-female ratio.

We use Monllau's classification (8) of the DLM type in our study, and the complete type was the dominant one (7 cases; 50%), followed by incomplete (5 cases; 35.71%) and then by Wrisberg type (1 case; 7.14%) and "ring-shaped" meniscus (1 case; 7.14%). In a Greek study analysing discoid dysmorphy among 39 patients with DLM, 23 cases were attributed to complete type DLM, 15 were incomplete and Wrisberg type was observed in one case. (6) A Scandinavian report on this subject indicated that the complete and incomplete types of DLM were dominant (23). In their study, Sriptahi et al. reported 69.4% complete type, 26.3% incomplete type, 4.2% "ring-shaped" discoid lateral menisci and no Wrisberg ligament type cases (9). Fabrizio et al. and O. Ahmet Atay et al. also reported similar incidences. (27, 28)

All patients in our study diagnosed with DLM reported preoperative problems involving popping, pain, occasional swelling and knee blockade. The dominant symptom was knee pain in 85.71% of individuals. Palpatory pain in the

projection of the lateral joint line was more frequent than knee popping, but no statistically important differences were observed in distribution. Palpatory pain and knee pain in general could be an indirect indicator of DLM lesions; therefore, the incidence of combined meniscus lesions with DLM findings was 85.72 %. Papadopoulos at al. also found knee pain to be the dominant symptom in 90% of cases. (6, 36, 37, 39)

Changes in the morphology and vascularization of DLM render the condition more prone to mechanical stress; therefore, arthroscopic findings of combined meniscus lesions are relatively common. (33) We detected DLM combined with lesions of the meniscus itself in 11 (85.72%) cases, and the incidence was statistically higher in patients over the age of 18 (90%). In our study, horizontal, vertical, radial, and transversal type lesions were present. Interestingly, only slightly more than half of the patients (57.14%) mention earlier knee trauma. Similar results were reported by Sripathi et al. (9)

Partial meniscectomy was performed on all patients because symptomatic DLM was identified. After the end of treatment and a standard rehabilitation protocol for meniscectomy, all patients reported subjective improvements in knee problems. The patients' IKDC score results were higher after surgery.

In 5 (35.71%) of our patients, in addition to DLM, combined lesions of the anterior cruciate ligament (ACL) in were diagnosed 3 patients (21.42%), chondral lesions in 1 (7.14 %) patient, and both ACL damage and chondral lesions in 1 (7.14%) patient.

CONCLUSION

The incidence of DLM in our study was 1.03%, and the dominant symptom was pain in the knee (85.71%). The most common lesions of the meniscus were a horizontal split, primarily in patients older than 18 years. Certain patients also presented with joint intra-articular lesions, the most common of which was ACL rupture. All patients reported improved IKDC scores after surgery.

The frequency of meniscal malformations in the population is difficult to determine because a number of these cases are asymptomatic. This study evaluated the incidence of DLM in knee arthroscopy and not the incidence in the general population. Arthroscopy remains an invasive surgical method. MRI screening may represent a better means of precisely determining the incidence of these anomalies in the general population.

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THE ANALYSIS OF ANTIBIOTIC CONSUMPTION AND BACTERIAL RESISTANCE AS AN INDICATOR OF THEIR PROPER USE AT THE UROLOGY DEPARTMENT IN THE HEALTH CENTRE "STUDENICA" KRALJEVO

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ANALIZA POTROŠNJE ANTIBIOTIKA I BAKTERIJSKE REZISTENCIJE KAO INDIKATORA NJIHOVE RACIONALNE UPOTREBE NA ODELJENJU UROLOGIJE ZDRAVSTVENOG CENTRA "STUDENICA" U KRALJEVU

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ABSTRACT

The objective of the study was to analyze antibiotic consumption and determine bacterial resistance rates as an indicator of the rational utilization of this drug group at the urology department in the Health Centre "Studenica" Kraljevo.

Over a two-year period, the average antibiotic consumption was 104.55 DDD/100BD. Of the total financial assets used for medical treatment, the antibiotic group JO1 had a share of 49.52% in 2011 and 47.53% in 2012. Antibacterial drugs from a group of β-lactamic antibiotics were consumed most commonly, at 54.02% (2011) and 43.44% (2012). First-generation cephalosporins, quinolones and aminoglycosides were the most frequently used drug groups, while cephalexin was the antibiotic with the highest individual consumption. E. coli was the most frequently isolated bacterium in 2011, while in 2012, Klebsiella pneumoniae was the most frequently isolated bacterium. The total bacterial resistance both in 2011 and 2012 was above 50%. Gram-negative bacteria showed a higher resistance rate (2011, 59.3%; 2012, 58.9%) than Gram-positive bacteria (2011, 44.4%; 2012, 40.6%). Klebsiella pneumoniae was the bacterium with the highest resistance (75.3%) in 2011, while in 2012, there was a resistance increase in Pseudomonas aeruginosa (71.4%), especially to carbapenems. A correlation was determined between the consumption of individual antibiotics and bacterial strain resistance in 2011 (r=0.433, p=0.044) and in 2012 (r=0.478, p=0.024).

The high resistance rate shown in the bacterial strains, which was correlated with antibiotic consumption, as well as the great financial assets used for this group of drugs suggest the necessity for the rationalization of their utilization. Empirical therapy with Gram-negative bacteria should be based on carbapenems utilization, except with Pseudomonas aeruginosa, where piperacillin/tazobactam should be used.

Key words: *antibiotics, bacterial resistance, DDD methodology, urology departments.*

SAŽETAK

Cilj ovog istraživanja je analiza potrošnje antibiotika i utvrđivanje stepena rezistencije bakterijskih sojeva kao indikatora racionalne primene ove grupe lekova na odeljenju urologije Zdravstvenog centra "Studenica" u Kraljevu.

U toku dvogodišnjeg perioda prosečna potrošnja antibiotika iznosila je 104,55 DDD/100BD. Od ukupnih novčanih sredstava utrošenih za medikamentoznu terapiju na antibiotike grupe J01 izdvojeno je 49,52% u 2011. odnosno 47,53% y 2012. Antibakterijski lekovi iz grupe β-laktamskih antibiotika beleže najveći udeo u potrošnji sa 54,02% (2011) i 43,44% (2012). Cefalosporini prve generacije, hinolonski antibiotici i aminoglikozidi su najčešće korišćene grupe antibiotika, a cefaleksin je antibiotik sa najvećom pojedinačnom potrošnjom. E. Coli je bila najčešće izolovana bakterija u 2011, dok je u 2012. Klebsiella pneumoni bila najzastupljenija. Ukupna rezistencija bakterijskih sojeva je u 2011. i u 2012. bila veća od 50%. Gram negativne bakterije su pokazale veći stepen rezistencije (2011-59,3%, 2012- 58,9%) u odnosu na Gram pozitivne (2011-44,4%, 2012-40,6%). Klebsiella pneumoni je bakterija sa najvećom rezistencijom u 2011. (75,3%), dok se u 2012. beleži porast rezistencije Pseudomonas aeruginosa (71,4%), posebno na karbapeneme. Utvrđena je korelacija potrošnje pojedinačnih antibiotika i rezistencije bakterijskih sojeva u 2011. (r=0,433, p=0,044) i 2012. (r=0,478, p=0,024).

Visok nivo rezistencije koji pokazuju bakterijski sojevi, a koji je u korelaciji sa potrošnjom antibiotika, kao i velika novčana sredstva koja se izdvajaju za ovu grupu lekova, sugerišu na potrebu racionalizacije njihove primene. Empirijska terapija kod Gram negativnih bakterija bi trebalo da se zasniva na primeni karbapenema, osim kod Pseudomonas aeruginosa gde prednost treba dati piperacilin/tazobaktamu.

Ključne reci: *antibiotici, bakterijska rezistencija, DDD metodologija, urologija.*

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ABBREVIATIONS

ATC – Anatomical Therapeutic Chemical;
DDD – defined daily dose;
WHO – World Health Organization;
SPSS – Service Provisioning System Software;
BD – Bed-days;
ALIMS – Medicines and Medical Devices Agency of Serbia;
INN – International Nonproprietary Names.

INTRODUCTION

The antibiotic share of a hospital's consumption of all prescribed drugs goes up to 30%, which makes them one of the most frequently used groups of drugs (1, 2). This study showed that 22.9% of inpatients used at least one antibiotic. Of those patients, 5.1% had two antibiotics included in the therapyand 1.1% used three antibiotics. With 37.4% of patients, antibiotic therapy was inappropriate because it was unjustified; the choice of antibiotic was wrong or there was a utilization mistake (2). Inappropriate antibiotic utilization is one of the main reasons for microorganism resistance which, aside from increasing hospitalized treatment costs, leads to more serious consequences, such as an increase in morbidity and mortality rates (3, 4). The utilization of amoxicillin/clavulanate and quinolones was more frequently inappropriate, while the utilization of small-therapeutic-amplitude penicillins, cephalosporins, meropenem, metronidazole, and rifampicin was significantly appropriate. The inadequacy of use was connected with both the type of antibiotic and the specialist department. Urology, nephrology, otolaryngology and geriatrics were the specialist departments with risk factors for inappropriate antibiotic utilization, while paediatrics was isolated because of a significantly high rate of adequate antibiotic use (2).

Approximately 56% of the hospitalized patients at the urology department used antibiotics. According to the study, the highest percentage of patients at a urology department that used antibiotics was recorded in Turkey (approximately 70%), whereas in Hungary (49%) and Germany (47%), this number was statistically significantly lower (5), which suggests the important regional differences in the utilization of these drugs. A multicenter centre study conducted in European countries between 2003 and 2010 showed an antibiotic resistance increase in urologic patients. Imipenem was the only antibiotic whose resistance to the total bacterial spectrum was below 10% (5). *Escherichia coli*, the most frequently isolated bacterium in urologic patients, had a significant resistance rate to ampicillin, amoxicillin + clavulanic acid and fluoroquinolones (6). Monitoring and analysis of antibiotic consumption are highly important for antibiotic use rationalization in urologic patients. Due to differences in demographic characteristics, this monitoring should be conducted at regional and local levels (6).

Taking into consideration the increased bacterial strain resistance isolated in urologic patients (5), the existence of risk for inadequate antibiotic use at the urology department (2) and the necessity for local analysis of antibiotic consumption (5), the aim of this paper was to:

- analyze the individual antibiotic presence at the urology department;
- determine the presence and resistance rate expressed by bacterial strains isolated from the clinical material of inpatients at this department;
- determine the correlation between antibiotic consumption and bacterial resistance rate; and
- compare the given results with the available results of similar studies in our country and region.

MATERIAL AND METHODS

This study was based on data collected in the Health Centre "Studenica" that provides primary health care at the Health Centre Kraljevo and secondary health care in the General Hospital Kraljevo. The General Hospital has 580 beds and provides secondary health care for approximately 250,000 patients.

In accordance with the Anatomical Therapeutic Chemical (ATC) Classification System, the analysis was conducted on the antibiotic groups for systemic use, J01, used between January 2011 and December 2012 at the urology department. The data concerning total patient number and average hospitalization length at the urology department were obtained from the planning and analysis department. The central pharmacy provided the data concerning antibiotic consumption at the urology department, while bacteriological analysis results of samples were obtained from The Institute of Public Health.

All antibiotics were classified according to the ATC Classification System and their generic names; their total consumption was calculated and presented in units of measurement (mg and ij) which were then presented in DDD per 100 bed-days (BD), using the ATC/DDD methodology of the World Health Organization (WHO) (8).

The sensitivity of all of the bacterial strains isolated from the clinical material of hospitalized patients was estimated by the disc-diffusion method on Mueller-Hinton Agar.

During the study, data on individual patients were not collected. Thus, it was determined that approval by the Ethical Committee was not necessary.

The collected data were statistically analyzed. The descriptive statistical data were expressed as a percentage or as the arithmetic mean with the standard deviation. The data distribution normality was checked by the Kolmogorov-

Table 1: The systemic use of different pharmacotherapeutic groups of antibiotics in the department of urology.

ATC*	Pharmacotherapeutic group of antibiotics	2011		2012	
		DDD/100BD#	%	DDD/100BD#	%
J01C	Penicillins	3.18	3.09	2.90	2.73
J01DB	Cephalosporins 1st generation	28.58	27.81	24.41	22.95
J01DC	Cephalosporins 2nd generation	15.21	14.80	7.10	6.68
J01DD	Cephalosporins 3rd generation	6.74	6.56	10.78	10.13
J01DE	Cephalosporins 4th generation	0.00	0.00	0.00	0.00
J01DH	Carbapenems	1.80	1.75	1.01	0.95
J01E	Sulfonamides	1.81	1.76	11.23	10.56
J01F	Macrolides	1.78	1.73	0.87	0.82
J01G	Aminoglycosides	18.33	17.84	21.66	20.37
J01M	Quinolones	19.44	18.92	19.17	18.03
J01XA	Glycopeptides	0.06	0.06	0.12	0.11
J01A	Tetracyclines	0.07	0.07	0.47	0.44
J01XD	Imidazole derivatives	5.67	5.52	6.63	6.23
J01B	Amphenicol	0.09	0.09	0.00	0.00
In total		102.76	100	106.35	100

* Anatomical Therapeutic Chemical; # Defined daily dose/100 bed-days.

Smirnov and Shapiro-Wilk tests. Student's t-test i.e., and its non-parametric alternative, Mann-Whitney U test, were used for determining statistically significant differences between the values of two continuous variables. The comparison between categorical variables was conducted using the Chi-square test. The relationship between antibiotic consumption and bacterial resistance was determined using Pearson's correlation. A value of $p<0.05$ was considered statistically significant. All statistical analyses were performed with the computer programme SPSS, version 18.

RESULTS

The urology department of the General Hospital Kraljevo has 22 beds. In 2011, there were 1484 hospitalized patients with an average hospitalization length of 4.83 days; therefore, the number of achieved bed-days was 7167 (BD). In 2012, there were 6326 bed-days. The number of hospitalized patients for this year was 1451, and the average hospitalization length was 4.36 days.

Of the total financial assets used for medical treatment in this department in 2011, antibiotics had a share of 49.52%, while in 2012 that share was 47.53%.

During this two-year period, the average antibiotic consumption was 104.55 DDD/100BD. The increase of total antibiotic consumption from 102.76 DDD/100BD in 2011 to 106.35 DDD/100BD in 2012 was not statistically significant ($p=0.645$, $U=81.000$). Antibacterial drugs for systemic use that belonged to the β-lactamic group were consumed most frequently at: 54.02% (2011) and 43.44% (2012). The decreased consumption of β-lactamic antibiotics by slightly more than 10% was not statistically significant ($p=0.781$, $t=0.288$). The consumption of first-generation cephalosporins was the highest when compared to other drug groups. The second most frequently consumed antibiotic was quinolones in 2011, and aminoglycosides in 2012 (Table 1).

During the monitored period at the urology department, 24 generically different antibiotics were used. During

Table 2: Ranking of the antibiotics composing 90% of the total DDDs prescribed in the department of urology.

ATC*	INN¥	2011		2012	
		DDD/100BD#	%	DDD/100BD#	%
J01DB01	Cephalexin	25.34	24.66	18.59	17.48
J01MA02	Ciprofloxacin	12.95	12.60	14.74	13.86
J01GB06	Amikacin	9.91	9.64	13.20	12.41
J01EE01	Co-trimoxazole	/	/	11.23	10.56
J01DD04	Ceftriaxone	6.25	6.08	10.43	9.81
J01GB03	Gentamicin	8.42	8.19	8.46	7.95
J01DC02	Cefuroxime	15.21	14.80	7.10	6.68
J01XD01, P01AB01	Metronidazole	5.67	5.52	6.63	6.23
J01MB04	Pipemidic acid	6.49	6.32	4.43	4.17
In total		90.24	87.82	90.38	89.15

* Anatomical Therapeutic Chemical; # Defined daily dose/100 bed-days; ¥ International Nonproprietary Names.

Table 3: Resistance of all of the isolates to individual antibiotics in the department of urology.

Antibiotics	Resistance N (%)	
	2011	2012
Amikacin	105 (57.1)	63 (57.8)
Amoxicillin	6 (31.6)	5 (29.5)
Amoxicillin/clavulanate	81 (54.7)	39 (40.2)
Ampicillin	142 (78.9)	73 (70.2)
Cefaclor	4 (40.0)	2 (20.0)
Cefalexin	9 (64.3)	17 (53.1)
Cefepime	2 (100)	4 (80.0)
Cefixime	101 (67.3)	58 (64.4)
Cefotaxime	105 (66.9)	57 (65.5)
Ceftazidime	18 (46.2)	20 (66.7)
Cefrixsone	7 (70.0)	13 (76.5)
Ciprofloxacin	169 (81.6)	108 (85.0)
Erythromycin	0 (0.0)£	0 (0.0)£
Fusidic acid	1 (100)	/
Gentamicin	140 (71.4)	79 (66.4)
Chloramphenicol	1 (50.0)	1 (33.3)
Imipenem	20 (10.7)	16 (14.7)
Meropenem	17 (9.1)	17 (15.3)
Clindamycin	0 (0.0)£	/
Nalidixic acid	/	0 (0.0)£
Norfloxacin	170 (75.9)	105 (77.2)
Ofloxacin	108 (68.4)	52 (71.2)
Penicillin G	8 (30.8)	9 (33.3)
Pipemidic acid	/	18 (75.0)
Piperacillin	127 (73.8)	69 (70.4)
Piperacillin/tazobactam	12 (22.6)	12 (35.3)
Tetracycline	16 (69.6)	13 (76.5)
Co-trimoxazole	8 (57.1)	18 (60.0)
Vancomycin	0 (0.0)	0 (0.0)
Ertapenem	0 (0.0	/
In total	1377 (57.8)	868 (56.6)

£ One to two probes

both years, the same antibiotics comprised 90% of the total antibacterial drugs for systemic use, except cotrimoxazole, which was not included in 2011. Cephalexin was the antibiotic with the highest individual consumption (Table 2).

The total bacterial resistance was higher than 50% both in 2011 and in 2012. Table 3 shows the resistance for each individual antibiotic.

In 2011, 218 isolates (urine, 98.6%) from the urology department were analyzed. Twenty different species of bacteria were isolated; two bacteria were isolated from each of 13 isolates. *Escherichia coli* was the most frequently isolated bacterium. In 2012, 141 isolates (urine, 98.7%) were processed. Two bacteria were isolated from each of 9 isolates. *Klebsiella pneumoniae* was the most frequently isolated from 14 different isolated species of bacteria.

Gram-negative bacteria showed a higher resistance (2011, 59.3%; 2012, 58.9%) rate than Gram-positive bacteria (2011, 44.4%; 2012, 40.6%). The lowest level of Gram-negative bacterial resistance was to carbapenems, whereas Gram-positive bacteria were sensitive to glycopeptide antibiotics. Table 4 shows the resistance of the most frequently isolated bacteria.

There was a correlation between the consumption of individual antibiotics and bacterial strain resistance in 2011 ($r=0.433$, $p=0.044$) and in 2012 ($r=0.478$, $p=0.024$). There was no statistically significant correlation between antibiotic consumption during 2011 and bacterial resistance in 2012 ($r=0.367$, $p=0.093$).

DISCUSSION

One of the main objectives of the study was to determine which antibiotics were used most commonly at the urology department and whether their consumption correlated significantly with bacterial strain resistance rates.

The consumption of all antibiotics for systemic use was analyzed up to level five of the ATC/DDD classification, and then, a comparison was performed. All antibiotics used at the urology department and included in the study belonged to group J01, except metronidazole for oral use, as it belongs to group P (antiparasitic products). The average antibiotic consumption during the two-year period was 104.55 DDD/100BD, which was significantly lower than consumption at the urology departments of the Surgical Clinics in Niš and Novi Sad. The results of a two month study in 2004 showed that the consumption of group J anti-infective drugs at the urology department was 263.54 DDD/100BD in Niš and 224.85 DDD/100BD in Novi Sad (9). Lower antibiotic utilization at the urology department in Kraljevo compared with antibiotic utilization in Niš and Novi Sad was evident, even though our study included only J01 antibiotics because they were consumed the most frequently consumed antibiotic from group J (10). The average consumption of antibiotics for systemic use at the Surgical Clinic of Clinical Hospital Centre – Priština in Gračanica from 2007 to 2008 was 124.22 DDD/100BD (11), which was closer to the results of our study than to the study conducted in Niš and Novi Sad (9). According to our data, cephalosporins, quinolones and aminoglycosides had the highest utilization. In Niš and Novi Sad, cotrimoxazole had the highest consumption, followed by quinolones and aminoglycosides in Niš and cephalosporins and aminoglycosides in Novi Sad (9). The study of the Medicines and Medical Devices Agency of Serbia (ALIMS) showed that from 2004 to 2006 in health institutions in Serbia, penicillin with an extended spectrum (J01CA) was consumed

Table 4: Resistance within the most frequently isolated bacteria.

Antibiotics	Resistance N (%)							
	Escherichia coli		Klebsiella pneumoniae		Proteus mirabilis		Pseudomonas aeruginosa	
	2011	2012	2011	2012	2011	2012	2011	2012
Amikacin	9 (28.1)	2 (12.5)	24 (64.9)	16 (43.2)	14 (53.8)	16 (72.7)	27 (71.1)	25 (86.2)
Amoxicillin	/	0 (0.0)[£]	/	/	/	/	/	/
Amoxicillin/clavulanate	5 (20.8)	1 (5.9)	23 (63.9)	17 (45.9)	18 (72.0)	17 (70.8)	4 (100)	1 (100)
Ampicillin	27 (69.2)	15 (68.2)	39 (100)	29 (93.5)	26 (86.7)	18 (81.8)	3 (100)	2 (100)
Cefalexin	1 (50.0)	2 (28.6)	/	7 (100)	0 (0.0)[£]	3 (75.0)	/	/
Cefepime	/	/	/	0 (0.0)[£]	/	/		2 (100)
Cefaclor	/	1 (100)	/	/	/	/		/
Cefixime	6 (16.2)	4 (17.4)	36 (94.7)	32 (84.2)	20 (69.0)	19 (76.0)	4 (100)	1 (100)
Cefotaxime	6 (15.8)	2 (10.0)	37 (94.9)	29 (90.6)	18 (64.3)	17 (73.9)	4 (100)	1 (33.3)
Cefriaxone	0 (0.0)[£]	1 (33.3)	1 (100)	5 (100)	0 (0.0)[£]	1 (33.3)	/	/
Ceftazidime	/	/	/	/	/	/	15 (45.5)	17 (63.0)
Ciprofloxacin	14 (42.4)	9 (52.9)	38 (100)	33 (89.2)	20 (76.9)	19 (86.4)	35 (94.6)	30 (100)
Fusidic acid	1 (100)	/	/	/	/	/	/	/
Gentamicin	15 (35.7)	4 (17.4)	32 (84.2)	26 (74.3)	16 (57.1)	16 (64.0)	32 (91.4)	27 (96.4)
Chloramphenicol	/	/	/	/	/	1 (100)	1 (100)	/
Imipenem	0 (0.0)	0 (0.0)	2 (5.3)	0 (0)	3 (11.5)	0 (0)	11 (28.2)	15 (50.0)
Meropenem	0 (0.0)	0 (0.0)	2 (5.3)	0 (0)	0 (0.0)	0 (0)	11 (28.9)	14 (48.3)
Ofloxacin	12 (30.0)	8 (44.4)	37 (94.9)	27 (96.4)	19 (70.4)	15 (75.0)	5 (83.3)	1 (100)
Norfloxacin	14 (33.3)	10 (41.7)	38 (97.4)	34 (89.5)	20 (69.0)	18 (75.0)	35 (94.6)	28 (100)
Nalidixic acid	/	0 (0.0)[£]	/	/	/	/	/	/
Piperacillin	13 (59.1)	5 (38.5)	35 (97.2)	26 (92.9)	20 (83.3)	16 (80.0)	23 (63.9)	18 (60.0)
Penicillin G	/	0 (0.0)[£]	/	/	/	/	/	/
Pipemidic acid	/	4 (57.1)	/	10 (83.3)	/	4 (80.0)	/	/
Piperacillin/tazobactam	0 (0.0)	/	1 (50.0)	/	1 (50.0)	/	8 (23.5)	10 (35.7)
Tetracycline	0 (0.0)[£]	1 (25.0)	/	0 (0.0)	/	0 (0.0)[£]	/	/
Co-trimoxazole	1 (50.0)	2 (25.0)	/	8 (80.0)	2 (66.7)	5 (83.3)	/	/
Ertapenem	0 (0.0)[£]	/	/	/	/	/	/	/
Vancomycin	/	0 (0.0)[£]		/		/		/
In total	124 (29.2)	71 (27.4)	345 (75.3)	299 (65.7)	197 (59.5)	185 (63.1)	218 (62.5)	192 (71.4)
Frequently isolated bacteria N (%)	43 (18.6)	25 (16.7)	39 (16.9)	38 (25.3)	31 (13.4)	26 (17.3)	37 (16.0)	30 (17.3)

[£] One to two probes

most frequently, followed by aminoglycosides and cephalosporins of the third and first generation (10). However, at the urology department of the Health Centre "Studenica" during 2011 and 2012, penicillin (J01C) consumption was only approximately 3% of total antibiotic consumption (Table 1). High consumption of this antibiotic group, as opposed to others from J01, was not recorded at the urology departments in Niš or Novi Sad (9).

In 2011 as well as 2012, cephalexin was the antibiotic consumed most frequently (Table 2). This antibiotic, according to the study by the ALIMS, was included in the ten most frequently used antibiotics in health institutions from 2004 to 2006, but it was not among the first three for its consumption (10). Based on the three-year study conducted in the Clinical Centre Niš, ceftriaxone had the highest consumption in 2003 and 2007, whereas in 2005, ampicillin and ciprofloxacin preceded it. Cephalexin was not among the ten most frequently used antibiotics for its consumption at the Clinical Centre Niš (12). At the Surgical Clinic of the Clinical Hospital Centre – Priština in Gračanica, cephalexin was the third most frequently consumed antibiotic in 2007, after cefuroxime and ceftriaxone, whereas in 2008, both cotrimoxazole and gentamicin had were consumed more than cephalexin (11).

According to our study, the average bacterial resistance both in 2011 and 2012 was above 50% (Table 3). A high resistance rate (70.3%) was also shown in a study that followed the prevalence and resistance rate of urinary tract infection agents in patients treated in the Clinical Centre Kragujevac from 2009 to 2011 (13). As expected, the resistance rate was positively correlated with antibiotic consumption. If we ignored antibiotics that had only one to

two tests, the lowest resistance rate was for carbapenems and glycopeptide antibiotics, i.e., vancomycin. In Table 3, it can be observed that in 2011 there was lower resistance to meropenem and imipenem in 2012. However, both antibiotics had a tendency to increase resistance. The results showing a low resistance rate to vancomycin (Table 3) should be observed with caution because there were few tests of bacterial sensitivity for this antibiotic, and these tests were not conducted on the most frequently isolated bacteria (Table 4). The results of a multicentre study conducted in Europe showed that the resistance rate to all tested antibiotics, except imipenem, was above 10% (6).

Our results showed that, of the four most frequently isolated bacteria, *Escherichia coli* had the lowest resistance rate in the monitored period. This bacterium showed the highest sensitivity to carbapenems and piperacillin/tazobactam, which is in congruence with other studies (12-14). If the number of tests was taken into consideration, *Escherichia coli* developed the highest resistance to ampicillin (69.2%, 2011; 68.2%, 2012) (Table 4). According to a study conducted in a tertiary care hospital in Spain, *Escherichia coli* resistance to ampicillin was 48.1% (7), whereas according to results from Iran, the resistance to the same antibiotic was 63.2% (14). *Escherichia coli* showed higher resistance to ampicillin in the Clinical Centre Kragujevac (13) than in the results of our study. The *Escherichia coli* resistance rate to ampicillin in the Clinical Centre Niš decreased in 2007 (57.61%) as opposed to 2003 (73.08%), but it still remained the antibiotic to which this bacterium showed the highest resistance (12).

In 2011, of all the most frequently isolated bacteria, *Klebsiella pneumoniae* showed the highest resistance, especially to quinolones and amikacin (Table 4). In a ten-year study, this bacterium showed a statistically significant increase in resistance to ceftadizime, ciprofloxacin and cotrimoxazole, whereas the sensitivity to carbapenems remained at a very high level (15), which was supported in our study.

In 2012, there was a resistance increase in *Pseudomonas aeruginosa*. The resistance increase of this bacterium to carbapenems presented a unique problem, which was also seen observed in other studies (13, 16, 17). In our study, *Pseudomonas aeruginosa* had the highest sensitivity to piperacillin/tazobactam, which was in accordance with other studies (18).

CONCLUSION

According to the results of this study, antibiotic consumption (DDD/100BD) at the urology department of the Health Centre "Studenica" was lower than in other areas of the country (9). However, the high resistance rate that the bacterial strains showed, which was in correlation with antibiotic consumption, as well as the great financial assets used for this group of drugs, suggests the necessity for rationalization of their utilization. Gram-negative bacteria were more frequently isolated and had a higher resistance rate than Gram-positive bacteria. Empirical therapy with Gram-negative bacteria should be based on carbapenem utilization, except with *Pseudomonas aeruginosa*, where piperacillin/tazobactam should be used. When choosing antibiotics, empirically or based on the results of an antibiogram, one should consider both pharmacokinetic and pharmacodynamic drug characteristics, individual patient characteristics, and the possibility of interactions with other drugs (19).

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INFLUENCE OF THE NUMBER OF PLATELETS AND HEMOGLOBIN CONCENTRATIONS IN PREDICTING THE DEVELOPMENT OF PROTEINURIA INDUCED BY THE ADMINISTRATION OF BEVACIZUMAB

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UTICAJ BROJA TROMBOCITA I KONCETRACIJE HEMOGLOBINA U PREDVIĐANJU RAZVOJA PROTEINURIJE IZAZVANE PRIMENOM BEVACIZUMABA

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ABSTRACT

Bevacizumab neutralizes a key stimulation factor in tumour angiogenesis - vascular endothelial growth factor (VEGF). The origin of VEGF is related to platelets, and the basic stimulus for its production is hypoxia. By neutralizing VEGF, changes occur in the blood that are manifested at the platelet and haemoglobin levels. Study results indicate that proteinuria is a side effect of bevacizumab treatment. The aim of the present study was to examine the percentages of proteinuria in patients with metastatic colorectal cancer after the administration of bevacizumab. The correlation between the degree of proteinuria and changes in the number of platelets and concentration of haemoglobin in patients, as well as in relation to the age (< 60 i ≥ 60) and gender of the patients, was examined.

The study group of respondents included 20 patients with metastatic colorectal cancer (9 men and 11 women) ranging in age from 43 to 73 years. Proteinuria was tested using "Laboquick" test strips. The levels of platelets and haemoglobin were measured using an autoanalyser, the type Cell Dyn 3700 – Abbott, according to the protocol prescribed by The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

Statistically significant differences were recorded for the platelet measurements before and after therapy in the overall group of respondents (p= 0.025). Statistically significant decreases in platelet counts and increases in haemoglobin concentrations were also noted in women (Plt: p=0.0036; Hb: p=0.0286) and in patients < 60 years old (Plt: p=0.0270; Hb: p=0.0553). No significant differences were identified in men or in patients ≥ 60 years of age. Proteinuria was detected in one case in which demonstrating an increase in only the recorded number of platelets and a decrease in the level of haemoglobin.

Platelets and haemoglobin may serve as prognostic biomarkers of the risk of proteinuria. Based on the initial values of these parameters, we can predict the target group, in terms of gender and age, that may obtain the most benefit from bevacizumab therapy.

Key words: *angiogenesis, bevacizumab, VEGF, platelets, haemoglobin.*

SAŽETAK

Bevacizumab neutrališe ključni faktor stimulacije tumorske angiogeneze vaskularni endotelni faktor rasta (VEGF). Poreklo VEGF-a se vezuje za trombocite, a osnovni stimulans za njegovu produkciju je hipoksija. Neutralizacijom VEGF-a nastaju promene na krvnoj slici manifestovane promenama nivoa trombocita i hemoglobina. Rezultati studija ukazuju na proteinuriju kao neželjeni efekat primene bevacizumaba. Cilj studije je bio ispitati u kom procentu će se pojaviti proteinurija kod ispitanika sa metastatskim kolorektalnim karcinomom nakon primene bevacizumaba. Studija analizira da li postoji korelacija između stepena proteinuruje i promena u broju trombocita i koncentraciji hemoglobina kod ispitanika kao i promene u odnosu na starosnu dob (< 60 i ≥ 60) i pol ispitanika.

Grupa ispitanika je obuhvatala 20 pacijenata sa metastatskim kolorektalnim karcinomom, 9 muškaraca i 11 žena, starosne dobi od 43 do 73 godina. Proteinurija je testirana "Laboquick" test trakama. Nivoi trombocita i hemoglobina su određene na autoanalizatoru tipa Cell Dyn 3700 – Abbott, po protokolu propisanom od strane Međunarodne federacije za kliničku hemiju i laboratorijsku medicinu (IFCC).

Evidentirane su statistički signifikantne razlike u nivoima izmjerenih trombocita pre i nakon terapije u ukupnoj test grupi (p= 0,025). Statistički značajne razlike, u smislu smanjenja broja trombocita i povećanja koncentracije hemoglobina, evidentirane su kod žena (Plt: p=0,0036; Hb: p=0,0286) i kod ispitanika < 60 godina (Plt: p=0,0270; Hb: p=0,0553). Nije bilo statističke značajnosti kod muškaraca i ispitanika ≥ 60 godina. Proteinurija se pojavila kod jednog pacijenta kod kojeg je jedino evidentirano povećanje broja trombocita i smanjenje nivoa hemoglobina.

Trombociti i hemoglobin mogu poslužiti kao prognostički biomarkeri koji mogu ukazati na rizik od pojave proteinurije. Na osnovu inicijalnih vrijednosti ovih parametara može se predvideti koja ciljna grupa pacijenata, u odnosu na pol i dob, može dobiti maksimalnu efikasnu korist od terapije bevacizumabom.

Ključne reči: *angiogeneza, bevacizumab, VEGF, trombociti, hemoglobin.*

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INTRODUCTION

Angiogenesis is the process of creating new blood vessels from existing ones; it is manifested by the activation, proliferation and migration of endothelial cells and is rapidly activated in response to hypoxic or ischaemic conditions. It is of crucial importance during physiological and pathological processes that occur in humans. Angiogenesis is regulated by numerous paracrine growth factors that are divided into stimulators and inhibitors and that are in a constant equilibrium. In response to prevailing factors that cause stimulation, excessive angiogenesis occurs, resulting in pathological processes such as tumour growth, atherosclerosis, chronic inflammatory diseases (rheumatoid arthritis, Crohn's disease), diabetes, psoriasis, endometriosis and adiposity (1). Small tumours with a diameter of less than 2 mm represent so-called dormant tumours and provide nutrients via diffusion. Larger tumours generate their own blood vessels from existing ones in the host through an increase in the expression of growth factors. This elevated expression is further increased under conditions of hypoxia. The blood vessels of tumours are structurally different from those of healthy tissues. They have an irregular shape and inconsistent diameter, are tortuous and permeable, and they are not organized into final venules, arterioles and capillaries. As a result of the irregular structure of tumour blood vessels, the blood flow to the tumour is variable, often providing a sub-optimal supply of nutrients to tumour cells (2). VEGF *(vascular endothelial growth factor)* is a specific mitogen for the vascular endothelium and an important stimulator of angiogenesis (3). Angiogenesis that is conditioned by VEGF enables tumour metastasis. The basic stimulus for the production VEGF is hypoxia, which develops in the tumour (2). Because there is a correlation between anaemia and tumour hypoxia, we can expect an increased level of VEGF in the serum of anaemic patients. Thus, anaemia, i.e., a low level of haemoglobin, can stimulate angiogenesis (4).

Platelets are the main source of VEGF in the blood. VEGF is important in two processes in vertebrates: angiogenesis and haematopoiesis (5). VEGF increases the activity of haematopoietic stem cells and has the capacity to suppress stem cell apoptosis. In this way, platelet production is increased in the bone marrow in response to VEGF (6). Platelets contribute to angiogenesis by releasing VEGF from alpha granules after activation by ADP (7).

Angiogenesis plays a significant role in tumour growth and hence represents an attractive therapeutic goal. In clinical trials, more than 30 substances have been shown to inhibit angiogenesis by neutralizing VEGF, by inhibiting VEGF receptors or by inhibiting matrix degradation (8). The drugs approved by the FDA as anti-angiogenic agents are as follows: the bevacizumab and tyrosine kinase inhibitors sorafenib, sunitinib and pazopanib.

Bevacizumab is a humanized monoclonal IgG antibody (93% human, 7% murine) that was produced by recombinant DNK technology in Chinese hamster ovary cells. The mechanism of action of bevacizumab is based on its binding to VEGF receptors on the surface of endothelial cells. By neutralizing VEGF activity, tumour vascularisation decreases, resulting in regression of the existing tumour blood cells, inhibition of new tumour vasculature and, consequently, a decrease in the tumour size (9). Bevacizumab also normalizes the tumour vasculature, reduces interstitial pressure in the tumour, and thus increases the flow into and effects of chemotherapeutic agents in the tumour (10). Bevacizumab has been approved by the FDA in combination with chemotherapy for the treatment of patients with metastatic colorectal, lung, breast and kidney cancer (11). The safety and effectiveness of the recommended dose of bevacizumab in combination with chemotherapy in metastatic cancer has been examined in randomized, active-controlled clinical studies. The results of these studies have demonstrated high rates of objective responses, overall survival and duration of survival without disease progression compared with chemotherapy. The most common adverse reactions were hypertension, proteinuria, gastrointestinal perforation, fistula, haemorrhage, thromboembolism, congestive heart failure, and hypersensitivity reactions. In clinical studies, proteinuria occurred in 0.7% to 38% patients who received bevacizumab. The severity of the proteinuria ranged from clinically asymptomatic, transient, trace proteinuria to nephritic syndrome. Proteinuria has been noted in all studies; it was not related to renal dysfunction and rarely necessitated permanent discontinuation of therapy (12).

Proteinuria includes the occurrence of protein in the urine in amounts that exceed physiological concentrations (150-200 mg/24 hours), which is indicative of a number of renal disorders. Proteinuria was clinically significant. Proteinuria is the cause of progressive glomerular dysfunction and progressive renal insufficiency. Renal diseases are chronic and progressive. Most renal diseases lead to gradual and irreversible damage to kidney function that results in a state of renal insufficiency (13). The terminal stage of kidney insufficiency inevitably leads to dialysis or kidney transplantation. The excessive filtration of serum proteins contributes directly to the progression of renal damage (14).

The main objective of this study was to analyse the percentage of proteinuria in patients with metastatic colorectal cancer after the administration of bevacizumab. We assessed whether there was any relationship between the degree of proteinuria and changes in platelet counts and haemoglobin concentrations in the whole group of patients and with respect to age (<60 and ≥ 60) and sex.

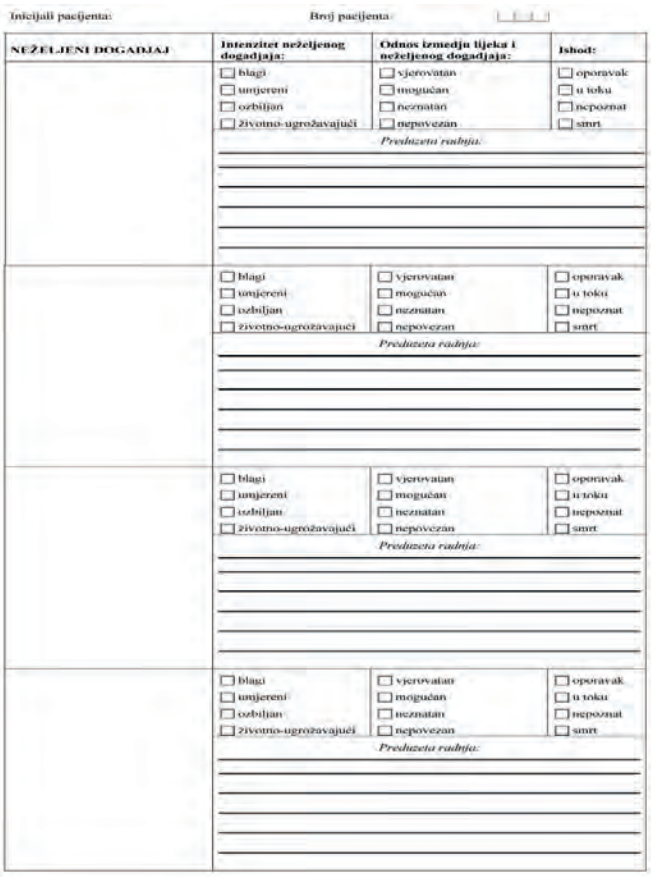
MATERIALS AND METHODS

Patients tested

This prospective non-randomized study included 20 patients from the Oncology Clinic, Clinical Center of

Montenegro, consisting of 9 men and 11 women aged 43 to 73 years. Before monitoring the results, whether the patients suffered from a chronic disease or received an accompanying treatment was considered in terms of the potential for proteinuria induction. The study was designed in accordance with the standards of good clinical practice. The criterion to include patients in the study was a set patho-histological diagnosis of colorectal cancer. The patients provided written consent to participate in the study. One patient was released from the study after one month. In 11 patients, the first line of chemotherapy was FOLFOX (5-fluorouracil/leukovorin/oxaliplatin), and in 9 patients, it was XELOX (capecitabine/oxaliplatin). In the second-line treatment for metastatic colorectal cancer, the patients received chemotherapy in combination with bevacizumab. Before starting the treatment with bevacizumab, the patients were tested for proteinuria. After introducing bevacizumab, the presence of protein in the urine was tested using a qualitative screening for protein in urine collected over 24 hours. Side effects that occurred during the study were documented on a form used to monitor side effects.

The indicated form is filled out by the doctor every other week. Security data were reported in accordance with Volume 9[a] EU Clinical Trials. The protocol used for the clinical study describes the method that was used to



Inicijali pacijenta: Broj pacijenta:

NEŽELJENI DOGADJAJ	Intenzitet neželjenog dogadjaja:	Odnos izmedju lijeka i neželjenog dogadjaja:	Ishod:
	☐ blagi ☐ umjereni ☐ ozbiljan ☐ životno-ugrožavajući	☐ vjerovatan ☐ mogućan ☐ neznatan ☐ nepovezan	☐ oporavak ☐ u toku ☐ nepoznat ☐ smrt
	Preduzeta radnja:		
	☐ blagi ☐ umjereni ☐ ozbiljan ☐ životno-ugrožavajući	☐ vjerovatan ☐ mogućan ☐ neznatan ☐ nepovezan	☐ oporavak ☐ u toku ☐ nepoznat ☐ smrt
	Preduzeta radnja:		
	☐ blagi ☐ umjereni ☐ ozbiljan ☐ životno-ugrožavajući	☐ vjerovatan ☐ mogućan ☐ neznatan ☐ nepovezan	☐ oporavak ☐ u toku ☐ nepoznat ☐ smrt
	Preduzeta radnja:		
	☐ blagi ☐ umjereni ☐ ozbiljan ☐ životno-ugrožavajući	☐ vjerovatan ☐ mogućan ☐ neznatan ☐ nepovezan	☐ oporavak ☐ u toku ☐ nepoznat ☐ smrt
	Preduzeta radnja:		

Figure 1. Form to monitor adverse effects

monitor side effects. The clinical study protocol describes the method used to monitor adverse events. The intensity ratio between the drug and the adverse effects and the outcomes of the adverse effects were assessed. Serious adverse events were any adverse event, regardless of the dose, that met at least one of the following criteria (15):

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those events that may not be immediately life-threatening but are clearly of major clinical significance. Such events may jeopardize the subject and may require intervention to prevent other serious outcomes noted above.

The stated study was approved by the Ethical Committee of the Clinical Center of Montenegro in which the stated protocol was applied, as well as the Agency for Medications and Medical Devices of Montenegro.

Biochemical analyses:

The platelets counts and haemoglobin concentration were performed separately for each patient during the first visit (initial visit before treatment with bevacizumab), during treatment (every two months) and after completion of the treatment (final visit). The final visit (fifth visit) was recorded at ten months after the date of bevacizumab initiation in all but six patients. During the study, five serious adverse events were recorded that were not associated with bevacizumab according to the physician:

- In two patients, disease progression occurred, leading to death.
- One patient was hospitalized due to ileus.
- One patient was hospitalized because of aggressive behaviour.
- One patient was hospitalized because of neurovegetative dystonia.

In addition to the adverse events mentioned above, events associated with the study drug bevacizumab were noted, such as hypertension and proteinuria. In three patients, an increase in blood pressure was detected, which was successfully controlled during the study with antihypertensive agents and did not result in discontinuation of therapy. After eight months of bevacizumab treatment, one patient presented proteinuria and haematuria. The patient was hospitalized in the Department of Nephrology, and further investigations were performed.

To obtain a more accurate diagnosis, the results were analysed for all 19 patients included in the study as well as for the 14 patients who completed the study. The platelet count and haemoglobin concentration were determined using a Cell Dyn 3700 autoanalyzanalyser from Abbott according to the protocol prescribed by IFCC.

Protein quantification

Protein in the urine was quickly detected using "Laboquick" test strips. If any shade of green colour developed, protein was present in the urine.

In cases with a positive identification of protein in the urine using the test strips, a quantitative assessment of the protein concentration was conducted using the biuret method. This method is based on a compound with at least two peptide bonds that react with copper salts under alkaline conditions to yield a purple product. The colour intensity is proportional to the protein concentration and was measured photometrically using a Humalyzer 2000 spectrophotometer at 546 nm. The measured parameter (R) represents the amount of protein in the urine. The amount of protein in the urine over 24 hours is expressed in g/l and was calculated according to the following formula:

$$\frac{R \bullet diuresis}{1000}$$

The amount of protein in urine over 24 hours was assessed for 3 days. A proteinuria grade was assigned to the samples. According to the toxicity criteria of the National Institute for Cancer version I and II (National Cancer Institute Common Toxicity Criteria version I or II), proteinuria comprises four grades: Grade1 (+) – protein amounts from 0.1 g to 1 g/24 h; Grade 2 (++ to +++) – protein amounts from 1 g to 3.5 g/24 h; Grade3 (++++) – protein amounts greater than 3.5 g/24 h; Grade 4 – nephric syndrome.

Statistical analysis

The collected laboratory data were entered into and converted using the SPSS programme.

RESULTS

The test results for the presence of proteinuria

Eight months after the initiation of bevacizumab, proteinuria and haematuria were recorded in one patient. The quantitative analysis of the protein concentrations was conducted over 3 days. The measured values were based on daily diuresis in patients and ranged from 2.91 g/L, 2.32 g/L and 2.52 g/L (proteinuria grade 2). After the presence of proteinuria was determined, the patient was admitted to the Nephrology Division, and treatment with bevacizumab was terminated, after which the protein levels in the urine decreased.

Test results for platelet counts and haemoglobin concentrations before and after treatment with bevacizumab

The platelet counts and haemoglobin concentrations were determined for each patient separately during the initial and final visits. Differences in the platelets and haemoglobin during the initial visit prior to bevacizumab administration and during the final visit after the completion of treatment are shown in Table 1.

Table 1 Differences in platelet counts and haemoglobin levels between the initial and final visit in the 19 patients who entered the study

INITIAL VISIT (IV)		FINAL VISIT (FV)			
Measured values		Measured values		% differences IV and FV	
Hb (g/L)	Plt (g/L)	Hb (g/L)	Plt (g/L)	Hb (g/L)	Plt (g/L)
131	323	154	264	18	-18
120	242	136	202	13	-17
118	322	123	186	4	-42
123	128	118	200	-4	56
161	203	173	204	7	0
151	183	163	259	8	42
80	338	109	265	36	-22
133	224	156	184	17	-18
134	268	135	112	1	-58
131	268	176	339	34	26
151	313	133	247	-12	-21
140	136	123	93	-12	-32
164	236	141	196	-14	-17
116	203	116	142	0	-30
133	203	151	181	14	-11
138	290	134	207	-3	-29
117	197	119	217	2	10
118	289	114	232	-3	-20
147	193	158	145	7	-25
Excluded from the study					
SEM*				5.947368	-11.9847
p-value**				0.11	0.022

SEM* - Standard error of the mean
p-value** - Statistical significance

Analysis of the whole group of 19 patients revealed a statistically significant decrease in the average platelet counts from the initial to the final measurements (p=0.022). Similar results were obtained for the 14 patients who continued the study (p=0.025). There were no statistically significant differences in the haemoglobin concentrations before or after initiation of bevacizumab, either in the overall population (p=0.11) or in the 14 patients who continued the study (p=0.179).

Analysis of platelet counts before and after bevacizumab administration by gender

In terms of gender, the test group was divided into two subgroups, and differences in platelet counts were assessed during the initial and final visit by gender. This analysis was performed in all 19 patients who entered the study and in the 14 patients who continued the study. Statistical analyses of the average platelet counts are presented in Table 2.

There were no significant differences in platelet counts between the initial and final visits in male patients in either tested subgroup. However, in the female patients in both subgroups, there was a very statistically significant reduction in platelet counts after compared with before bevacizumab treatment.

Analysis of platelet counts before and after treatment by age

The patients were divided into two subgroups by age: patients ≥60 and <60 years old. The platelet counts were determined during the initial and final visits for the subgroup of all patients and for those who continued the study. Statistical analysis of the average values for the platelet counts are presented in Table 3.

From Table 3, we can conclude that there were no statistically significant differences in platelet counts from the initial to the final visit in patients ≥60 years of age; however, in the patients <60 years old, a statistically significant reduction in the platelet counts was identified in the group of 19 patients. The haemoglobin concentration was also assessed before and after treatment by age.

Table 4 shows that there were no significant differences in average haemoglobin concentrations between the initial and the final visit in male patients, either in the test group or in the group that continued treatment. In both female subgroups, there was a statistically significant increase in haemoglobin concentrations after compared with before the treatment.

Analysis of haemoglobin concentrations before and after treatment by age

Table 5 shows the statistical analysis of the average haemoglobin concentrations by subgroups.

Table 5 shows that there were no statistically significant differences in the haemoglobin concentrations between the first and final visits in patients ≥60 years old; however, a significant difference was identified in those <60 years old. In the present study, we examined the correlation between platelet counts and haemoglobin concentrations by gender for the overall population. The correlations were performed to evaluate the association of platelets and haemoglobin before and after initiation of bevacizumab treatment by gender and for comparison with the results of clinical studies demonstrating an association of these parameters. These correlations were performed using the Pearson coefficient (r) and statistical value p.

Based on these parameters (r=0.590; p=0.095), there was a significant positive correlation between the platelet counts and haemoglobin concentrations after bevaci-

Table 2. Comparison of statistical parameters determined for platelet counts by gender

Gender / Parameters	Male gender (entered the study)	Male gender (continued the study)	Female gender (entered the study)	Female gender (continued the study)
$\bar{x}$	-7	0.8	-21.9	-19.66667
p-value	**0.3444**	**0.4657**	**0.0036**	**0.0081**

Table 3. Comparison of statistical parameters for platelet counts by age

Age / Parameters	≥ 60 years old (entered the study)	≥ 60 years old (continued the study)	< 60 years old (entered the study)	< 60 years old (continued the study)
$\bar{x}$	-7.44444	-12.16667	-15.9	-12.5
p-value	**0.1103**	**0.1058**	**0.0270**	**0.0859**

Table 4. Comparison of statistical parameters for haemoglobin levels by gender

Gender / Parameters	Male gender (entered the study)	Male gender (continued the study)	Female gender (entered the study)	Female gender (continued the study)
$\bar{x}$	4.666667	2.6	7.1	7.444444
p-value	**0.2403**	**0.3983**	**0.0286**	**0.0385**

Table 5. Comparison of statistical parameters for haemoglobin levels by age

Age / Parameters	≥ 60 years old (entered the study)	≥ 60 years old (continued the study)	< 60 years old (entered the study)	< 60 years old (continued the study)
$\bar{x}$	3.555556	-1.83333	**8.1**	11.375
p-value	**0.1575**	**0.7080**	**0.1181**	**0.0553**

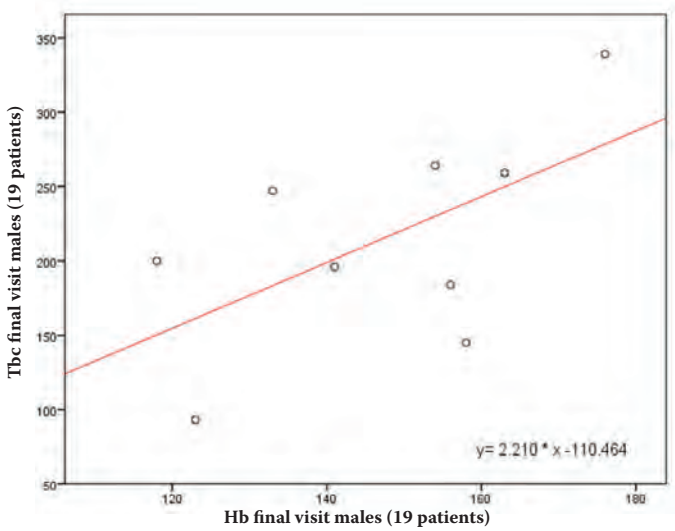


Figure 1.

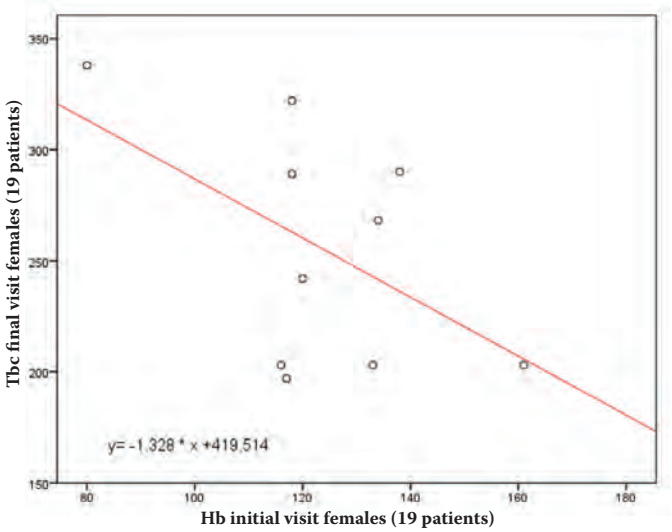


Figure 2.

zumab treatment in males in the overall population (Figure 1). The platelet counts and haemoglobin concentrations before bevacizumab treatment in females in the overall population showed a tendency toward towards a negative correlation, which may indicate an important difference in larger samples (r=-0.517; p=0.126) (Figure 2).

DISCUSSION

To date, there are no published data regarding the incidence of the type and grade of proteinuria in patients in Montenegro treated with bevacizumab, nor are there data related to the success of therapy. The purpose of this study was to determine the percentage of proteinuria in patients with metastatic colorectal cancer who were treated with bevacizumab in Montenegro. By evaluating platelet counts and haemoglobin levels by age and gender, we were able to predict which target population has a greater risk of developing proteinuria. In the present study, proteinuria occurred in one of the 14 patients (7%). This percentage is consistent with the available literature and the results of clinical trials showing proteinuria in proportions of patients ranging from 0.7 % to 38 % (12).

It is known that chemotherapy destroys the cells in the body that are rapidly dividing, as well as those in the bone marrow. In patients who had already received a combination of chemotherapeutic agents as first-line chemotherapy, it was necessary to control basic laboratory blood parameters prior to the introduction of second-line chemotherapy. In the present study, emphasis was placed on examining the platelet counts and haemoglobin concentrations because platelets are the main source of VEGF in the blood, and the production of VEGF is greatest during episodes of hypoxia or low levels of haemoglobin. Application of the anti-VEGF neutralizing antibody bevacizumab resulted in changes in the blood concentrations of these two elements. Studies have indicated that bevacizumab administered as mono or combination therapy leads to increased haemoglobin levels due to the inhibition of VEGF (16) as well as the reduced numbers of platelets (17).

In the present study, after the five bevacizumab treatments, increased haemoglobin concentrations were noted in 57% of the patients (ranging from 2% to 36%). There were no statistically significant differences in the haemoglobin levels before compared with after the bevacizumab treatment in the total population.

A comparison between the platelet counts during the initial visit before bevacizumab treatment and the final visit revealed a significant decrease in 10 out of 14 patients (71 %), ranging from 11 % to 58 %. The largest increase in platelet counts (56%) was recorded in a patient who had platelet counts that were lower than the reference value at the beginning of treatment. This patient developed proteinuria. The present study results revealed a statistically significant difference in the platelet counts measured before treatment compared with after the injection of bevacizumab in the overall population of patients who began the study, as well as in the population that completed the study (p = 0.025). Our results confirm previous research and mechanisms to date showing an association among VEGF, platelets and bevacizumab.

The decreased risk of colorectal cancer in postmenopausal women on hormone replacement therapy and increased incidence in patients with breast cancer suggest that sex hormones play a role in the pathogenesis and development of colorectal cancer and, therefore, may have prognostic value (18). Oestrogen stimulates the production of antiangiogenic factors through a mechanism that involves the activation of PAF-mediated NF-κB factor (19). Studies have shown that oestrogen plays a major role in the epithelisation of the colon. The isolation of colorectal cancer cells has revealed that a significant number of cells are positive for beta oestogenetic receptors (20). Hendifar and colleagues showed that among post-surgical patients with colorectal cancer, women younger than 55 years of age have a better

survival rate than young males. In contrast, women older than 55 years of age demonstrated a worse overall survival compared with older men (21). All of the above findings suggest that oestrogen has a prominent role in preventing the development of colorectal cancer. Moreover, antibodies have a longer half-life in women, resulting in an improved response to treatment with monoclonal antibody (22). Wakelee and associates examined the effects of age and gender on the survival of patients with NSCLC with and without bevacizumab. The administration of bevacizumab resulted in a significantly different average survival time in women under 60 years of age (the longest survival time) without affecting males (23). The administration of bevacizumab in combination with chemotherapy in patients with colorectal cancer improved overall survival and the period without disease progression similarly in patients under 65 years of age, patients from 65 to 70 years old and patients older than 70 years of age (24).

The present results for gender and age provided interesting information. Differences in the number of platelets in the male population were not statistically significant, whereas in women, statistically significant differences were obtained, in terms of reduced platelet counts ($p = 0.0036$, $p = 0.0081$) and increased haemoglobin concentrations after bevacizumab treatment ($p = 0.0286$, $p = 0.0385$)

Regarding age, there was a significant reduction in platelet counts ($p = 0.0270$) and an increase in haemoglobin concentrations ($p = 0.0553$) after treatment with bevacizumab in the population under the age of 60 years, while the results for those over 60 years of age were not statistically significant. It must be noted that the average age of the patients assessed herein was 58 years; 58 % of the patients were under 65 years of age, 32 % were between 65 and 70 years of age and only 10 % were older than 70 years. Considering the total number of female patients, 30 % of the women older than 60 years of age and previous research on the importance of oestrogen, we can conclude that our results confirm previous study findings and mechanisms associated with oestrogen, colorectal cancer and bevacizumab.

The present study has some limitations, among which the small study population was prominent. The significant difference in platelet counts in the tested population suggests the potential for even more meaningful results with a larger number of specimens. Furthermore, there was a significant difference in haemoglobin concentrations in females, which would probably be more evident in a larger patient cohort. Another limitation of this study was the exclusion of patients after the identification of grade 2 proteinuria; thus, the application of a larger dose of bevacizumab was not tested to determine whether it would lead to an increased degree of proteinuria.

CONCLUSION

The present results demonstrate that proteinuria developed in one patient, thus representing 7% of the tested population. These data are consistent with the published literature, although a smaller percentage was obtained herein due to the small number of patients in the tested population.

In the total population, there was a statistically significant difference between the platelet counts before and after the initiation of bevacizumab. In females, but not in males, a highly significant difference in platelet counts was obtained significant difference. Furthermore, significant differences were even noted in patients under 60 years of age. The haemoglobin concentrations before and after bevacizumab treatment were not significantly different in the overall population. However, analyses by gender and age revealed statistically significant differences in female patients and in patients <60 years old. A significant positive correlation was evident between the platelet counts and haemoglobin concentrations in male patients after bevacizumab treatment. Based on the present results, proteinuria occurred in only one patient, who presented an apparent deviation in laboratory parameters from the expected values in terms of a significant increase in platelet counts and a significant decrease in haemoglobin levels.

Because the study was performed in a small group of patients, it will be necessary to perform detailed analyses in a larger cohort to confirm the hypothesis that platelet counts and haemoglobin concentrations may serve as a prognostic biomarker for the development proteinuria, and furthermore, that based on the initial values determined for these biological parameters, a target group of patients with respect to gender and age may be predicted to obtain optimal benefits from bevacizumab therapy.

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QUALITY OF LIFE AMONG PATIENTS WITH DEPRESSION

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KVALITET ŽIVOTA PACIJENATA SA DEPRESIJOM

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ABSTRACT

Depression is a disease of great social and medical importance. Quality of life can correlate with severity of manifested depression. The aim of our study was to determine whether people with unipolar depression have a poorer quality of life than healthy individuals, in what areas they have poorer quality of life and how socio-demographic characteristics and different therapies impact quality of life.

The survey was conducted among 110 subjects, of which 55 were patients diagnosed with depression using ICD-10 criteria at the Psychiatric Clinic in Kragujevac and 55 were healthy subjects. Quality of life was evaluated by The Quality of Life Questionnaire compiled by the WHO. Quality of life was compared between the two groups and within research groups, depending on the applied therapy.

There were statistically significant differences in quality of life between the groups: physical health - 49.64 versus 70.84, p=0.000; psychological health - 38.69 versus 69.85, p=0.000; social relations - 53.73 versus 64.89, p=0.004; living conditions - 54.58 versus 66.7, p=0.000, and in overall quality of life - 75.41 versus 96.00, p=0.000.

The results showed that there was no statistically significant difference in quality of life between applied therapies.

The overall quality of life of depressed patients did not depend on marital status or gender of the respondents.

Depressed patients generally have a low quality of life in all domains and in overall quality of life. To improve of mental health, oOne of the primary goals to improve mental health should be to improve quality of life among depressed patients.

Keywords: *quality of life, depression, antidepressant therapy*

SAŽETAK

Depresija je bolest od velikog društvenog i medicinskog značaja. Kvalitet života može biti u korelaciji sa težinom manifestovane depresije. Cilj našeg istraživanja bio je da se utvrdi da li osobe sa unipolarnom depresijom imaju lošiji kvalitet života u poređenju sa zdravim pojedinacima, u kojim oblastima, kao i kako određene socio-demografske karakteristike i različite terapije utiču na kvalitet života.

Istraživanje je sprovedeno među 110 ispitanika: 55 pacijenata kojima je dijagnostikovana depresija korišćenjem ICD-10 kriterijuma na psihijatrijskoj klinici u Kragujevcu i 55 zdravih ispitanika. Kvalitet života je procenjen korišćenjem Upitnika o proceni kvaliteta života Svetske zdravstvene organizacije. Poređen je kvalitet života istraživačke i kontrolne grupe, kao i u okviru istraživačke grupe, u zavisnosti od primenjene terapije.

Pokazano je da postoji statistički značajna razlika u kvalitetu života između grupa: fizičko zdravlje 49.64 prema 70,84, p = 0,000; psihičko zdravlje-38.69 prema 69.85, p = 0,000; društveni odnosi-53.73 prema 64,89, p = 0,004; životni uslovi 54.58 prema 66,7, p = 0,000, kao i ukupan kvalitet života-75.41 prema 96,00, p = 0,000.

Rezultati pokazuju da ne postoji statistički značajna razlika u kvalitetu života u zavisnosti od primenjene terapije.

Vrednosti ukupnog kvaliteta života depresivnih pacijenata ne zavise od bračnog statusa ili pola ispitanika.

Depresivni pacijenti imaju generalno niske vrednosti kvaliteta života u svim pojedinačnim domenima, kao i u ukupnom kvalitetu života. U ime unapređenja mentalnog zdravlja, jedan od primarnih ciljeva treba da bude poboljšanje kvaliteta života depresivnih pacijenata.

Ključne reči: *kvalitet života, depresija, antidepresiva terapija*

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INTRODUCTION

Depression can occur as a symptom or syndrome in different psychiatric diseases or as an independent entity. The concept of depression involves a large number of clinical phenomena, such as feelings of sadness, hopelessness and helplessness, sleep disturbance, loss of appetite, decreased libido, and suicidal thoughts, that, depending on intensity and quality, determine whether a depressive disorder is present at the neurotic or psychotic level [1].

The World Health Organization defines quality of life as an individual's perception of their station in life in terms of the culture and value system in which they live, which is related to their goals, expectations, standards and concerns. This concept consists of physical health, psychological health, level of the independence, social relationships, beliefs and relationships with the environment [2].

According to the forecasts of experts, depression will be the most common cause of morbidity by 2030, indicating a growing need to invest effort in the prosperity and conservation of mental health [3].

The strategy for the development of mental health lists prevention and early diagnosis of mental disorders as one of the main goals [4].

The first requirement of an adequate therapeutic approach for a depressed patient is accurate diagnosis. All therapeutic strategies have the same goals: to reduce or eliminate symptoms, to increase the extent to which a subject "feels good", to increase quality of life, and to prevent recurrence of the disease, i.e., relapse. The main antidepressant drug groups, classified by their mechanism of action, are tricycle and similar antidepressants (TCA), selective serotonin reuptake inhibitors (SSRI), monoamine oxidase inhibitors (MAOI), and "atypical" antidepressants [5-7].

The aim of this study was to determine whether unipolar depression patients undergoing the appropriate therapy have a poorer quality of life that healthy people, in what areas they suffer a poorer quality of life, and how certain socio-demographic characteristics and different therapies have on impact quality of life of these patients.

PATIENTS AND METHODS

Subjects

This study was conducted in July 2011 at the Psychiatric Clinic in Kragujevac by interviewing respondents to assess their quality of life. This study included 110 patients; the research group consisted of 55 patients suffering from unipolar depression, and the control group consisted of 55 healthy subjects. The groups were matched by sex, place of residence, age and marital status.

The research group included patients of both sexes, with ages of 24-78 years, who were diagnosed with unipolar depression and, therefore, had previous ambulance or hospital treatment. Patients were included who fulfilled the ICD-10 criteriathe, were classified as having a major depressive episode (F32.0, F32.1 and F32.2) and recurrent depressive disorder (F33.0, F33.1 and F33.2) without psychotic elements and who had been using antidepressant drugs for at least three months were included in the study. They are divided into two groups; the first group was of patients taking selective serotonin reuptake inhibitors (SSRI), and the second group was of patients using drugs from other groups of antidepressants.

We tested whether selective serotonin reuptake inhibitors were more effective in improving the quality of life of the patients than other antidepressants.

Statistics

The required number of patients was calculated using a calculator and based on a t-test with $\alpha = 0.05$, $\beta = 0.5$ nd $R = 0.8$. We found that the minimum total sample size was 102, and the minimum number of participants per group was 51.

The two groups of patients were compared using the $\chi 2$ test. To obtain more valid results, it was essential that the two groups were as similar as possible. There were no statistically significant differences in socio-demographic characteristics between the two groups (all p values were >0.05).

A general questionnaire about the patient's general socio-demographic and therapeutic data in addition to the World Health Organization Quality of Life-Brief (WHOQOL-BRIEF) scale, a short version of the WHOQOL-100, were used to obtain the necessary data. The WHOQOL-BRIEF is a questionnaire that contains 26 questions to determine quality of life in four domains: (a) physical health (activities, need for treatment, amount of energy, mobility, presence of pain, sleep quality and capacity to work associated with health), (b) psychological well-being (satisfaction with self-body image, presence of negative emotions and positive emotions, self-esteem, religiosity, spirituality, learning, memory and concentration), (c) social relationships (personal relationships, social support, and sexual activity) and (d) living conditions (financial resources, a feeling of freedom, physical safety, social and medical security, home environment and physical environment including pollution, noise, traffic and air transport). The answer to each question was scored from 1 to 5. Each domain is determined by the values of the relevant responses and then converted to new values using special tables [17].

Thus, it allows comparison between the domains and the WHOQOL-100 scale.

Data obtained from the scale for assessing quality of life were statistically analysed using SPSS 18 for Windows. A $\chi 2$ test was used to compare certain quality of life variables between groups. The results were analysed using parametric t-tests and the nonparametric Mann-Whitney tests, depending on the presence of a normal distribution for each individual group.

Table 1. Socio-demographic characteristics of the study subjects.

		Control group	Research group							x^2	p	d.f.
			SSRI			Other antidepressants		Summary				
		No	Percentage	No	Percentage	No	Percentage	No	Percentage			
Gender	male	19	35%	13	38%	6	29%	19	35%	0.000	1.000	1
	female	36	65%	21	62%	15	71%	36	65%			
Age	20-29	4	7%	1	3%	2	10%	3	5%	0.723	0.982	5
	30-39	4	7%	3	9%	2	10%	5	9%			
	40-49	5	9%	4	12%	3	14%	7	13%			
	50-59	26	48%	17	49%	9	42%	26	47%			
	60-69	9	16%	5	15%	3	14%	8	15%			
	70-79	7	13%	4	12%	2	10%	6	11%			
Marital status	married	43	78%	24	71%	15	71%	34	76%	0.431	0.511	1
	Single/divorced/ widowed	12	22%	10	29%	6	29%	11	24%			
Place of living	Urban place	38	69%	19	56%	16	76%	35	36%	0.000	1.000	1
	Rural place	17	31%	15	44%	5	24%	20	64%			

d.f.- degrees of freedom; SSRI - selective serotonin reuptake inhibitors; x2 - chi square test value

RESULTS

The frequencies and percentages of the socio-demographic characteristics of subjects and the values of χ2 tests between the research and control groups are shown in Table 1.

The subjects in the research group have used antidepressants from 3 months to 30 years.

Of the 55 patients, 34 (62%) had used selective serotonin reuptake inhibitors, where 7 used paroxetine (20 mg), 22 used sertraline (50 mg) and 5 patients used citalopram (20 mg).

Table 2. Values from the WHOQOL-BRIEF scale, statistical parameters for domains and overall quality of life between depressed patients and healthy subjects.

	Physical health (mean)	s.d..	Psychological health (mean)	s.d.	Social relations (mean)	s.d.	Life conditions (mean)	s.d.	General quality of life (mean)	s.d.
Research group-mean	49.64	21.82	38.69	22.54	53.73	18.93	54.58	14.27	75.41	16.86
Control group-mean	70.84	19.68	69.85	16.27	64.89	21.96	66.7	15.34	96.00	15.91
Mann Whitney-test value	-4,475		-6.588		-2.885		-4.058		-5.777	
P value	0.000***		0.000***		0.004**		0.000***		0.000***	

s.d.- standard deviation *p<0.05 ** p<0.01 *** p<0.001

Twenty-one subjects in the research group had used drugs from other groups of antidepressants, where 13 (15%) had used venlafaxine (75mg, 150mg), 1 (2%) used mianserin (30 mg), 2 (4%) used trazodone (150mg) and 5 patients (11%) used mirtazapine (15mg).

The physical health, psychological (mental) health, social relations and living conditions scores for the research group (depressed patients) and the control group (healthy subjects) are presented in Table 2.

Within the research group, the patients' quality of life was also analysed between the antidepressant therapies. In the research group, we tested whether the absence of a spouse further affected the quality of life.

DISCUSSION

There are several published studies with results similar to our findings. A study published in 1997 that compared quality of life between depressed patients and control subjects showed that depressed patients have generally lower scores and that scores do not depend on sex, age or other variables [8].

This study showed that patients with unipolar depression generally have poorer scores in all quality of life domains compared with the healthy population (Table 2).

The most significant predictors of depression in a study that used the SF 36 scale with 89 subjects were two items: the assessment of mental health and the assessment of general health [9].

In this study, we used the quality of life questionnaire issued by the World Health Organization. The lowest level of quality of life in depressed subjects from this study was in the domain of psychological well-being (38.69), although high statistical significance was also present in the areas of physical health, living conditions and overall quality of life. (Table 2)

The six-month study examined quality of life associated with health and the impact of antidepressants on quality of life [10].

This was a European observational study designed to assess the quality of life in 3468 adult patients with diagnosed episodes of depression before and after 3 or 6 weeks of treatment with antidepressants. In all groups of drug treatment, the greatest improvements occurred after 3 months of therapy. Another study dealt with the assessment of changes in depressive symptoms and monitoring quality of life during treatment with antidepressants [11].

Eighty-seven patients were followed in that study during hospitalization and after hospital discharge. The researchers also used the World Health Organization Quality of Life scale. The results showed that there was an increase in quality of life and reduction of symptoms during therapy. However, more than half of the patients had 10 or more symptoms at discharge.

Because our study was a cross-sectional design, the improvement of quality of life after administered therapy could not be shown. Another limiting factor of our study was that most of the patients were not hospitalized therefore adherence to therapy could not be assessed.

Other limiting factors were a small sample size and usage of other drugs in addition to antidepressants that could affect quality of life.

Comparing the patient groups, there were no statistically significant differences in single domains or in overall quality of life between patients taking SSRIs and patients taking other antidepressants.

Other studies with depressed patients have shown that approximately 60% of the cases of depression were not receiving treatment for depression [12]. This is supported by a cohort study that was performed on patients with major depression for a period exceeding 9 months [13]. One hundred seventy-nine patients (mostly women, 73%), with an average age of 38 years were followed in that study. After 9 months, 42% of the patients still suffered from depression and 25% had complete remission, while only 9% were correctly treated with antidepressants. There was a statistically significant relationship at the beginning and after 9 months in terms of quality of life. This research showed poor quality of life among depressed patients, which suggests an inappropriate treatment of depression.

Although our study showed that quality of life was not affected by the type of antidepressant used, we did not compare quality of life between untreated depressive patients and those with antidepressants, which also could be a limitation of this study. A study that was conducted among 982 patients who did not use any antidepressants showed that quality of life is initially connected with depressive symptoms [14]. The primary care patients were followed at baseline and after 6 weeks, 3 months and 9 months. Patients who were diagnosed with depressive symptoms at baseline showed no improvement in any of the measures after nine months.

Our study included patients who were mostly female (65%), and 72% of the control patients were more than 50 years old; however, the results were not related to the patient's age. A study that dealt with the correlation between age, depressive symptoms and quality of life was conducted on 443 adults over 30 years of age [15]. Depression was negatively associated with quality of life in the psychological and social domains, but age was not.

The study also showed that environmental quality of life increased with age, whereas physical quality of life decreased.

Lots of studies have dealt with pain, depressive symptoms and quality of life. A study that was performed with premenopausal women examined quality of life and pain in women who were diagnosed with unipolar depression [16]. The results showed that premenopausal women experienced pain and had lower quality of life than controls, especially in the domains of emotional status and social well-being.

Another study that used a regression model was conducted among depressed women over 40 years of age and showed that both physical condition and severity of depression significantly influenced quality of life [17]. The intensity of depressive symptoms has been shown as the most important factor influencing quality of life. We can make the assumption that this study also demonstrates that depressive symptoms have great influence on quality of life. In our study, we did not analyse the effects of comorbidities, and the presence of other illness, especially of those associated with pain, can also affect quality of life.

A study that compared depressive symptoms and quality of life among patients with amyotrophic lateral sclerosis with the presence of pain dealt with that issue [18]. The results showed that depression scores significantly decreased quality of life, and that the effect remained significant after considering pain intensity as a covariate ($p<0.05$).

ALS patients with pain had lower quality of life and higher depressive symptoms than ALS patient without pain, but there was no significant difference between these two groups.

Our study compared quality of life between patients with unipolar depression that were treated in the Psychiatric Clinic in Kragujevac and healthy controls. A similar method was used and similar results were found in a study that was conducted among students in Turkey where the prevalence of depression was 21.8% [19]. Quality of life was lower in all domains among the students with depression compared to the students without depression. In terms of health-related quality of life, the students that presented with higher intensity of depressive symptoms obtained scores significantly lower in all domains and in general health-related quality of life in particular [20].

In a study that used a multilevel regression model, the presence of depressive symptoms in women was associated with poorer physical health related to quality of life, whereas depressive symptoms in men were associated with a decrease in mental health-related quality of life [21].

That study also showed that sex was not associated with quality of life, and quality of life was not different among students with and without an emotional partner.

CONCLUSION

A review of the literature revealed that unipolar depression leads to impairment in the quality of life in all areas. Our study has shown that patients with unipolar depression have a poorer quality of life than healthy individuals, especially in the domain of mental health. Selective serotonin reuptake inhibitors therapy was not more effective at improving quality of life in comparison with other antidepressants. The modern concept of protection and improvement of mental health defines the quality of life of mentally ill persons as one of primary goals, and this study supports that conclusion.

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VITAMIN C IN NEUROPSYCHIATRY

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VITAMIN C U NEUROPSIHIJATRIJI

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ABSTRACT

Vitamins are necessary factors in human development and normal brain function. Vitamin C is a hydrosoluble compound that humans cannot produce; therefore, we are completely dependent on food intake for vitamin C. Ascorbic acid is an important antioxidative agent and is present in high concentrations in neurons and is also crucial for collagen synthesis throughout the body. Ascorbic acid has a role in modulating many essential neurotransmitters, enables neurogenesis in adult brain and protects cells against infection. While SVCT1 enables the absorption of vitamin C in the intestine, SVCT2 is primarily located in the brain.

Ascorbate deficiency is classically expressed as scurvy, which is lethal if not treated. However, subclinical deficiencies are probably much more frequent. Potential fields of vitamin C therapy are in neurodegenerative, cerebrovascular and affective diseases, cancer, brain trauma and others. For example, there is some data on its positive effects in Alzheimer's disease. Various dosing regimes are used, but ascorbate is safe, even in high doses for protracted periods. Better designed studies are needed to elucidate all of the potential therapeutic roles of vitamin C.

Key words: *vitamin C, neurotransmitters, antioxidant, collagen*

SAŽETAK

Vitamin su neophodni faktori za razvoj i normalnu funkciju mozga kod ljudi. Vitamin C je hidrosolubilno jedinjenje koje ljudski organizam ne može da sintetiše tako da smo potpuno zavisni od unosa putem hrane. Askorbinska kiselina je važno antioksidativno sredstvo i prisutna je u neuronima u visokim koncentracijama. Takođe je od ključnog značaja za sintezu kolagena u celom organizmu. Askorbinska kiselina ima ulogu u modulaciji mnogih bitnih neurotransmitera, omogućava neurogenezu u mozgu odraslog i štiti ćelije od infekcije. Dok SVCT1 omogućava apsorpciju vitamina C u crevima, SVCT2 se nalazi uglavnom u mozgu.

Nedostatak askorbata klasično se ispoljava kao skorbut koji je letalan ako se ne leči, ali je supklinička deficijencija verovatno mnogo češća. Potencijalni terapijski domeni vitamina C terapije su neurodegenerativne, cerebrovaskularne i afektivne bolesti, karcinomi, traume mozga i drugi. Postoje na primer podaci o pozitivnim efekatima askorbinske kiseline u Alchajmerovoj bolesti. Koriste se razni režimi doziranja, ali je askorbat pokazao bezbednost čak i u visokim dozama tokom dugih perioda. Potrebne su bolje dizajnirane studije da se razjasne sve potencijalne terapijske uloge vitamina C.

Ključne reči: *vitamin C, neurotransmiteri, antioksidansi, kolagen*

INTRODUCTION

Some of the functions of vitamins in neurological and psychiatric diseases are well known (1). The growing body of evidence shows novel roles of vitamins, such as vitamins B, D and A, in the brain, with important neuropsychiatric implications (2). Vitamin C (ascorbic acid, ascorbate) is a hydrosoluble vitamin that is not produced in humans. Vitamin C was isolated in the early 1930s. Albert Szent-Györgyi won the Nobel Prize in 1937 for his discovery of vitamin C, and Linus Pauling, a double Nobel laureate, popularized its use for disease prevention and longevity (3).

Vitamin C has important functions in the synthesis of collagen (the main component of connective tissues) noradrenalin and carnitine, and in fat, peptide hormone and tyrosine metabolism. It is also involved in angiogenesis and cell survival (4, 5). Ascorbic acid has a prominent role in maintaining healthy bones, teeth, gums and muscles, as well as in wound healing, iron absorption and combating infections. Another significant role of ascorbic acid is its antioxidative protection of proteins, lipids, carbohydrates and nucleic acids.

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METABOLISM

Vitamin C is synthesized from glucose by many animals, with the exception of humans and some other species that are unable to synthesize this vitamin due to enzyme mutations that make them dependent on food resources (4). With oral vitamin C intake, the plasma and tissue concentrations are strictly controlled by absorption, tissue accumulation, renal reabsorption and the rate of utilization, which maintains plasma concentrations up to 100 mmol/l. With supplementation, the plasma concentrations do not exceed 250 mmol/l and are frequently less than 150 mmol/l (6).

Normally, the whole-body vitamin C content is approximately 20 mg/kg, or 1500 mg (7). In restricted intake, ascorbic acid is lost at a rate of 3% of the whole-body content per day. Plasma ascorbate levels between 20 and 80 mmol/l are considered normal. Leukocyte ascorbate is a better indicator of vitamin C status than the plasma levels.

The oxidized form of ascorbate is dehydroascorbic acid (DHA), which is transported to and from the cells via facilitated diffusion through the ubiquitous GLUT family (GLUTs) glucose transporters (5). In cells, DHA is reduced to ascorbate. The ascorbate transporters sodium-dependent vitamin C transporter 1 and 2 (SVCT1 and SVCT2) are fundamental for controlling the pools and cellular contents of vitamin C (8). SVCT1 enables vitamin C absorption in the intestine. Ascorbate cannot cross the blood-brain barrier unless it is converted to DHA. SVCT2 is primarily located in the brain and neuroendocrine tissues, enabling intracellular neuronal concentrations to be increased 20- to 60-fold compared to the plasma concentration. It is a potential protective factor in brain capillary endothelial cells under ischaemia (9). SVCT2 is also present in the peripheral nervous tissue, where it promotes myelination through the formation of the extracellular matrix (10). Trials with vitamin C in Charcot-Marie-Tooth type 1A patients did not show any benefit (11, 10). Ascorbic acid is important for the hydroxylation of lysine and proline residues in the collagen in connective tissues (12).

Vitamin C is an important regulator of intracellular redox status by maintaining sulphydryl compounds, particularly glutathione, in their reduced state (13). It can repair protein hydroperoxides by regenerating the parent amino acids through reduction. Free radicals convert ascorbic acid to DHA, which is reconverted via the glutathione enzyme complex. Vitamin C can regenerate other antioxidants, such as vitamin E, and protects folic acid. Vitamin C reduces copper and iron.

VITAMIN C DEFICIENCY

Scurvy is the manifestation of vitamin C deficiency and is a potentially fatal disease. It has been recognized for many centuries. Patients demonstrate pain in the extremities from a failure of osteoid formation, easily occurring gingival bleeding and other haemorrhagic manifestations, as well as hair and tooth loss, swelling, ulcerations, and ultimately death (4). Pseudoparalysis of the limbs occurs because of the extreme pain upon movement caused by haemorrhages under the periosteum. The early signs of vitamin C deficiency are impaired wound healing due to collagen-related pathology, follicular hyperkeratosis, petechial haemorrhages and low plasma and leukocyte concentrations. The lowest physiological requirement is approximately 10 mg/day. Symptoms occur when ingestion falls lower than 10 mg/day or when the whole-body content falls under 300 mg (4).

Between 1988 and 1994 in the United States of America, approximately 15% of adults were vitamin C-deficient with plasma levels less than 11 mmol/L, which was likely predominantly caused by being overweight (14).

The main foods with higher levels of ascorbic acid are fruit and vegetables, but many people take vitamin C supplements. To correct all signs and symptoms of ascorbic acid deficiency, it is necessary for the whole-body vitamin C level to reach 1000 mg (7).

VITAMIN C AND THE BRAIN

Vitamin C is crucial for brain function. Some evidence shows that vitamin C is active during brain development in stem cell division, neuronal and glial differentiation, maturation, and neurotransmission (15, 5). On the other hand, vitamin C deficiency impairs folic acid metabolism and presents the clinical manifestation of folic hypovitaminosis during pregnancy (15). Brain ascorbate concentrations are higher in the CSF than in plasma.

Ascorbic acid from the blood accumulates in the brain and is maintained at high concentrations in neuronal and glial cells (16). The ascorbate content in glial cells is substantially lower than in neurons. Ascorbate has different actions from other antioxidants, such as vitamin E and glutathione (GSH). The newly discovered functions for ascorbate are its role as a co-factor in several important enzyme reactions. Vitamin C reduces oxidative neuronal stress by protecting cell membranes and DNA, reducing beta-amyloid toxicity and regenerating other antioxidants, such as vitamin E (17).

The regional distribution of ascorbate in the brain is asymmetrical. Vitamin C acts as a neuromodulator of glutamatergic, dopaminergic, cholinergic and GABAergic transmission and the corresponding behaviours (5). Ascorbate mediates acetylcholine and catecholamine release from synaptic vesicles. Intracellular ascorbate is essential for the protection from oxidant stress.

The role of vitamin C in cognition, mood and behaviour has been most extensively studied in memory and locomotor activity in animal models. Age-related and toxic memory deficits in mice have been reversed by intraperitoneal ascorbate (18). Another effect is anxiety reduction (5). There are also some inconclusive studies about vitamin C's cognitive effects. Acute vitamin C and D deficiency is highly prevalent among patients in acute care (19), and these patients often have mood disturbance and cognitive dysfunction due to these hypovitaminoses. In a random-

ized, double-blind study of short-term 1000 mg vitamin C supplementation was associated with a 71% reduction in mood disturbance and a 51% reduction in psychological distress with the normalization of vitamin C plasma levels. However, daily 5000 IU vitamin D intake increased the plasma levels, but not to the normal level, and did not improve mood or decrease distress (19).

As oxidative stress is involved in the pathophysiology of neurodegenerative diseases, ascorbic acid has therapeutic potential in ischaemic stroke, Alzheimer's disease (AD), Parkinson's disease and Huntington's disease. It protects neurons from glutamatergic excitotoxicity. In many studies, ascorbic acid protected cerebral tissue, which was attributed to its antioxidant capacity (20). In an experimental model, vitamin C dose-dependently inhibited interleukin-1β (IL-1β)-mediated PGE2 synthesis in a human neuronal cell line, which was increased synergistically in combination with aspirin (20).

The main etiopathogenetic factor in Alzheimer's disease is amyloid beta, which is derived from the amyloid precursor protein (APP) and exists in an oligomeric soluble form as well as in deposits called senile plaques (21). The physiological role of APP is different after cleavage of the N-terminal ectodomain, which acts as a growth factor, while the C-terminal endodomain has a role in cell adhesion and gene regulation (22). It is not clear whether amyloid beta has any physiological roles. Vitamin C plasma and CSF levels are reduced in AD despite regular nutrition and supplement intake reduces the AD incidence (5).

Data from the Cache County Study showed that use of vitamin E and vitamin C supplements in combination reduces the prevalence and incidence of Alzheimer's disease (23). Additional analysis from the same study showed that higher vitamin C intake alone was associated with higher cognitive function at baseline on a Modified Mini-Mental State examination (3MS) and the patients had lesser cognitive decline compared with those with lower vitamin C intake (24).

Oxidative stress causes lipid peroxidation in neuronal membranes; the reactive products have cytotoxic and genotoxic properties that alter proteins and DNA and interact with other risk factors to promote neurodegeneration leading to AD (25). Ascorbate is involved in oligosaccharide metabolism and could preclude the formation of toxic amyloid beta oligomers, which are potentially produced in excess with lower plasma levels of vitamin C (22). In a study of patients with moderate AD, the CSF: plasma ascorbic acid ratio predicted cognitive decline, possibly as an index of vitamin C availability to the brain (17). The use of combined antioxidants (vitamins C and E as well as non-steroidal anti-inflammatory drugs) in elderly patients in the Cache County Study had a protective effect on cognitive decline and the development of Alzheimer's disease, but only in participants with the APOE epsilon 4 allele (26).

In a murine model of AD, the addition of vitamin C can improve spatial learning (27). A meta-analysis of the large population-based studies using a self-reported questionnaire produced inconclusive results, with the majority unable to decisively confirm the association of ascorbate intake or supplementation and AD. However, there were considerable methodological limitations (25). Avoiding vitamin C deficiency with adequate food intake is more beneficial than taking supplements, but more well-designed studies are needed (28). The likely scenario in AD is that there is a shortage of vitamin C. Therefore, the brain uses ascorbate from the peripheral pools to ensure the adequate antioxidant capacity of the brain and reduce the oxidative stress involved in AD pathophysiology (25).

Very high doses of vitamin C have been shown to be toxic to tumour cells in clinical and experimental studies (29). Pharmacologic ascorbate has cytotoxic properties against cancer cells in experimental murine brain tumour (glioblastoma) models (6).

Oxidative injury is considered to be an important pathogenic mechanism in Parkinson's disease, with severe decreases in dopaminergic transmission. Ascorbate improves the bioavailability of levodopa and partially protects dopaminergic neurons from MPTP toxicity (5).

Ischaemic small vessel disease of the brain with white matter lesions correlates with cognitive status in both older and younger populations (30). Common vascular risk factors are present, but some risk factors still unknown. Vitamin C may have favourable actions in ischemic brain disease as well. A meta-analysis of prospective studies revealed a significant inverse association between dietary vitamin C intake, blood levels of vitamin C and stroke risk (31). Data about ascorbic acid supplements are sparse. The likely mechanisms are its strong antioxidant action, anti-inflammatory action, inhibition of smooth muscle proliferation, membrane protection but also its blood pressure-lowering effects that slow the progression of atherosclerosis (31).

In patients with severe head injury, administration of high doses (up to 10 grams) of vitamin C led to a significant stabilization of the perilesional edema and a reduction of hospital mortality (32).

In adults, neurogenesis only occurs in limited, but crucial brain areas (the hippocampus, olfactory bulb, periventricular area and subependymal hypothalamus) (1). It is of utmost importance that vitamin C is present in adequate quantities for stem cell generation, proliferation and differentiation (33). It is also crucial for neural stem cell generation, proliferation and differentiation during embryonic development.

INDICATIONS AND DOSING

The recommended dietary allowance (RDA) is primarily based on the prevention of deficiency disease and not on prevention of chronic disease. The RDAs are listed below.

Infants
0-6 months 40 mg/day
7-12 months 50 mg/day
Children
1-3 years 15 mg/day
4-8 years 25 mg/day
9-13 years 45 mg/day
Adolescents 14-18 years
Males 75 mg/day Females 65 mg/day

Adults 19 years and older
Male non-smokers 90 mg/day Female non-smokers 75 mgMale smokers125 mg/day
Female smokers 110 mg/day **In pregnancy**
up to 18 years 80 mg/day
19 years or older 85 mg/day
While breast feeding
up to 18 years 115 mg/day and
19 years or older 120 mg/day

Higher doses (400 mg/day or more) of vitamin C supplementation may be cardioprotective (34). The effects on brain disease have been presented above. The brain is the most difficult organ to deplete of ascorbate. Ascorbic acid inhibits the synthesis of some animal carcinogens in the gastric contents at doses of approximately 1000 mg per day (35). Many studies have tested the potential of vitamin C as a chemotherapeutic agent using intravenous doses of up to 65 grams, but the results are controversial. Even with such high doses, the side effects are minimal and primarily include diarrhoea. There are absorption-limiting factors in the intestine, which raises questions about the utility of high dose vitamin C supplementation (28).

There is some evidence that vitamin C intake can reduce the prevalence of cataracts (36). Other possible therapeutic areas are type 2 diabetes, immune disturbances, adrenal hormone disorders, tumours, depression, neurodegenerative disorders, motoneuron disease, the common cold, antagonizing the negative effects of nicotine on the vascular system, promoting wound healing, combating complex regional pain syndrome, arterial hypertension, osteoarthritis and others (5).

SAFETY

Hypervitaminosis C is very mild (diarrhoea of osmotic origin in doses over 10 g per day) and occurs if large doses are taken for many years; therefore, vitamin C is practically non-toxic. However, toxicity has been observed when doses of 3,000 mg per day or higher were taken at once (37). Symptoms can be circumvented by taking buffered salt and not free acid. For instance, doses of 10,000 mg per day have been taken for more than a year without any side effects (38). Some phenomena, such as conditioned scurvy, oxalate or urate kidney stones and pro-oxidant effects, have not been confirmed (39). Vitamin B12 is crucial for central and peripheral nervous system function (40). The observation that ascorbic acid can destroy vitamin B12 *in vitro* has been quite concerning, but subsequent studies showed no damage (39). The most frequently used dosages of vitamin C in adults are 100–3,000 mg/day, and some advocate "bowel tolerance" doses in acute viral infections, which are doses slightly lower than those causing diarrhoea (41). Taking ascorbic acid in 3 to 6 daily doses or taking it with food increases abortion.

CONCLUSION

Vitamin C is crucial for normal development and bodily functions in human adulthood; however, humans are not capable of producing vitamin C. It has many important functions. Ascorbic acid is one of the main antioxidative agents in the human body, particularly the brain. Its deficiency is classically expressed as scurvy, but subclinical deficiencies are probably much more frequent. This opens a potential field of interest for vitamin C therapy in neurodegenerative, cerebrovascular and affective diseases, as well as cancer, brain trauma and others. Various dosing regimes are used; however, ascorbate is safe, even in high doses for protracted periods. Better designed studies are needed to elucidate all of the potential therapeutic roles of vitamin C.

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FRACTURES OF THE HAMATUM AND CAPITATUM IN A CHILD: A CASE REPORT

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PELOMI GLAVIČASTE I KUKASTE KOSTI KOD DETETA: PRIKAZ SLUČAJA

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ABSTRACT

Fractures of the carpal bones are rarely seen in children, particularly in the first decade of life. Scaphoid fractures are the most common carpal bone injuries seen during this period of life. A 5-year-old boy was referred to our clinic with right hand and wrist pain and massive swelling. The patient showed limited wrist extension and flexion with pain and swelling, but there was no neurovascular damage. Conventional X-ray and CT scans were performed. The CT results in particular showed clear non-displaced capitatum and hamatum fractures, and a short arm cast was applied. At the 18-month follow-up visit, the patient's fractures were healed with no displacement, and full ROM was achieved with a pain-free motion.

SAŽETAK

Prelomi karpalnih kostiju su retki kod dece, posebno u prvoj deceniji života. Prelomi čunaste kosti su najčešće povrede karpalnih kostiju u ovom periodu života. Petogodišnji dečak je primljen na našu kliniku zbog bola u desnoj ruci i zglobu ručja, kao i zbog masivnog otoka u tom predelu. Kod pacijenta je registrovana ograničena ekstenzija i fleksija u zglobu ručja, praćena bolom i otokom, ali bez znaka neurovaskularnih povreda. Sprovedeno je snimanje X-zracima i CT. Na CT snimcima su se uočili jasni prelomi glavičaste i kukaste kosti, bez dislokacije.i primenjena je gipsana imobilizacija zgloba ručja. Pri pregledu nakon 18 meseci, prelomi su u potpunosti zarasli bez dislokacija i postignut je potpuni opseg pokreta bez osećaja bola.

INTRODUCTION

Fractures of the carpal bones are rarely seen in children, particularly in the first decade of life. Scaphoid fractures are the most common carpal bone injuries reported during this period of life (1-4), whereas fractures of the other carpal bones are rarely seen and have generally been described as case reports or small series of patients in the literature (1,2)(5,6).

The main difficulty associated with the diagnosis of paediatric carpal bone fractures is that conventional X-ray radiographies can be inadequate; diagnosis is especially difficult at the time of injury (6). Because of the skeletal immaturity of paediatric patients, it is difficult to visualize the cartilaginous carpal bones with conventional X-ray radiographies (6). Instead, these cartilaginous components are more effectively visualized using imaging techniques such as ultrasonography (USG), CT and MRI (7). Nevertheless, all visualization techniques present different advantages, such as the absence of radiation up-take for USG and MRI and easy accessibility and low cost for CT. Moreover, few reports have described the value of these imaging techniques in young children (8,9).

In this paper, we report the case of a 5-year-old child with a combination of hamatum and capitatum fractures, which is a rare combination in childhood. The purpose of the study was to emphasize the diagnostic value and importance of CT when MRI cannot be achieved because of patient discomfort associated with carpal bone injuries in children.

CASE PRESENTATION

A 5-year-old boy was referred to our clinic with right hand and wrist pain and massive swelling. The injury occurred when he was playing football at school and fell on his hand. After receiving emergency care at the local hospital, the boy was transferred to our orthopaedic clinic for consultation.

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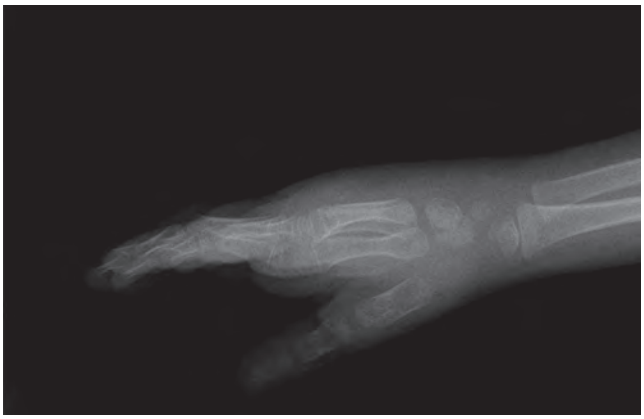

Figure 1: Antero-posterior X-ray of the wrist

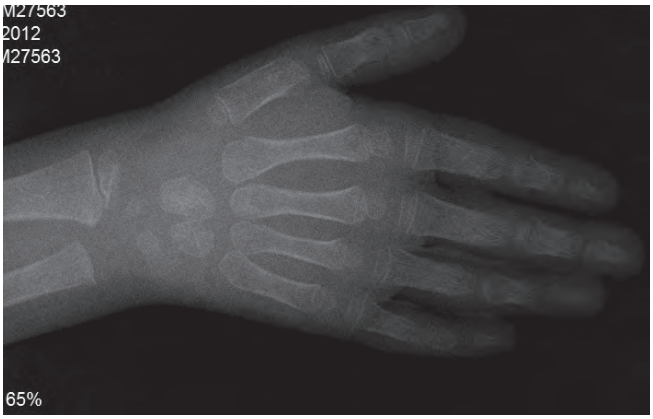

Figure 2: Lateral X-ray of the wrist

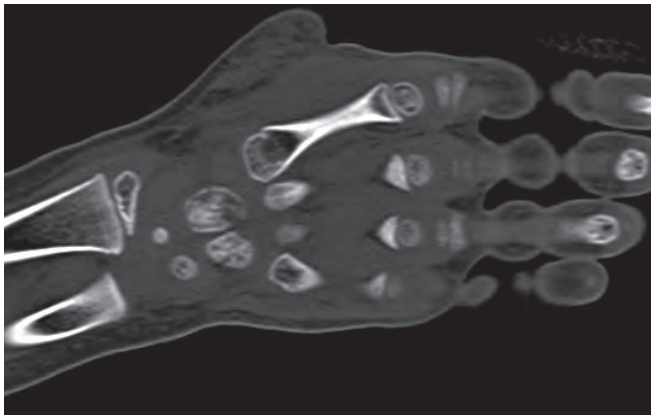

Figure 3: Axial CT image of the wrist

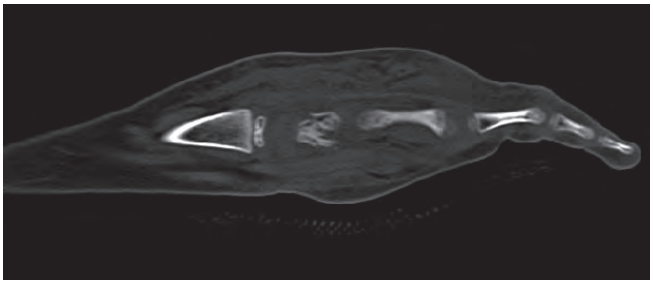

Figure 4: Sagittal CT image of the wrist

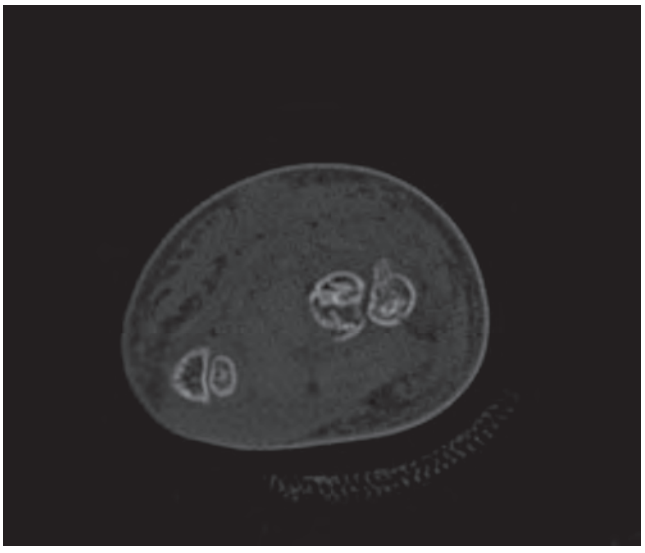

Figure 5: Coronal CT image of the wrist

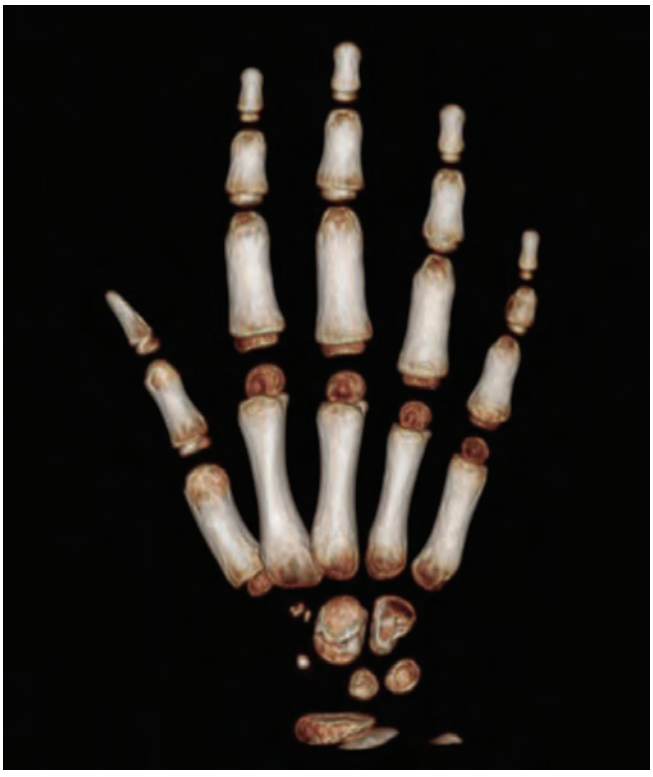

Figure 6: 3D reconstructed axial CT image of the wrist

The patient showed limited wrist extension and flexion with pain and swelling. There was no tenderness in the scaphoid fossa and no neurovascular damage. Conventional X-ray images were taken, although MRI could not be performed because of patient discomfort. Thus, CT images were acquired. The CT results clearly showed non-

displaced capitatum and hamatum fractures (Figures 1-6), and a short arm cast was applied.

The patient was hospitalised, and after applying the short arm cast, the arm was elevated to prevent compartment syndrome. After 24 hours of observation, there was no evidence of compartment syndrome, and the patient was discharged with recommendations. Four weeks after the trauma, the short arm cast was removed, and exercises to increase the range of wrist motion were recommended. One week later, there was no restriction on wrist range of motion. For this reason, there was no need for additional physical therapy. At the 18-month follow-up visit, the patient showed no wrist pain, and full range of wrist motion was achieved.

DISCUSSION

Carpal fractures in children are rare, and the scaphoid is the most frequently affected area, whereas the triquetrum is less commonly affected (5, 6, 10-13). The majority of such cases involve fractures of the scaphoid and the capitatum and often occur in combination with scaphocapitate syndrome (14). Combined fractures of the capitatum and hamatum are extremely rare. There may be 'weak zones' in the capito-hamate and piso-triquetral joints in adults (15), and these bones are often the failure points, rather than the soft tissues, in paediatric peri-articular injuries. Rotational displacement of the capitatum is rare in children when associated with scaphoid fractures. In our patient, the mechanism of injury was usual, as it was associated with a fall onto a dorsiflexed wrist (6).

MRI is a sensitive, reliable and important technique for detecting carpal bone injuries in children, in whom osseous development is progressing and the cartilaginous structure is predominant, particularly when there is a clinical suspicion. MRI may reveal bone marrow changes and cortical disruption and can clearly delineate fracture lines, particularly if the imaging is performed in more than one plane (2). However, we could not use MRI because of age-dependent discomfort in our case. Thus, CT may play a role in cases of complex fractures, as this approach is valuable in defining the extent of displacement of the physeal component and in determining the need for internal fixation (16). We used CT to diagnose the extension fracture because of age-dependent discomfort for MRI.

Paediatric delays in diagnosis are common, and the majority of paediatric carpal injuries heal uneventfully with simple cast immobilization. However, displaced fractures may require open reduction and temporary immobilization using either percutaneous Kirschner wires, absorbable pins or conventional screw fixation (17). In our case, the fractures of the hamatum and capitatum were not displaced; therefore, simple cast immobilization treatment was performed.

CONCLUSION

MRI is the most useful diagnostic tool for the diagnosis of carpal lesions in the skeletal immature child. CT can also be useful for diagnostic studies of paediatric carpal bone fractures because of age-dependent discomfort associated with MRI.

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Author. (Year of publication). Article title. *Journal Title. Volume* (issue), range of pages. DOI.

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Nikora, V. (2006). Hydrodynamics of aquatic ecosystems: spatial-averaging perspective. *Acta Geophysica*, 55(1), 3-10. DOI: 10.2478/s11600-006-0043-6.

b. Journal Article (two or more authors)

Format:

Author1, Author2 & Author3. (Year of publication). Article title. *Journal Title. Volume* (issue), range of pages. DOI.

Example:

Cudak, M. & Karcz J. (2006). Momentum transfer in an agitated vessel with off-centred impellers. *Chem. Pap.* 60(5), 375-380. DOI: 10.2478/s11696-006-0068-y.

c. Journal article from an online database

Format:

Author(s). (Year of publication). Article title [Electronic version]. *Journal Title. Volume* (issue), range of pages. Retrieved date of access, from name of database. DOI.

Example:

Czajgucki Z., Zimecki M. & Andruszkiewicz R. (2006, December). The immunoregulatory effects of edeine analogues in mice [Abstract]. *Cell. Mol. Biol. Lett.* 12(3), 149-161. Retrieved December 6, 2006, from PubMed database on the World Wide Web: http://www.pubmed.gov. DOI: 10.2478/s11658-006-0061-z.

d. Newspaper article (no author)

Format:

Article title. (Publication date). Journal Title. page.

Example:

Amazing Amazon region. (1989, January 12). New York Times, p. D11.

e. Encyclopedia article

Format:

Author. (Year of publication). Article title. In Encyclopedia title (volume number, pages). Place of publication: Encyclopedia name.

Example:

Bergmann, P. G. (1993). Relativity. In The new encyclopedia britannica (Vol. 26, pp. 501-508). Chicago: Encyclopedia Britannica.

4. Other formats

a. Web page

Format:

Author/Sponsor. (last update or copyright date). *Title*. Retrieved date of access, from URL.

Example:

Walker, J. (1996, August). *APA-style citations of electronic resources*. Retrieved November 21, 2001, from http://www.cas.usf.edu/english/walker/apa.html

b. Lecture note

Format:

Author(s). (Date of presentation). *Lecture title*. Lecture notes distributed in the unit, at the name of the teaching organisation, the location.

Example:

Liffers, M. (2006, August 30). *Finding information in the library*. Lecture notes distributed in the unit Functional Anatomy and Sports Performance 1102, University of Western Australia, Crawley, Western Australia.

c. Patent

Format:

Author. (Year). Patent number. The location. Issue body.

Example:

Smith, I. M. (1988). U.S. Patent No. 123,445. Washington, D.C.: U.S. Patent and Trademark Office.

d. Standard

Format:

Issue body. (Year). Standard name. Standard number. The location.

Example:

Standards Association of Australia. (1997). Australian standard: Pressure equipment manufacture. AS4458-1997. North Sydney.

e. Video

Format:

Producer, P. P. (Producer), & Director, D.D. (Director). (Date of publication). Title of motion picture [Motion picture]. Country of origin: Studio or distributor.

Example:

Zhang, Y. (Producer/Director). (2000). Not one less [Motion Picture].China: Columbia Pictures Industries, Inc.

f. Audio recording

Format:

Songwriter, W. W. (Date of copyright). Title of song [Recorded by artist if different from song writer]. On Title of album [Medium of recording]. Location: Label. (Recording date if different from copyright date).

Example:

Taupin, B. (1975). Someone saved my life tonight [Recorded by Elton John]. On Captain fantastic and the brown dirt cowboy [CD]. London: Big Pig Music Limited.

g. Mailing list

Format:

Author. (Exact date of posting). Subject line of message. Message posted to followed by name of mailing list, archived at followed by address for the archived version of the message

Example:

Hammond, T. (2000, November 20). YAHC: Handle Parameters, DOI Genres, etc. Message posted to Ref-Links electronic mailing list, archived at http://www.doi.org/mail-archive/ref-link/msg00088.html

h. Computer software

Format:

Author(s). (Year). Title [computer software]. The location: Company.

Example:

Ludwig, T. (2002). PsychInquiry [computer software]. New York: Worth.

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DOI:10.2478/s11533-007-0023-3.

Book:

Jones, E., Farina, A., Hastorf, A., Markus, H., Miller, D., & Scott, R. (1984). *Social stigma: The psychology of marked relationships*. New York: W. H. Freeman.

Chicago style[2]

Article in a journal:

Spitzer, Steven. Review of *The Limits of Law Enforcement*, by Hans Zeisel. *American Journal of Sociology* 91 (1985): 726-29; DOI:10.2478/s11533-007-0023-3.

Book:

Lloyd, Donald A., and Harry R. Warfel. *American English and Its Cultural Setting*. New York: Alfred A. Knopf, 1956.

Harvard style[3]

Article in a journal:

Conley, TG & Galenson, DW 1998, 'Nativity and wealth in mid-nineteenth century cities', *Journal of Economic History*, vol. 58, no. 2, pp. 468-493, DOI:10.2478/s11533-007-0023-3.

Book:

Hodgson, A 1998, *Accounting theory*, John Wiley & Sons, Brisbane.

Oxford style[4]

Article in a journal:

KHOO, G.K. Accounting for leases. *The Chartered Accountant in Australia*, 46(5): Nov. 1975: 19-23: DOI:10.2478/s11533-007-0023-3.

Book:

GIBBS, Graham. *Teaching students to learn: a student-centred approach*. Milton Keynes, Open University Press, 1981.

MLA style[5]

Article in a journal:

Joyce, Michael. "On the Birthday of the Stranger (in Memory of John Hawkes)." Evergreen Review 5 Mar. 1999. 12 May 1999 http://www.evergreenreview.com/102/evexcite/joyce/nojoyce.html. DOI:10.2478/s11533-007-0023-3.

Book:

Bird, Isabella L. A Lady's Life in the Rocky Mountains. New York, 1881. Victorian Women Writers Project. Ed. Perry Willett. 27 May 1999. Indiana U. 4 Oct. 1999

IEE style[6]

Article in a journal:

I.E. Sutherland, R.F. Sproull, and R.A. Schumaker, "A Characterization of 10 Hidden-Surface Algorithms," *ACM Computing Surveys*, Mar. 1974, pp. 1-55, DOI:10.2478/s11533-007-0023-3.

Book:

W.M. Newman and R.F. Sproull, *Principles of Interactive Computer Graphics*, McGraw-Hill, 1979, p. 402.

Vancouver style[7]

Article in a journal:

You CH, Lee KY, Chey WY, Menguy R. Electrogastrographic study of patients with unexplained nausea, bloating and vomiting. Gastroenterology 1980;79:311-4; DOI:10.2478/s11533-007-0023-3.

Book:

Eisen HN. Immunology: an introduction to molecular and cellular principles of the immune response. 5th ed. New York: Harper and Row; 1974.

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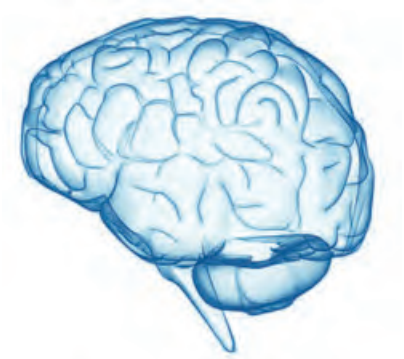

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