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CLINICAL AND ECONOMIC OUTCOMES OF NEW ORAL ANTICOAGULANTS IN ORTHOPAEDICS

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KLINIČKI I EKONOMSKI ISHODI PRIMENE NOVIH ORALNIH ANTIKOAGULANASA U ORTOPEDIJI

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ABSTRACT

Venous thromboembolism, including pulmonary embolism and deep vein thrombosis, is a significant factor in morbidity and mortality of patients. New oral anticoagulants, such as apixaban, dabigatran, and rivaroxaban, have recently demonstrated their safety and efficacy in patients undergoing major orthopaedic surgery. Selection of the appropriate drug should be adjusted according to patient needs. Major bleeding is rare with new oral anticoagulants and is comparable with the bleeding rate associated with low-molecular-weight heparins. Clinical data indicate that therapy with apixaban and rivaroxaban was more effective compared to enoxaparin, while dabigatran has a similar efficacy to enoxaparin. Cost-effectiveness studies of new oral anticoagulants showed that these medicines offer higher efficacy with acceptable costs for the healthcare system, even saving costs in certain cases. Clinical practice in Serbia reflects considerably more frequent use of traditional anticoagulant medication therapy compared to new oral anticoagulants.

Keywords: *new oral anticoagulant, orthopaedic surgery, clinical outcomes, economic outcomes*

SAŽETAK

Venska tromboembolija, koja uključuje plućnu emboliju i duboku vensku trombozu, predstavlja značajan faktor morbiditeta i mortaliteta pacijenata. Novi oralni antikoagulansi, poput apiksabana, dabigatrana i rivaroksabana, su nedavno pokazali svoju bezbednost i efikasnost kod pacijenata koji se podvrgavaju velikim ortopedskim intervencijama. Izbor odgovarajućeg leka treba prilagoditi individualnim potrebama pacijenta. Velika krvarenja su relativno retka u toku primene novih oralnih antikoagulanasa i uporedivi su sa stopom krvarenja prilikom primene nisko-molekularnih heparina. Klinički podaci ukazuju da je terapija apiksabanom i rivarokabanom efikasnija u odnosu na enoksaparin, dok dabigatran ima slične efekte kao enoksaparin. Troškovna isplativost novih oralnih antikoagulanasa potvrđuje veću efikasnost sa prihvatljivim troškovima za zdravstveni sistem, u nekim slučajevima čak i sa uštedama.

Ključne reči: *novi oralni antikoagulansi, ortopedska hirurgija, klinički ishodi, ekonomski ishodi*

INTRODUCTION

Venous thromboembolism (VTE), which includes pulmonary embolism (PE) and deep vein thrombosis (DVT), is a significant factor in morbidity and mortality of patients. The incidence of VTE in the world is approximately 1 person per 1,000 in one year, while 180 000 deaths are annually recorded in the United States as a result of VTE (1-3). In Europe, 1.66 million cases of non-fatal symptomatic VTE are recorded per year (4). In the majority of hospitalized patients, clot predisposing factors are present, especially in orthopaedic and surgical patients. High risk of VTE is present in patients who are undergoing knee and/or hip surgery. According to clinical studies, the percentage of venography that confirmed DVT in major joint surgery without the use of prophylaxis is between 40% and 85% (5, 6). Therefore, pharmacological prophylaxis represents a very important factor in the reduction of morbidity and mortality in patients who are undergoing major orthopaedics surgeries. For many years, warfarin and low-molecular-weight heparins were applied with prophylactic purposes. However, all of these drugs have certain limitations in modern clinical practice (7). In the last several years, several new oral anticoagulants were approved for the prevention of VTE in these patients (8).

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CLINICAL OUTCOMES OF NEW ORAL ANTICOAGULANTS

New oral anticoagulants (NOA), such as apixaban, dabigatran, and rivaroxaban, have recently demonstrated their safety and efficacy. These NOAs are currently considered to be an alternative to warfarin. The new oral anticoagulants have a predictable and stable pharmacokinetics with low potential for interaction. These medicines do not need continuous monitoring or frequent adjustment of dose, and there is a low probability that they will cause intracranial bleeding (8). The development of NOA that is used in orthopaedic surgery has great clinical significance.

Apixaban

Apixaban is a direct and reversible factor Xa inhibitor (9). Studies for the prevention of DVT in animal models showed that apixaban has adequate efficiency. The recommended daily dose is 2.5 mg orally twice daily. To minimize undesirable outcomes, the starting dose should be initiated 12-24 h after the surgery (10). Apixaban has good bioavailability, low clearance and low potential for interactions with other drugs and is eliminated by urine, bile and faeces (11). In 2011, apixaban was approved in Europe for prevention of DVT in patients who had elective hip or knee replacement surgery. For this indication, apixaban is available in more than 50 countries (12). Use of apixaban in the prevention of DVT is supported by 3 clinical trials, known as the ADVANCE clinical studies (13-15). The studies were designed to assess the safety and efficacy of apixaban in more than 11,500 patients, of which 5770 patients were on apixaban and 5,755 patients were on enoxaparin.

ADVANCE-1 (13) and ADVANCE-2 (14) studies have evaluated the use of apixaban in patients who underwent elective knee replacement surgery, while the ADVANCE-3 study (15) evaluated the effects in patients who underwent elective hip replacement surgery. During Phase III in all three ADVANCE studies, the primary efficacy endpoint composed of all VTE and all-cause mortality were followed, while for the safety assessment, the occurrence of bleeding during treatment was followed. Apixaban showed a statistically superior reduction in the primary endpoint compared to enoxaparin in both elective hip or knee replacement surgery. The risk of major bleeding after administration of apixaban was significantly lower compared to enoxaparin (OR = 0.55; 95% CI 0.32, 0.96) (16). Apixaban parameters of efficacy and safety compared to warfarin have been published in 2012, and these results are even more convincing compared to enoxaparin. Incidence of VTE and all-cause mortality were 9% in the apixaban group compared to 26.6% in the warfarin group. The incidence of bleeding was comparable between groups (17).

Dabigatran

Dabigatran is a direct thrombin inhibitor, which consequently results in antiplatelet activity. Dabigatran is indicated for the primary prevention of VTE in adult patients undergoing elective total hip or knee replacement surgery. Key studies have shown that dabigatran has similar efficacy and safety as enoxaparin. Compared to vitamin K antagonists (warfarin), dabigatran provides a number of advantages, including the lack of interaction with food, oral administration, no risk of thrombocytopenia and a predictable anticoagulant effect. Dabigatran clinical trials in the US and Europe estimated its importance for prevention of thromboembolic events. In 2008, dabigatran was approved by the EMA for the primary prevention of VTE in patients who underwent total hip or knee replacement (18). The results of post-marketing studies (RE-study) additionally confirmed the validity of dabigatran use in prevention of VTE in orthopaedic patients compared to low-molecular-weight heparin (19).

Later meta-analysis of the RE-MODEL, RE-NOVATE and RE-MOBILIZE studies showed that there were no significant differences in efficacy between dabigatran and enoxaparin, including the total VTE and mortality (20).

The latest study from 2015 estimated the safety and efficacy of dabigatran for the prevention of VTE in patients undergoing total hip or knee replacement, but for those previously treated with low-molecular-weight heparins. The authors concluded that switching patients from low-molecular-weight heparin to dabigatran is a safe and effective pharmacotherapy. In addition, switching from a subcutaneous to an oral therapy offered an easier, less expensive, and more convenient route of administration for the patients and healthcare professionals (21). As with the other NOA, the major concerns regarding the safety profile are the frequency and severity of bleeding. In all of the abovementioned studies, the safety of dabigatran was comparable to that of the low-molecular-weight heparins. In the RE-studies, a similar safety profile of dabigatran and warfarin for risk of major bleeding was observed. The incidence of relevant bleeding, especially cerebral bleeding, was low in patients who were treated with dabigatran compared to other anticoagulants in the Phase III study. This supports once more a desirable safety profile of dabigatran (22).

Rivaroxaban

Rivaroxaban is a direct and selective factor Xa inhibitor (23). Rivaroxaban is approved in the United States and the European Union for the prevention of VTE in patients undergoing a total hip or knee replacement (24). In four RECORD studies, which included more than 12,500 patients, the efficacy of rivaroxaban was superior compared to enoxaparin in major orthopaedic surgery (25-28). The incidence of DVT and non-fatal PE was significantly lower in the rivaroxaban group compared to the enoxaparin group, regardless of whether it was an elective knee (30% to 50% reduction in incidence of VTE) or hip surgery (decreased for more than 70%). In addition, the safety profiles of these drugs were very similar. The latest international study XAMOS (Xarelto®, use in prophylaxis of surgical venous thromboembolism after major orthopaedic surgery

of the hip or knee) intended to provide additional information on the assessment of the benefits and risks of use of rivaroxaban compared with standard VTE prophylaxis. More than 17,000 patients from 37 countries participated in this study between February 2009 and August 2011. Rivaroxaban was associated with a significantly lower rate of thromboembolic events. The relevant bleeding rate was similar between the two groups. Therefore, it was concluded that the non-intervention study XAMOS confirmed the results of the RECORD clinical trial (29).

ECONOMIC OUTCOMES OF NEW ORAL ANTICOAGULANTS

The cost-effectiveness of therapy certainly plays an important role in the selection of an appropriate anticoagulant drug. There is a continued need for saving time and money in addition to maintaining the simplicity of the drug application. Use of aspirin, due to its low price and simple administration, is shown to be a good alternative to low-molecular-weight heparins after hip or knee surgery (30). New oral anticoagulants play a significant role in reducing cost when compared to low-molecular-weight heparins (31).

Apixaban

A Canadian study analysed the cost-effectiveness of apixaban compared to enoxaparin in prophylactic therapy for DVT in patients undergoing total hip or knee replacement. In the study, two economic models, decision tree and Markov model, were combined. The results showed that apixaban was better (more effective and less expensive) than enoxaparin in treating patients undergoing major orthopaedic surgery. Savings of USD180 to USD270 per patient could be expected with apixaban treatment compared to enoxaparin treatment (32). Budgetary impact for the Spanish health system of apixaban prophylaxis of VTE in patients undergoing total knee or hip replacement showed that the inclusion of apixaban did not have any positive monetary impact, but led to a reduction in the rate of VTE and bleeding (33). In the British guidance of apixaban, it is stated that apixaban was more clinically effective and less expensive than enoxaparin; however, it was also concluded that there was insufficient clinical evidence to determine whether apixaban was more or less clinically effective than another NOA (34).

Dabigatran

For in- or out-patients in need for thromboprophylaxis after orthopaedic surgery, the total cost of enoxaparin administration becomes higher than that of dabigatran, making dabigatran therapy cost-effective for these groups of patients (22). The British guidance from 2008 concluded that dabigatran was likely to be of equivalent clinical and cost effectiveness to low-molecular-weight heparins for the prevention of VTE but had advantages in route of administration since it did not have a need for monitoring, and demonstrated a reduction in administration costs and better adherence to treatment (35).

Rivaroxaban

The cost-effectiveness of rivaroxaban was assessed in several countries based on the data from the RECORD studies. In the Germany study, prophylaxis with rivaroxaban reduced the number of VTE events and provided cost savings (EUR27.3 saving per patient treated) compared with enoxaparin (36). A cost-effectiveness study conducted for the Swedish health system showed that in patients undergoing total hip replacement, the extended prophylaxis for 35 days with rivaroxaban was cost-effective compared to 14 days of prophylaxis with enoxaparin or dalteparin. In total knee replacement patients, 14 days of rivaroxaban treatment was more effective and less expensive than 14 days of low-molecular-weight heparin prophylaxis for VTE (37).

CLINICAL PRACTICE IN SERBIA

Despite the availability of NOA, the dominant place in clinical practice in Serbia still use low-molecular-weight heparins and older anticoagulants, such as warfarin or acenocoumarol. The main reason for this is the limited availability of NOA in Serbia. Although all three NOAs have marketing authorization in Serbia, only dabigatran and rivaroxaban are on the reimbursement list (38). The supply of these two drugs is based on a centralized public procurement procedure.

Table 1 shows the IMS (IMS Health, IMS Health Database) consumption data of pharmacological agents that are used in the prophylaxis of VTE during the four-year period (2012-2015). All data are presented according to the original packaging. A low-molecular-weight heparin, nadroparin, had the highest consumption over all four years.

Figure 1 shows the consumption of drugs used in the prophylaxis of VTE according to the WHO/DDD methodology. The defined daily dose (DDD) is a unit of measurement that was proposed by the World Health Organization (WHO). DDD is defined as "the assumed average maintenance dose per day for a drug used for its main indication in adults." Unit DDD represents the average dose for an adult weighing 70 kg in primary indication of the ATC classification system. This dose is independent from the price of the pharmaceutical form, while the concentration is in a single form and size of the package (39). The number of inhabitants each year is taken from the website of the Republic Institute for Statistics (40).

The highest consumption of pharmacotherapy for prophylaxis of VTE was recorded during 2015 with a total 8.21 DDD/1000 inhabitants/day. During the four years, the largest share in the consumption belonged to warfarin (41% in 2014; 58% in 2013). During the observed period, NOA consumption growth was recorded.

Table 1. Consumption of drugs used in the prophylaxis of VTE during the period 2012 - 2015 (IMS Health Database)

INN	Package, pharmaceutical form, strength	2012	2013	2014	2015
warfarin	30 tablets, 5 mg	372,867	404,686	325,543	461,714
acenocoumarol	20 tablets, 4 mg	134,614	103	163,665	253,596
heparin	5 ampules, 5000IU/0.25 ml	36,154	35,315	4,850	7
heparin	5 ampules, 5000IU/ml	28,849	34,158	35,743	46,633
heparin	10 ampules, 25000IU/5 ml	27,828	34,890	32,510	31,638
dalteparin	10 prefilled syr., 2500IU/0.2 ml	15,831	19,168	13,550	13,880
dalteparin	10 prefilled syr., 5000IU/0.2 ml	15,061	12,834	14,478	10,660
dalteparin	10 prefilled syr., 10000IU/ml	60	74		50
enoxaparin	2 prefilled syr., 2000IU/0.2 ml	24,552	27,528	4,048	27
enoxaparin	10 prefilled syr., 2000IU/0.2 ml		3	3,499	3,679
enoxaparin	2 prefilled syr., 4000IU/0.4 ml	103,176	117,359	10,162	3
enoxaparin	10 prefilled syr., 4000IU/0.4 ml		807	23,925	27,017
enoxaparin	2 prefilled syr., 6000IU/0.6 ml	72,912	84,697	5,621	
enoxaparin	10 prefilled syr., 6000IU/0.6 ml		548	18,659	20,696
enoxaparin	2 prefilled syr., 8000IU/0.8 ml	47,661	55,532	989	1
enoxaparin	10 prefilled syr., 8000IU/0.8 ml		402	9,766	10,455
nadroparin	10 prefilled syr., 2850IU/0.3 ml	118,549	131,788	156,425	161,072
nadroparin	10 prefilled syr., 3800IU/0.4 ml	20,050	26,674	30,968	42,319
nadroparin	10 prefilled syr., 5700IU/0.6 ml	41,299	52,753	64,467	73,911
reviparin	10 prefilled syr., 1750IU/0.25 ml	1.407			
reviparin	10 prefilled syr., 3436IU/0.6 ml	1,463			
fondaparinux	10 prefilled syr., 2.5 mg/0.5 ml	883	1,460	1,036	1,370
rivoroxaban	10 tablets, 10 mg	95	860	1,944	2,577
rivoroxaban	28 tablets, 15 mg		48	1,153	4,357
rivoroxaban	42 tablets, 15 mg		13	76	64
rivoroxaban	28 tablets, 20 mg		189	2,942	8,798
dabigatran	10 capsules, 110 mg	102	101	296	236
dabigatran	30 capsules, 75 mg	131	243	356	357
dabigatran	10 capsules, 75 mg	40	254	354	379
dabigatran	30 capsules, 110 mg	93	254	225	316
dabigatran	60 capsules, 150 mg		493	4,032	9,610
dabigatran	60 capsules, 110 mg		662	5,053	12,235

Table 2 shows the volume of drugs used in the prevention of VTE, which was obtained through public procurement. The table shows data on an annual basis for 2014 and adjusted data for 2015, considering the different time durations of public procurement.

CONCLUSIONS

In the future, an increase in the number of hip and knee operations is expected. For this reason, safe and effective prophylaxis is essential to reduce mortality and morbidity related to VTE. The NOAs have the potential to reduce the incidence of VTE after total hip and knee replacement. Therefore, NOAs can contribute to significant savings through the reduction of VTE rate, improved safety and reduced costs of monitoring and administration.

Implementation of NOAs in prophylaxis of VTE is easy and straightforward, but there are specific pharmacological differences between apixaban, rivaroxaban and dabigatran. Selection of the appropriate drug should be adjusted according to the patient needs. Major bleeding is rare with NOA and comparable with the bleeding rate with low-molecular-weight heparins. All NOAs have predictable pharmacokinetics with a short half-life. Clinical data indicate that therapy with apixaban and rivaroxaban was more effective compared to enoxaparin, while dabigatran has a similar efficacy to that of enoxaparin. Cost-effectiveness studies of NOAs showed that these medicines offer higher efficacy with acceptable

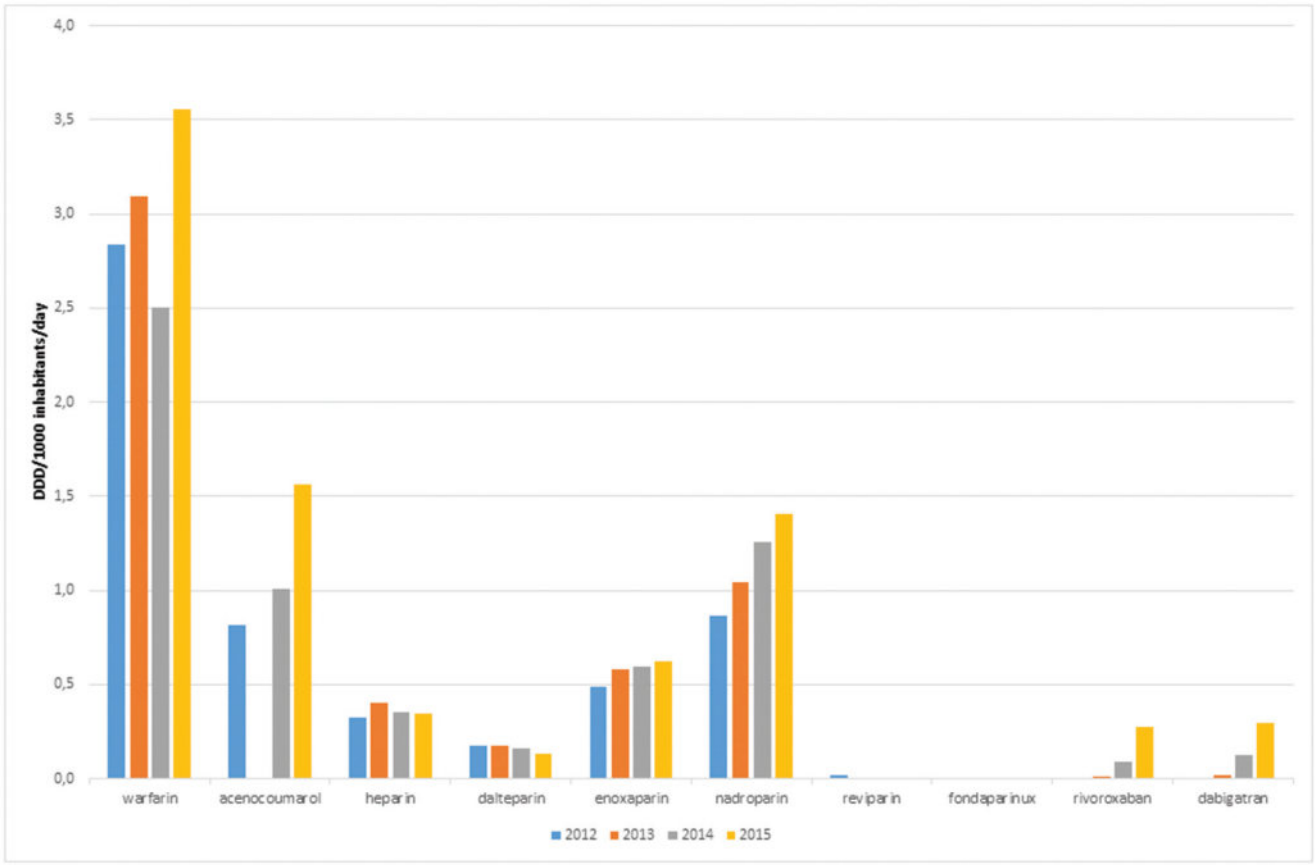

Figure 1. Consumption of drugs used in the prophylaxis of VTE according to the WHO/DDD methodology from 2012 to 2015

Table 2. Consumption of drugs used in the prophylaxis of VTE through centralized public procurement in 2014 and 2015

INN	Pharmaceutical form, strength	Number of unit doses in 2014	Number of unit doses in 2015
warfarin	30 tablets, 5 mg (original pack)		140,000*
acenocoumarol	20 tablets, 4 mg (original pack)		45,250*
heparin	ampule, 5000IU/0.25 ml	140,600	74,666
heparin	ampule, 5000IU/1 ml	99,500	111,376
heparin	ampule, 25000IU/5 ml	146,800	253,960
dalteparin	prefilled syr., 2500IU/0.2 ml	119,400	200,464
dalteparin	prefilled syr., 5000IU/0.2 ml	95,600	223,664
dalteparin	prefilled syr., 10000IU/ml	1,000	104
enoxaparin	prefilled syr., 2000IU/0.2 ml	32,700	25,096
enoxaparin	prefilled syr., 4000IU/0.4 ml	154,900	160,000
enoxaparin	prefilled syr., 6000IU/0.6 ml	115,000	144,512
enoxaparin	prefilled syr., 8000IU/0.8 ml	63,600	78,400
nadroparin	prefilled syr., 2850IU/0.3 ml	897,000	1,160,808
nadroparin	prefilled syr., 3800IU/0.4 ml	139,000	229,784
nadroparin	prefilled syr., 5700IU/0.6 ml	287,000	469,776
rivoroxaban	tablet, 10 mg		3,000
dabigatran	capsule, 75 mg		200
dabigatran	capsule, 110 mg		800

* - Refers to the original packaging and not to individual forms (tablet)

costs for healthcare system and, in certain cases, even presented with savings. NOAs represent an alternative to traditional anticoagulants due to the following advantages: no titration of the dose, low capacity for interactions and no need for monitoring. In that line, NOAs are considered to be effective, acceptable in safety profile and a cost-effective alternative to parenteral pharmacological prophylaxis. Clinical practice in Serbia reflects considerably more frequent use of traditional anticoagulant medication therapy compared to NOA.

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THE EFFECTS OF CHRONIC ADMINISTRATION OF CISPLATIN ON OXIDATIVE STRESS IN THE ISOLATED RAT HEART

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EFEKTI HRONIČNE PRIMENE CISPLATINE NA OKSIDACIONI STRES IZOLOVANOG SRCA PACOVA

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ABSTRACT

Taken into consideration that molecular and cellular mechanisms involved in cardiotoxicity are still not clear the aim of this study was to compare the production of oxidative stress parameters in the isolated rat heart between animals chronically treated with cisplatin and saline. The hearts of male Wistar albino rats (n = 24, 12 per group, age 8 weeks, body mass 250±50 g) were excised and perfused according to the Langendorff technique at gradually increased coronary perfusion pressures (40-120 cmH_2O). We followed the production of superoxide anion radicals, hydrogen peroxide, and nitrites and also index of lipid peroxidation during the changes of coronary perfusion pressure (CPP) (from 40 to 120 cm H_2O) in coronary venous effluent. Modifications CPP were performed in order to determined if oxidative stress is involved in coronary endothelium response in conditions of hypoxia (lower than 60 cm H_2O) and hyperoxia (higher than 80 cm H_2O).

Based on the results of this research we can conclude that with enhancement of CPP the values of oxidative stress statistically increased. However, this increment is more prominent in control group as a result of preserved endothelium and its more powerful response to hyperoxia. On the other hand, damaged endothelium of cisplatin-treated animals had weaker response to hyperoxia, and also lower antioxidant capacity.

Keywords: *cisplatin, change of coronary perfusion pressure, isolated rat heart, oxidative stress*

SAŽETAK

Uzimajući u obzir činjenicu da molekulski i ćelijski mehanizmi nastanka kardiotoksičnosti nisu u potpunosti poznati cilj ovog istraživanja bio je da se uporedi nastajanje i oslobađanje parametara oksidacionog stresa kod izolovanog srca pacova između grupa tretiranih cisplatinom i fiziološkim rastvorom. Srca Wistar albino pacova (n = 24, 12 po grupi, starosti 8 nedelja, telesne mase 250±50 g) su izolovana i perfundovana po Langendorff tehnici pri rastućem koronarnom perfuzionom pritisku. Praćena je produkcija superoksid anjon radikala, vodonik-peroksida, nitrita i indeksa lipidne peroksidacije pri promeni koronarnog perfuzionog pritiska (CPP) (od 40 do 120 cm H_2O). Promena CPP je sprovedena sa ciljem da se utvrdi da li je oksidacioni stress uključen u odgovor koronarnog endotela u uslovima hipoksije (pritisci niži od 60 cm H_2O) i hiperoksije (pritisci viši od 80 cm H_2O).

Na osnovu rezultata ove studije možemo zaključiti da sa porastom vrednosti CPP vrednosti oksidacionog stresa statistički značajno rastu. Međutim ovo povećanje je izraženije u kontrolnoj grupi usled bolje očuvanosti endotela i njegovog jačeg odgovora na hiperoksiju. Sa druge strane endotel kod životinja tretiranih cisplatinom je oštećen i ima lošiju sposobnost da odgovori na hiperoksiju kao i smanjen antioksidacioni kapacitet.

Ključne reči: *cisplatina, promena koronarnog perfuzionog pritiska, izolovano srce pacova, oksidacioni stress*

ABBREVIATIONS

CK - creatine kinaze
CPP - coronary perfusion pressure
cTnI - Cardiac Troponin I
GSH - glutathione
H_2O_2 - hydrogen peroxide
NADPH - nicotinamide adenine dinucleotide phosphate hydrogen
NO - nitric oxide
NO_2^- - nitrites
Nrf2 - nuclear factor erythroid 2-related factor 2
$O_2^{\cdot-}$ - superoxide anion radical
ROS - Reactive oxygen species
SOD - superoxide dismutase
TBARS - thiobarbituric acid reactive substances

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INTRODUCTION

Platinum chemotherapeutic agents are the principal therapeutics in the treatment of various cancers, including ovarian, testicular, and bladder cancer. Cis-diamminedichloroplatinum(II) (cisplatin), as the parent compound, is one of the most-used and the most effective platinum-derived agents in treatment of malignancies. Cisplatin binds to DNA, forming inter and intra-strand cross-links, resulting in defective DNA templates, arrest of DNA synthesis in rapidly dividing cancer cells (1). However, its therapeutic use is limited by cellular resistance and severe side-effects in normal tissues (2-4). In the literature the most commonly mentioned side-effects are nephrotoxicity, ototoxicity and peripheral neuropathy (5-7). Although not often cardiotoxicity is very serious and difficult side effect associated with cisplatin use. Acute vascular events were recorded during the drug administration, and may be associated with an increased long-term cardiovascular risk (8, 9). According to literature data cardiovascular events associated with cisplatin treatment are electrocardiographic changes, arrhythmias, myocarditis, cardiomyopathy and congestive heart failure (10). Toxic effects of cisplatin may be due to inhibition of protein synthesis, DNA damage, peroxidation of the cell membrane, mitochondrial dysfunction (11).

The mechanism of antitumor effects of cisplatin is almost fully known, but molecular and cellular mechanisms involved in cardiotoxicity are still not clear. Some experimental and clinical studies support the opinion that an increase of biomarkers of oxidative stress is involved in cisplatin's cardiotoxicity (12). It's well known that toxicity in numerous tissues and organ system such as liver, kidney, ear, and cardiovascular and nervous systems, induced by drugs is mediated with oxidative stress. There are a lot of evidences that oxidative stress had important role in acute kidney injury induced by cisplatin usage. Reactive oxygen species (ROS) directly act on cell components, including lipids, proteins and DNA, destroying their structure (13, 14).

The aim of this study was to compare the production of oxidative stress parameters in the isolated rat heart in animals chronically treated with cisplatin. On this way, we wanted to assess the influence of this antitumor drug on ROS generation and potential oxidative damages. Modifications of coronary perfusion pressure were performed in order to determined if oxidative stress is involved in coronary endothelium response in conditions of hypoxia (lower than 60 cm H_2O) and hyperoxia (higher than 80 cm H_2O).

MATERIAL AND METHODS

Experimental protocol

This was chronic experimental study, conducted on male Wistar albino rats, (body weight 250±50g) aged 8 weeks. The animals were divided into two groups (12 animals per group): experimental and control. Experimental group was treated with cisplatin for 4 weeks (4mg/kg body weight, once a week, intra-peritonealy). Control group was treated with saline for 4 weeks, once week, intra-peritonealy. After the four weeks of experimental protocol, the animals were anasthetized with ketamine (10mg/kg) and xylazine (5mg/kg) and then euthanized via cervical dislocation (Schedule 1 of the Animals/Scientific Procedures, Act 1986, UK).

All research procedures were carried out in accordance with European Directive for welfare of Laboratory animals N^0 86/609 EEC and principles of Good Laboratory Practice (GLP), and approved by Ethical committee of the Faculty of Medical Science.

Isolated rat heart preparation

Following a quick thoracotomy and rapid cardiac arrest by superfusion with ice-cold isotonic saline, the hearts were promptly excised and attached to the Langendorff apparatus via aortic cannulation and then were retrogradely perfused under a constant perfusion pressure of 70 cmH_2O with complex Krebs-Henseleit solution. The composition of the Krebs-Henseleit buffer (perfusion medium) was as follows (in mmol/l): NaCl (118); KCl (4.7); $CaCl_2 \times 2H_2O$ (2.5); $MgSO_4 \times 7H_2O$ (1.7); $NaHCO_3$ (25); KH_2PO_4 (1.2); glucose (5.5). It was equilibrated with gas mixture (5% CO_2-95% O_2) at 37°C, (pH 7.4).

Perfusion of the isolated rat heart

After 30 minutes period of stabilization at constant CPP of 70 cm H_2O experimental protocol was conducted. The experimental protocol implied changing of perfusion pressure from 40 cm to 120 cm. The isolated hearts were stabilized at each perfusion pressure and then the samples of coronary venous effluent were collected for biochemical analyses. The recorded values during the first measure at each perfusion pressure (40, 60, 80, 100 and 120 cm H_2O) were marked as values of control conditions, while the second measure of parameters at each coronary perfusion pressure were marked as experimental conditions.

Biochemical assays

Index of lipid peroxidation (Thiobarbituric Acid Reactive Substances – TBARS)

The degree of lipid peroxidation in coronary venous effluent was estimated by measuring of thiobarbituric acid reactive substances (TBARS) using 1 % thiobarbituric acid (TBA) in 0.05 sodium hydroxide (NaOH) incubated with coronary effluent at 100 °C for 15 minutes and read at 530 nm. Krebs-Hensenleit solution was used as a blank probe (15).

Nitrite determination

Nitric oxide was assessed as nitrite and quantified by the spectrophometric method using the *Griess*-reagent. 0.5 ml of perfusate was precipitated with 200 µl of 30%

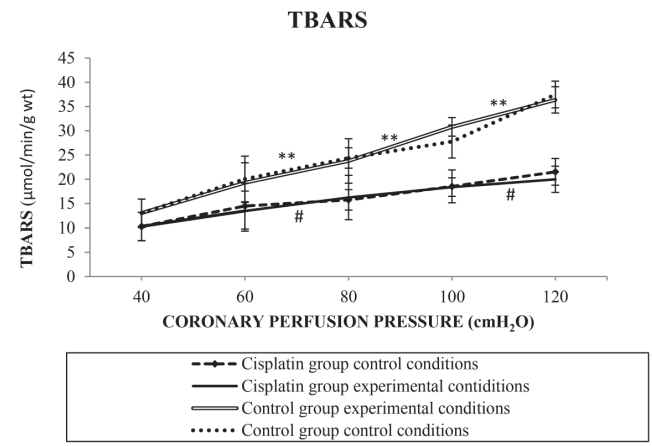

Figure 1 The effects of chronic adiministration of cisplatin and saline on index of lipid peroxidation in coronary venous effluent throughout changing the coronary perfusion pressure
All values are expressed as mean ± SD. Wilcoxon signed rank test were used in statistical analysis, *p* values less than 0.05 (marked with * or # depending on groups) were considered to be statistically significant and *p* values less than 0.01 (marked with ** or ## depending on groups) were considered to be statistically high significant.

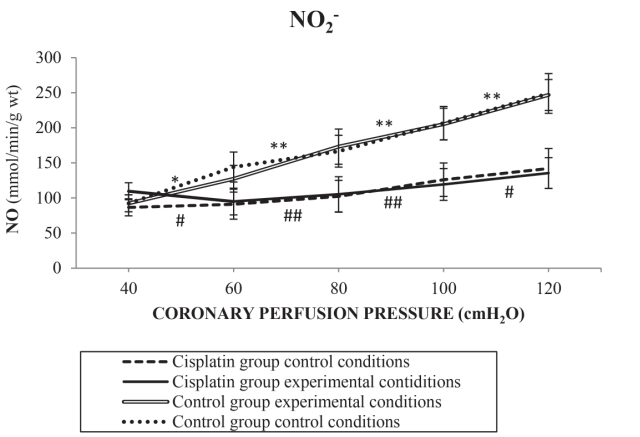

Figure 2 The effects of chronic adiministration of cisplatin and saline on production of nitrites in coronary venous effluent throughout changing the coronary perfusion pressure
All values are expressed as mean ± SD. Wilcoxon signed rank test were used in statistical analysis, *p* values less than 0.05 (marked with * or # depending on groups) were considered to be statistically significant and *p* values less than 0.01 (marked with ** or ## depending on groups) were considered to be statistically high significant.

sulfosalicylic acid, vortexed for 30 min and centrifuged at 3000 x g. Equal volumes of the supernatant and Griess's reagent, containing 1% sulfanilamide in 5% phosphoric acid/0.1% napthalene ethylenediamine-dihydrochloride was added and incubated for 10 min in the dark and read at 543 nmol/l. The nitrite levels were calculated by using sodium nitrite as a standard (16).

Superoxide determination

The level of superoxide anion radical ($O_2^{\cdot-}$) was measured using Nitro Blue Tetrazolium (NBT) reaction in TRIS-buffer with coronary venous effluent and read at 530 nm. Krebs-Hensenleit solution was used as a blank probe (17).

Hydrogen peroxide determination

The level of hydrogen peroxide (H_2O_2) was measured using phenol red oxidation with H_2O_2 from coronary venous effluent in the presence of horse-radish peroxidase and read at 610 nm (18).

Substances

All substances necessary for the preparation of Krebs-Henseleit buffer as well as Cisplatin were purchased from the company Sigma-Aldrich GmbH, Germany. For treatment of control group and dissolution of cisplatin was used saline (0.9% NaCl, Hemofarhospital Logica) comercially purchased.

Statistical Analysis

All values are expressed as mean ± SD. Wilcoxon signed rank test and Mann Whitney test were used in statistical analysis, *p* values less than 0.05 were considered to be statistically significant and *p* values less than 0.01 were considered to be statistically high significant. Wilcoxon signed rank test (for difference between related samples) was used for analyzed the difference between biochemical parameter at different coronary perfusion pressure. The Mann Whitney test was used for analyzed the difference between biochemical parameter in different groups at same coronary perfusion pressure. The statistical analysis was performed using SPSS 19.0 for Windows.

RESULTS

The effects of chronic adiministration of cisplatin and saline on TBARS values in coronary venous effluent throughout changing the coronary perfusion pressure

In a group treated with cisplatin with increase of CPP TBARS values increased, but that changes were statistically significant between 60 cm and 80 cm and also between 100 cm and 120 cm. On the other hand, in control group that increase was greater and statistically high significant (Figure 1). Comparing the effects between the groups it can be observed statistically high significant difference at higher CPP (from 80 to 120 cm, Table 1).

The effects of chronic adiministration of cisplatin and saline on production of nitrites in coronary venous effluent throughout changing the coronary perfusion pressure

In the both tested groups comparing the effects of changing CPP at production of nitrites there are statistically high significant differences (Figure 2). Also there were statistically high significant differences in production of nitrites at all examined CPP except at 40 cm between groups (Table 1).

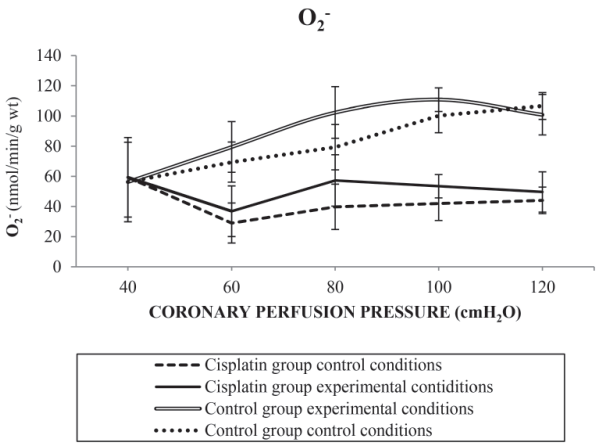

Figure 3 The effects of chronic adiministration of cisplatin and saline on production of superoxide anion radical in coronary venous effluent throughout changing the coronary perfusion pressure
All values are expressed as mean ± SD.

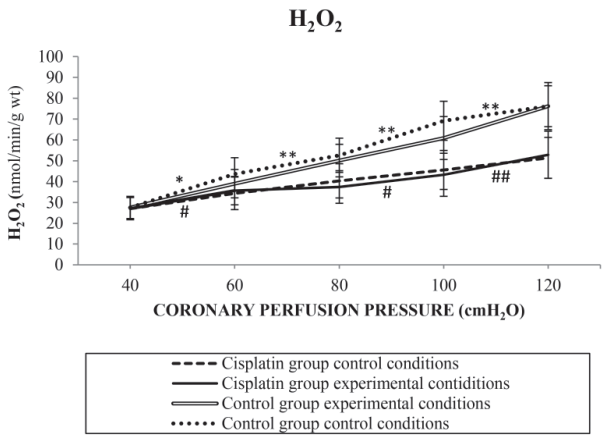

Figure 4 The effects of chronic adiministration of cisplatin and saline on production of hydrogen peroxide in coronary venous effluent throughout changing the coronary perfusion pressure
All values are expressed as mean ± SD. Wilcoxon signed rank test were used in statistical analysis, *p* values less than 0.05 (marked with * or # depending on groups) were considered to be statistically significant and *p* values less than 0.01 (marked with ** or ## depending on groups) were considered to be statistically high significant.

The effects of chronic adiministration of cisplatin and saline on production of superoxide anion radical in coronary venous effluent throughout changing the coronary perfusion pressure

There were no statistically significant changes in production of superoxide anion radical during the CPP changes in both groups (Figure 3). Also there was no statistically significant difference in production of superoxide anion radical between groups at same CPP values (Table 1).

The effects of chronic adiministration of cisplatin and saline on production of hydrogen peroxide in coronary venous effluent throughout changing the coronary perfusion pressure

In a group chronically treated with cisplatin statistically significant changes in production of hydrogen peroxide were existed. With an increase of CPP the production of hydrogen peroxide rised. On the other hand, in control group, statistically high significant changes in production of hydrogen peroxide were recorded with increase of CPP (Figure 4). Comparing the effects of changing of coronary perfusion pressure in these groups, we can notice that there were statistically high significant differences in production of hydrogen peroxide at higher CPP (from 80 to 120 cm, Table 1).

DISCUSSION

Previously was mentioned that cisplatin usage is associated with a numerous side effects, whereby one of the most commonly and detail characterized is nephrotoxicity. Cisplatin activates glucose-6-phosphate dehydrogenase and hemoxinase, which increase free radical production and decrease the production of antioxidative enzymes. It increases concentrations of calcium into cells, which leads to activation of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase and stimulating of ROS production. There are evidence that cisplatin-treated animals have increased levels of superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) (19). Free radicals, formed in this way, induced the damaging of lipid components in cell membrane by peroxidation and mitochondrial dysfunction (20, 21). Beside the increment of ROS production, the achievement of reactive nitrogen species was observed in

Table 1. Comparison of oxidative stress between cisplatin and control groups at different coronary perfusion pressure

Control group vs. Cisplatin group					
	40 CPP	**60 CPP**	**80 CPP**	**100 CPP**	**120 CPP**
Index of lipid peroxidation	> 0.05	0.020	0.004	0.001	0.000
Nitric oxide	> 0.05	0.000	0.000	0.000	0.000
Superoxide anion radical	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Hydrogen peroxide	> 0.05	> 0.05	0.001	0.000	0.000

Mann Whitney test were used in statistical analysis, p values less than 0.05 were considered to be statistically significant and p values less than 0.01 were considered to be statistically high significant.

cisplatin-induced nephrotoxicity. Concentration of nitric oxide and peroxynitrite in kidney were increased in animals treated with cisplatin. Peroxynitrite, which is generated by the reaction of nitric oxide (NO) with superoxide, is a strong oxidant that can damage subcellular organelles, membranes. Peroxynitrite induced changing of protein structure and function, lipid peroxidation, chemical cleavage of DNA and reduction in cellular defenses by oxidation of thiol pools. Aforementioned claims can serve as evidence that peroxynitrites are involved in cisplatin-induced nephrotoxicity. On the other hand it is still controversial if nitric oxide had toxic role in kidney injury (22, 23).

The fact that production of ROS is fundamental mechanism in nephrotoxicity, were rised attention of scientists to correlate production of ROS with occurrence of cardiotoxicity. There are a few papers which describe that concomitant use of antioxidants with cisplatin can reduce cardiotoxicity (24-26). Rosic and colleagues assessed the protective effects of N-acetylcysteine (NAC) on cisplatin-induced changes in myocardium (27). Results of their study showed that NAC coadministration with cisplatin mitigated cisplatin-induced disturbances of cardiodynamic and oxidative stress parameters, as well as morphological changes in myocardium and coronary blood vessels, by reduction of oxidative stress. As a results of increase production of ROS transcription and translocation of nuclear factor erythroid 2-related factor 2 (Nrf2) into the nucleus occurred. Activation of this factor induces the expression of many genes involved in synthesis of different antioxidative enzymes and heme oxygenase-1, which are important to protect the cells against oxidative stress and inflammation. Increased production of ROS can lead to increment in the expression of nuclear factor kappa B and production of pro-inflammatory cytokines such as tumor necrosis factor-alpha, chemokines such as monocyte chemoattractant protein-1. All these factors induce apoptosis and consequently myocardial injury (28, 29).

Results of our study showed that in both groups increment of coronary perfusion pressure values causes increase in production of oxidative stress biomarkers (Figure 1-4). The values of superoxide anion radical enhanced with increase of CPP, but that changes between two different CPP were not statistically significant (Figure 3). Also when we compare the values of superoxide anion radical between control and cisplatin group we didn't get any statistically significant difference (Table 1). On the other hand, the values of all other examined biomarkers of oxidative stress have statistically significant increased with enhancement of CPP (Figure 1, 2, 4) in both groups. Likewise, there was statistically significant difference in these parameters between groups. Based on the results of this study (Table 1) we can observe that there is no difference in production of oxidative stress biomarkers in conditions of hypoxia between groups.

Two groups of authors showed that animals treated with saline had greater levels of reduced glutathione and superoxide dismutase in heart tissue, than animals treated with cisplatin. These results demonstrated that animals treated with cisplatin had lower antioxidant capacity than animals treated with saline. In accordance to this we can assume that in our study animals treated for 4 weeks with cisplatin had relieved capacity for struggle with free radicals. So despite the fact that in coronary effluent of control group levels of free radical were higher, antioxidant capacity of these isolated hearts is preserved, and damages induced by changes of CPP will be lower than in cisplatin group. Also these authors confirm that administration of single dose of cisplatin induced increase of markers of heart injury, such as: levels of LDH and creatine kinaze (CK), cardiac troponin I (cTnI) in serum, as well as cardiac CK-MB index and CK-MB activities (24, 30). El-Sawalhi and coworkers also compare the effects of administration of cisplatin and saline on antioxidant capacity. These researchers found that administration of cisplatin induced a significant decrease of catalase and gluthatione peroxidase activity in postmitochondrial and mitochondrial fractions of heart tissue (25). Cardiac tissue generally had very low level of antioxidant enzymes such as superoxide dismutase (SOD) and catalase. Additional decreased of SOD activity in cisplatin-treated group can be explained by losing of copper and zinc which are essential for activity of this enzyme (31). On the other hand cisplatin had a great affinity to sulfur contained into glutathione (GSH), so conjucation of GSH by cisplatin caused depletion of GSH and decrease of redox state. Also the reduction of GSH may be explained by decrease activity of glutathione reductase induced by direct attack of cisplatin (32, 33). El-Sawalhi with coworkers in their research also showed that treatment with cisplatin induced increase of NADPH oxidase activity (25). NADPH oxidase is a major source of endoplasmic reticulum stress, and it be reported that plays an essential role in cisplatin-mediated ROS generation. So NADPH could initiate oxidative stress at early stages of cardiotoxicity and together with other enzymes acts synergistically to augment of oxidative stress (34).

Based on the results of this research we can conclude that with enhancement of coronary perfusion pressure the values of oxidative stress statistically significant increase. However, this increment is more present in control group as a result of preserved endothelium and its more powerful response to hyperoxia. On the other hand damaged endothelium of cisplatin-treated animals had weaker response to hyperoxia, and also lower antioxidant capacity. Finding of present study help in understanding of connection between oxidative stress and cisplatin usage, and thus elucidate molecular interactions involved in its mechanisms of action.

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THE EFFECT OF TIGECYCLINE ON THE BINDING OF FLUOROQUINOLONES TO HUMAN SERUM ALBUMIN

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DEJSTVO TIGECIKLINA NA VEZIVANJE FLUOROHINOLONA ZA HUMANI SERUMSKI ALBUMIN

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ABSTRACT

The co-administration of several drugs in multidrug therapy may alter the binding of each drug to human serum albumin (HSA) and, thus, their pharmacology effect. Therefore, in this study, the interaction mechanism between HSA and two fluoroquinolones (FQs), sparfloxacin (SPF) and levofloxacin (LVF), was investigated using fluorescence and absorption methods in the absence and presence of the competing drug-tigecycline (TGC). The the UV-Vis and fluorescence spectroscopy results showed that the fluorescence quenching of HSA was a result of the formation of the HSA-SPF and HSA-LVF complexes. The fluorescence quenching of HSA-TGC revealed that tigecycline can regulate the binding sites, binding mode and binding affinity of fluoroquinolones. The binding constants (K_A) and binding sites (n) of the interaction systems were calculated. The results confirmed that the K_A values of the HSA-FQ system decreased in the presence of TGC, indicating that TGC can affect the binding ability of FQ for HSA. This interaction may increase the free plasma concentration of unbound FQ and enhance their pharmacology effect.

Keywords: *Fluorescence, Human serum albumin, Fluoroquinolones, Tigecycline.*

SAŽETAK

Istovremena primena nekoliko lekova, u multilek terapiji, može izmeniti njihovo vezivanje za humani serumski albumin (HSA) i njihov farmakološki efekat. Zbog toga, u ovom radu je proučavan mehanizam interakcije između HSA i dva fluorohinolona (FQs): sparfloksacina (SPF) i levofloksacina (LVF) fluorescentnim i apsorpcionim metodama u odsustvu i prisustvu konkurentskog leka - tigeciklina (TGC). Rezultati UV-Vis i fluorescentne spektroskopije su pokazali da je gašenje fluorescencije u HSA rezultat formiranja HSA-SPF i HSA-LVF kompleksa. Gašenje fluoroscencije u HSA-TGC je pokazalo da tigeciklin može regulisati mesta vezivanja, način vezivanja i afinitet vezivanja fluorohinolona. Konstante vezivanja (K_A) i broj vezujićih mesta (n) za interakcije u sistemu su izračunate. Rezultati su potvrdili da su vrednosti K_A u HSA-FQ sistemu, smanjene u prisustvu TGC, a to ukazuje da TGC može da utiče na sposobnost vezivanja FQ za HSA. Ova interakcija može povećati slobodanu koncentraciju u plazmi nevezanog FQ i poboljšati njegov farmakološki efekat.

Keywords: *Fluorescencija, Humani serumski albumin, Fluorohinoloni, Tigeciklin.*

INTRODUCTION

Human serum albumin (HSA) presents the most abundant serum protein in human plasma. HSA plays an important role in controlling distribution, excretion, therapeutic efficacy and drug toxicity in the human body (1-3). HSA possesses several low- and high-affinity ligand binding sites that have been identified as site I and site II. Each domain can be subdivided into subdomains A and B and is responsible for the binding of most drugs with the HSA (4, 5).

Whenever co-administration of several drugs is needed, their competition for the same binding site or conformational changes in protein may occur. This leads to the increased or decreased concentration of the free drug fraction, which results in an enhanced or reduced therapeutic efficacy of the drug. Fluoroquinolones (FQs) belong to a group of antibacterial agents. Their mechanism of action consists of inhibiting different kinds of enzymes, such as homologous type 2, topoisomerase, DNA gyrase and DNA topoisomerase 4, which play a crucial role in chromosome function and replication (6). Fluoroquinolones have a wide range of use. Their application has been reported in an em-

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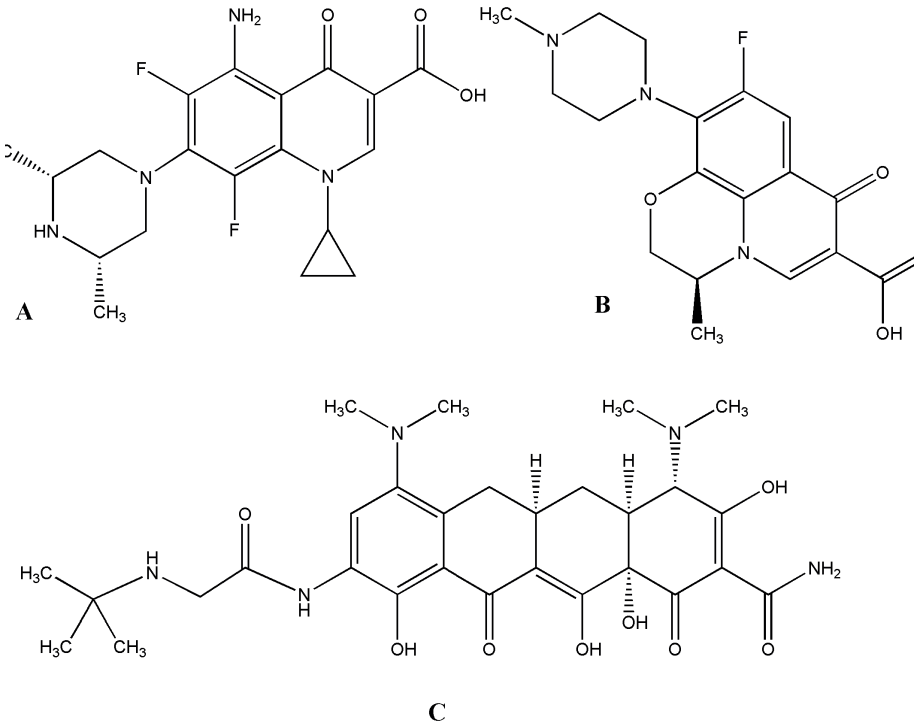

Fig. 1. Chemical structures of sparfloxacin (A), levofloxacin (B) and tigecycline (C).

pirical treatment of a variety of infections, especially in the genitourinary, gastrointestinal and respiratory tracts. Tigecycline possesses a similar mechanism of action to tetracyclines and acts by binding to the bacterial 30S ribosomal subunit and inhibiting protein synthesis (7, 8). Tigecycline has been approved for treating complicated intra-abdominal infections as well as skin and skin structure infections (9). The chemical structures of the investigated drugs are shown in Fig. 1.

Spectral methods can reveal the binding of drugs with albumin at low concentrations. The fluorescence quenching technique is often used to monitor molecular interactions because of its high sensitivity, reproducibility and relatively easy use (10, 11). In the present study, the interactions of sparfloxacin and levofloxacin with HSA and the effect of tigecycline on these interactions by UV-Vis and fluorescence spectroscopy were investigated. Additionally, the binding of TGC to HSA was determined in our previously published research (12).

MATERIAL AND METHODS

Chemical and reagents.

Human serum albumin (HSA, lyophilized powder, free fatty acid ≤0.007%, purity ≥96%, catalogue No. A1887), sparfloxacin (SPF, purity ≥98%, catalogue No. 56968), levofloxacin (LVF, purity ≥98%, catalogue No. 28266) and tigecycline (TGC, tigecycline hydrate, purity ≥98%, catalogue No. PZ0021) were purchased from the Sigma-Aldrich Chemical Company (St. Louis, MO, USA). The stock solutions of HSA (20 μmol/L), SPF (0.11 mmol/L), LVF (0.1 mmol/L) and TGC (85.38 μmol/L) were prepared fresh in phosphate buffer (pH 7.4; 0.1 mol/L) containing 0.15 mol/L NaCl, that which was selected to keep the pH value and maintain the ionic strength of the solution. All the above solutions were kept in the dark at 0-4°C in a refrigerator. The sample masses were accurately weighed on a microbalance (Sartorius, ME215S) with a resolution of 0.1 mg. pH measurements were performed with a Beckman F-72 pH meter. All reagents were of analytical reagent grade and used without further purification. Newly double-distilled water was used throughout the experiment.

UV-Vis and fluorescence spectroscopy.

The absorption spectra were recorded on a double beam Lambda 25 UV/Vis Spectrophotometer (PerkinElmer, USA) equipped with 1.0 cm quartz cells. Fluorescence spectra were recorded using a RF-1501 PC spectrofluorometer (Shimadzu, Japan) equipped with a 150 W Xenon lamp source, a 1.0-cm path-length quartz cell and a thermostatic cuvette holder. The excitation and emission bandwidths were both 10 nm.

In each combination, the quenching of HSA fluorescence by fluoroquinolone (FQ) was studied in the absence and presence of the competing drug, tigecycline (TGC). In the first set of experiments, the binding of each drug to HSA was studied under the same experimental conditions. The concentration of HSA was fixed at 2.0 μmol/L, and those of SPF and LVF were varied. In the second set of experiments, the binding of FQ to HSA was determined simultaneously with TGC. The concentrations of HSA and

TGC were fixed at 2.0 μmol/L, and the concentrations of SPF and LVF were varied. The same sets of samples were used to obtain the UV-Vis spectra. All measurements were performed at a temperature of 298 K.

RESULTS

Fluorescence studies.

The intrinsic fluorescence of HSA when it is excited at 295 nm is due primarily to the presence of the two tryptophan residues: Trp-134 and Trp-212. Trp-212 is located within a hydrophobic binding pocket in the IIA sub-domain of the protein, and Trp-134 is located on the surface of the albumin molecule and is more exposed to the environment (13). Although the tyrosine residue can also contribute to fluorescence, it presents a very weak emission (14). An important characteristic of the intrinsic fluorescence of HSA is that it is very sensitive to its microenvironment; it would be quenched if there is even a small change in the local surroundings of HSA, such as the biomolecular binding, protein conformation and denaturation (15). All fluorescence emission spectra were recorded from 300 to 450 nm, with excitation at 295 nm. It was necessary to distinguish between the static fluorescence quenching mode (resulting from the formation of a ground state complex between the fluorophore and the quencher) and the dynamic quenching mode (resulting from a collision between the fluorophore and the quencher). To confirm the quenching mechanism, the fluorescence data were analyzed at 298 K using the well-known Stern–Volmer Eq. (1) (16):

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q] = 1 + K_{SV}[Q] \quad (1)$$

where F and F_0 are the fluorescence intensities of the protein in the presence and absence of the quencher, respectively. K_q is the quenching rate constant of the biomolecule, [Q] is the quencher concentration, K_{SV} is the Stern–Volmer constant and τ_0 is the lifetime of the fluorophore in the absence of the quencher [τ_0 is approximately 10^{-8} s]. If the biomolecular quenching constants are larger than the limiting diffusion rate constant of the biomolecule [2.0 x 10^{10} L M^{-1} s^{-1}] (17), the quenching is initiated not by the dynamic collision, but by the protein complex.

For the static quenching process, when small molecules bind independently to a set of equivalent sites on a biomacromolecule, the equilibrium between the free and bound molecules could be represented by the well-known Eq. (2) (16-18):

$$\log\frac{F_0 - F}{F} = \log K_A + n\log[Q] \quad (2)$$

where K_A is the binding constant or the apparent association constant for drug-protein interaction, n is the number of binding sites per HSA molecule and [Q] is the concentration of quencher.

The quenching of the intrinsic fluorescence of HSA by sparfloxacin and levofloxacin was studied. The fluorescence spectra of HSA were recorded in the presence of increasing amounts of FQ (Fig. 2 and Fig. 3).

As shown in Fig. 2 and Fig. 3, the fluorescence intensity of HSA decreased regularly with increasing concentrations of FQ, but the emission maximum and shape of the peaks remained almost unchanged. This indicated that SPF and LVF could bind to HSA. Upon drug binding, the fluorescence spectra of HSA were accompanied by a shift in emission wavelength towards a lower wavelength (blueshift) in the case of SPF and towards a higher wavelength (redshift) in the case of LVF. These results are in accordance with the previous study (19). The blue/redshift in the emission wavelength (λ_{max}) suggests a change in the hydrophobicity of the microenvironment of the drug binding region (20, 21).

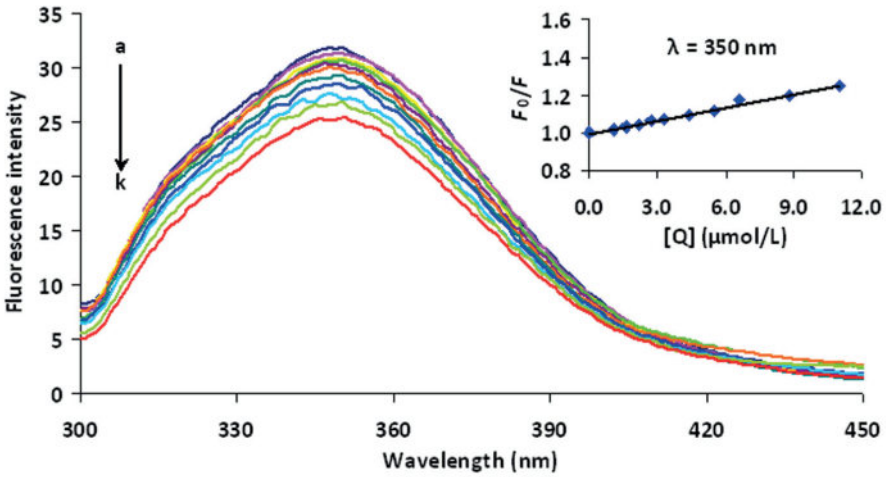

Fig. 2. The fluorescence quenching spectra of HSA by SPF (T = 298 K, pH = 7.4). [HSA] = 2.0 μmol/L; [SPF] (a-k): (0, 1.1, 1.65, 2.2, 2.75, 3.3, 4.4, 5.5, 6.6, 8.8, 11.0) μmol/L. The lower insert shows Stern–Volmer plots of the fluorescence quenching of HSA by SPF.

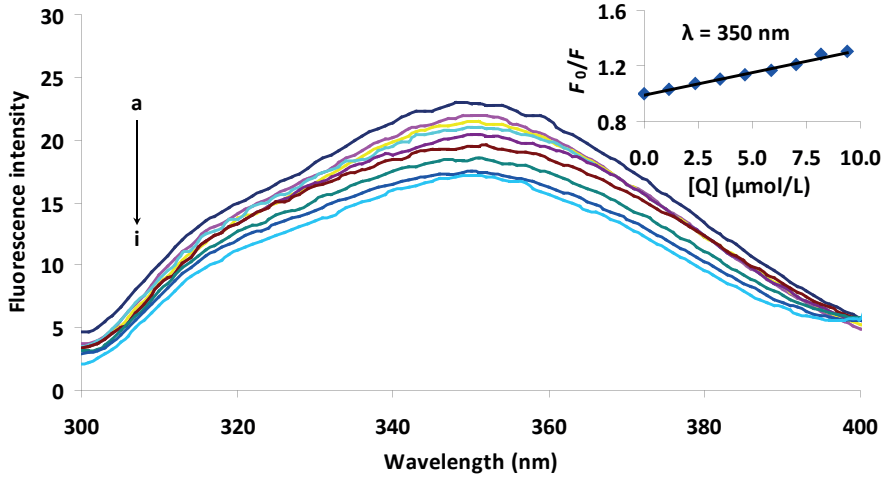

Fig. 3. The fluorescence quenching spectra of HSA by LVF (T = 298 K, pH = 7.4). [HSA] = 2.0 μmol/L; [LVF] (a-i): (0, 1.16, 2.34, 3.52, 4.68, 5.86, 7.03, 8.2, 9.38) μmol/L. The under insert is Stern–Volmer plots of the fluorescence quenching of HSA by LVF.

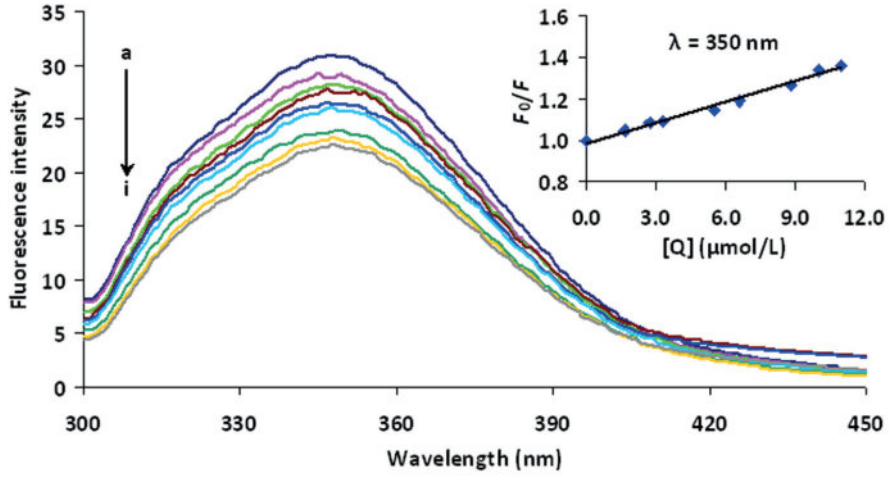

Fig. 4. The fluorescence quenching spectra of HSA by SPF in the presence of TGC (T = 298 K, pH = 7.4). [HSA] = 2.0 μmol/L; [SPF] (a-i): (0, 1.65, 2.75, 3.3, 5.5, 6.6, 8.8, 10.0, 11.0) μmol/L; [TGC] = 2.0 μmol/L. The lower insert shows Stern–Volmer plots of the fluorescence quenching of HSA by SPF in the presence of fixed TGC concentration.

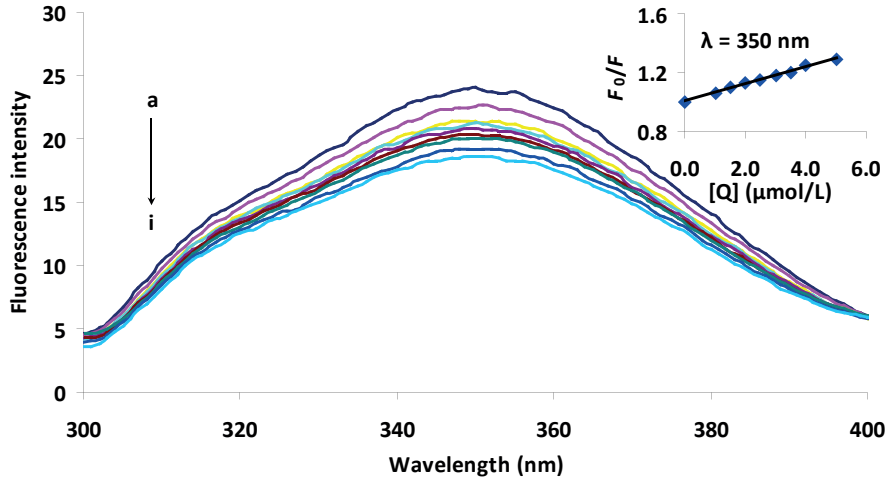

Fig. 5. The fluorescence quenching spectra of HSA by LVF in the presence of TGC (T = 298 K, pH = 7.4). [HSA] = 2.0 μmol/L; [LVF] (a-i): (0, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0) μmol/L; [TGC] = 2.0 μmol/L. The under insert is Stern–Volmer plots of the fluorescence quenching of HSA by LVF in the presence of fixed TGC concentration.

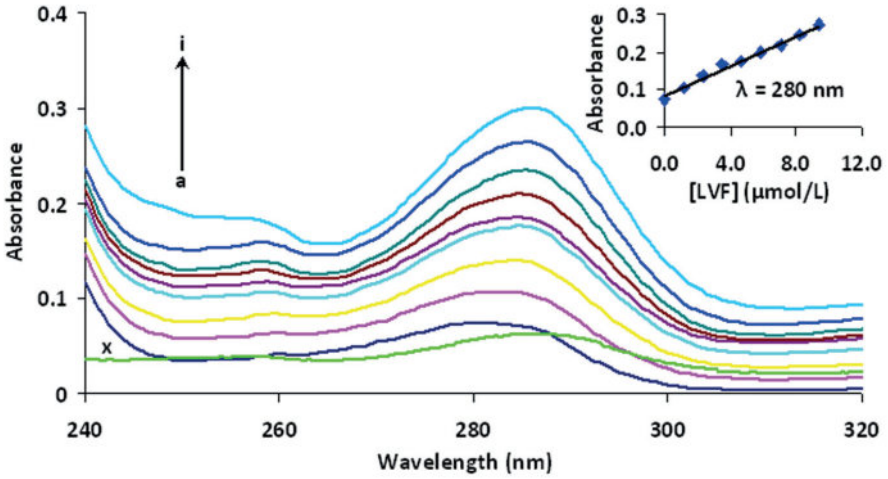

Fig. 6. Absorption spectra of HSA in the presence of various LVF concentrations (T = 298 K, pH = 7.4). [HSA] = 2.0 μmol/L; [LVF] (a-i): (0, 1.16, 2.34, 3.52, 4.68, 5.86, 7.03, 8.2, 9.38) μmol/L. x represents 2.0 μmol/L LVF only.

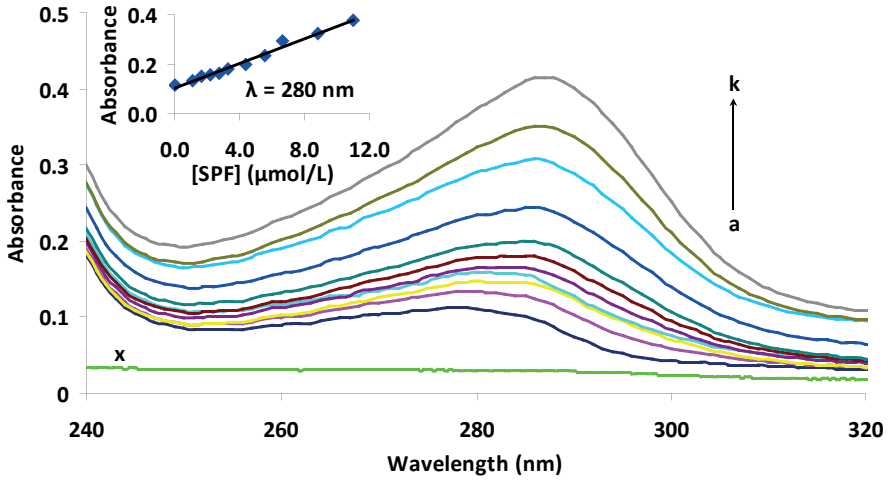

Fig. 7. Absorption spectra of HSA in the presence of various SPF concentrations (T = 298 K, pH = 7.4). [HSA] = 2.0 μmol/L; [SPF] (a-k): (0, 1.10, 1.65, 2.2, 2.75, 3.3, 4.4, 5.5, 6.6, 8.8, 11.0) μmol/L. x represents 2.0 μmol/L SPF only.

Drug–drug interactions at the protein-binding level can be useful for therapeutic purposes because an alteration in protein binding may change the volume of distribution, clearance, and elimination of a drug and may modulate its therapeutic effect (22, 23). The binding affinity of FQs for HSA in the presence of TGC and the competitive binding mechanism were investigated. The fluorescence spectra of the HSA-TGC complex were recorded in the presence of an increasing amount of FQs (Fig. 4 and Fig. 5). Increasing the concentration of FQs led to an increased quenching fluorescence signal of the HSA-TGC complex (Fig. 4 and Fig. 5), suggesting that FQs interact with the HSA-TGC complex. Therefore, sparfloxacin and levofloxacin can displace TGC from its binding site, which means that TGC and these drugs may share some common binding sites in HSA.

UV-Vis absorption studies.

UV-Vis absorption measurement is a very simple method and can be used to explore the structural changes and determine complex formations (15, 24). HSA has a weak absorption peak at approximately 280 nm because of cumulative absorption of three aromatic amino acid residues (Trp, Tyr and Phe). The absorbance peak around 280 nm is raised, which is mainly caused by the π–π^* transition of aromatic amino acid residues in HSA (25, 26). In the present study, the changes in the UV-Vis absorption spectra of the HSA-LVF system (Fig. 6) and HSA-SPF (Fig. 7) were measured under the simulated physiological conditions. Additionally, the UV-Vis absorption spectra of the HSA-SPF and HSA-LVF system were measured in the presence of the fixed TGC concentration (Fig. 8 and Fig. 9).

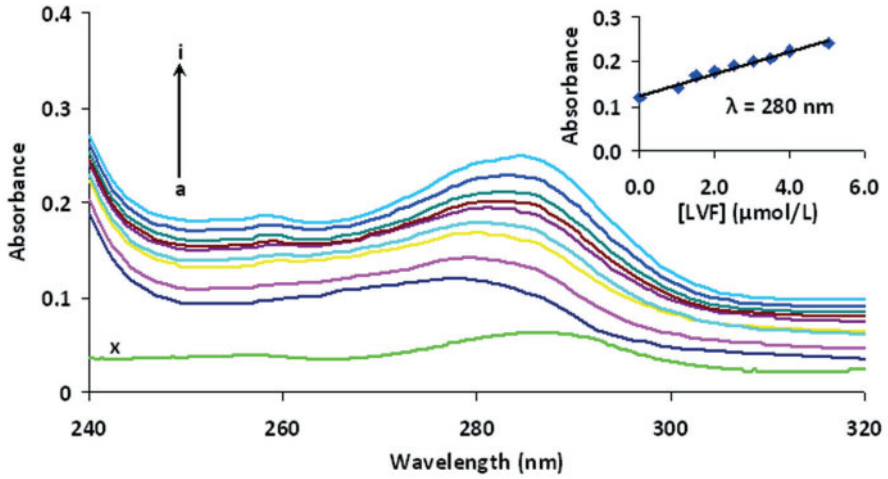

Fig. 8. Absorption spectra of HSA in the presence of various LVF concentrations and a fixed TGC concentration (T = 298 K, pH = 7.4). [HSA] = 2.0 μmol/L; [LVF] (a-i): (0, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0) μmol/L; [TGC] = 2.0 μmol/L. x represents 2.0 μmol/L LVF only.

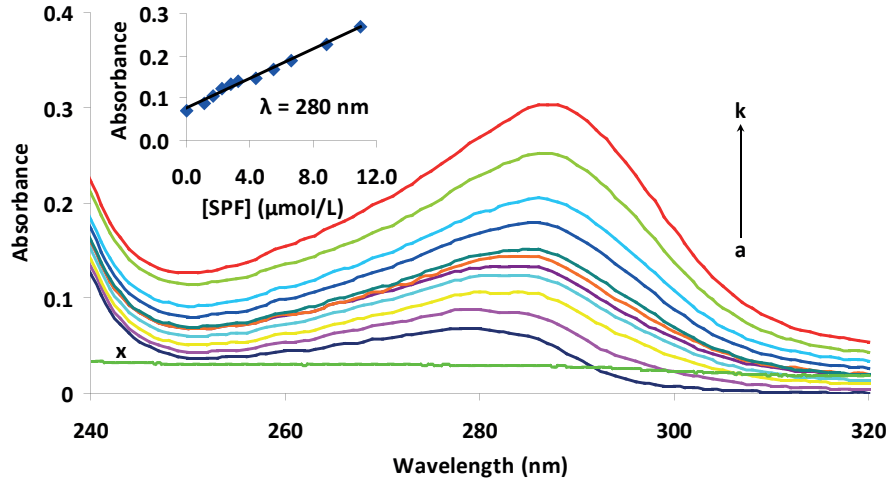

Fig. 9. Absorption spectra of HSA in the presence of various SPF and fixed TGC concentration (T = 298 K, pH = 7.4). [HSA] = 2.0 μmol/L; [SPF] (a-k): (0, 1.1, 1.65, 2.2, 2.75, 3.3, 4.4, 5.5, 6.6, 8.8, 11.0) μmol/L; [TGC] = 2.0 μmol/L. x represents 2.0 μmol/L SPF only.

DISCUSSION

Fluorescence and absorption spectra.

The fluorescence intensity of HSA and HSA-TGC decreased regularly with increasing FQ concentrations (Figs. 2, 3, 4 and 5). The quenching mechanism was determined to investigate whether FQs interact with HSA and HSA-TGC to form a complex. To confirm the quenching mechanism, the fluorescence quenching data were analyzed according to the Stern-Volmer Eq. 1. Eq. 1 was applied to determine K_{SV} via the linear regression of a plot of F_0/F against [Q]. The K_{SV} was then obtained from the slope. The values of K_{SV} and K_q were calculated and are given in Table 1. Stern–Volmer plots for the fluorescence quenching of HSA and HSA-TGC by SPF and LVF are shown in the inserts in Figs. 2 , 3, 4 and 5. The Stern–Volmer plots (Figs. 2, 3, 4 and 5) showed a linear curve for all of the investigated concentrations, which indicates that quenching type can be static or dynamic quenching because the characteristic Stern–Volmer plots of combined quenching (both static and dynamic) show an upward curvature (27). The linear plots, however, are insufficient to

Table 1. The quenching constants (K_q), binding constants (K_A) and number of binding sites (n) of HSA-FQ systems in the absence and presence of TGC at 298 K.

System	K_q (10^{12} L/mol·s)	R^2	K_A (10^4 L/mol)	n	R^2
HSA-TGC (12)	4.99	0.996	1.76	0.91	0.991
HSA-SPF	2.35	0.990	10.75	1.13	0.991
HSA-SPF-TGC	3.32	0.987	7.56	1.08	0.990
HSA-LVF	3.31	0.986	9.24	1.09	0.992
HSA-LVF-TGC	5.87	0.995	2.74	0.94	0.995

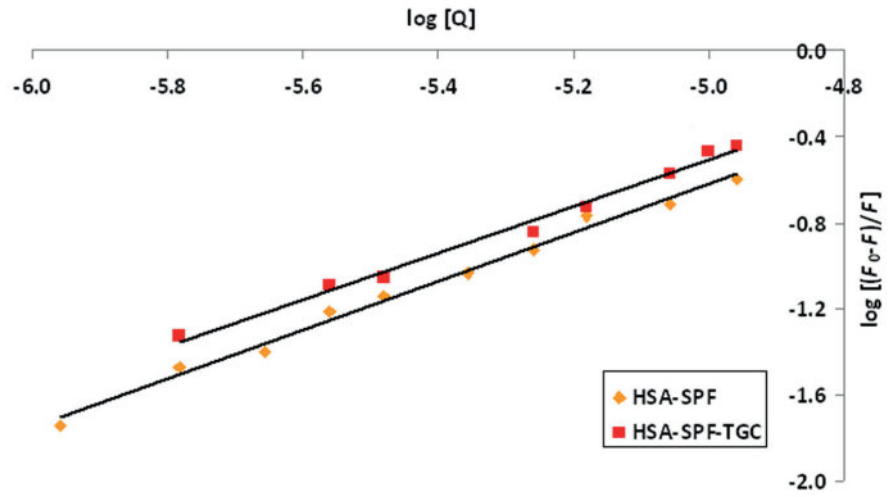

Fig. 10. Plots of log $(F_0–F)/F$ versus log [Q] of HSA-SPF and HSA-SPF-TGC system at 298 K.

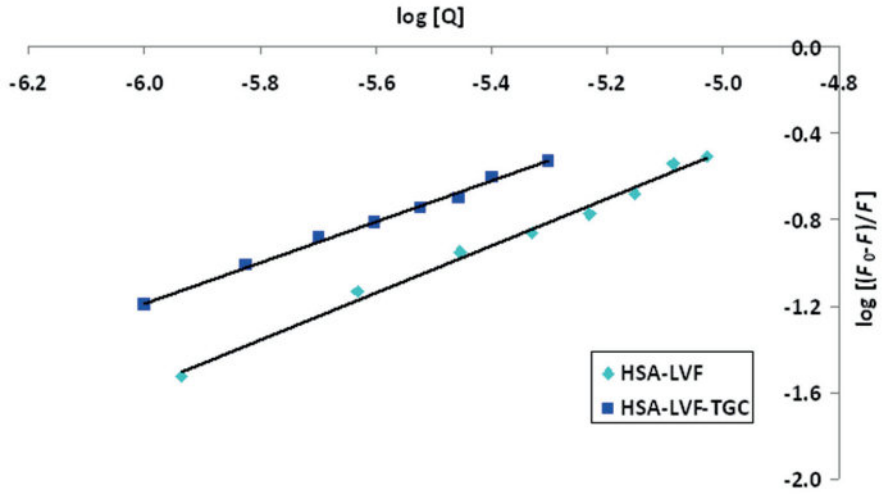

Fig. 11. Plots of log $(F_0–F)/F$ versus log [Q] of HSA-LVF and HSA-LVF-TGC system at 298 K.

define the quenching type. Thus, based on previous works (28-31), the possible quenching mechanism between HSA and fluoroquinolones was suggested to be static quenching resulting from the complex formation instead of dynamic quenching. As evident from the results (Table 1), the K_q values (>10^{12} L/mol·s) are higher than those of diverse kinds of quenchers that are used for biopolymer fluorescence (2.0 × 10^{10} L/mol·s). These results indicate that the quenching was initiated not from a dynamic collision, but from the formation of a complex in all of the tested systems.

The absorption spectra of HSA and HSA-TGC at different concentrations of FQ (Figs. 6, 7, 8 and 9) showed that in the visible region, the absorbance values of HSA and HSA-TGC increased regularly with an increase in the concentration of FQ, suggesting that a complex was formed between FQs and HSA and between FQs and HSA-TGC (32). The absorption peaks of these solutions showed moderate shifts towards a longer wavelength, indicating that with the addition of FQ, the peptide strands of the HSA molecules extended farther and the hydrophobicity was changed (33).

Binding parameters.

The quenching mechanism was determined to be static quenching; thus, the binding constant (K_A) and the number of binding sites (n) can be calculated according to Eq. 2. The values of K_A and n of all systems were obtained from the intercept and slope of the plots of log $(F_0–F)/F$ versus log [Q] (Figs. 10 and 11).

The values of the binding constant K_A and of n are given in Table 1. The number of binding sites (n) is approximately 1, indicating that there is one binding site in HSA for SPF and LVF. The binding constants of the HSA-SPF and HSA-LVF system that were found in this research are in agreement with previous studies (34, 35). The relatively high values of the stability constants suggest a strong interaction between

HSA and the experimental drugs. Additionally, the binding constants of the HSA-FQ system in the presence of TGC were determined (Table 1). As seen from Table 1, both the binding modes and binding affinities of these drugs underwent changes. In the presence of TGC, the binding affinity of HSA for FQs decreased compared to those in the absence of TGC (Table 1). The decreased binding constants of fluoroquinolone in the presence of tigecycline imply both that fluoroquinolone and tigecycline share some common binding sites in HSA and that FQ cannot displace TGC from its binding sites on albumin. The specific sites for LVF and SPF on the protein molecule are already occupied by TGC, so FQs cannot completely displace TGC, which results in a decreased binding affinity of FQ to HSA in the presence of TGC (competitive interference). If the fluoroquinolones and tigecycline bind at different sites, the binding constants of the fluoroquinolones should remain unchanged. In such a case, the binding of FQ will not be affected by the presence of TGC (independent binding). The simultaneous binding of two drugs may also cause structural changes in the albumin molecule (non-competitive interference), thereby creating more binding sites or increasing the accessibility of the existing sites and hence increasing the degree of binding of the parent drug (34).

The degree of binding to albumin may have consequences for the rate of clearance of the metabolites and for their delivery to cells and tissues (36). Based on the conventional concept, the cellular uptake is proportional to the unbound fraction of drugs. According to this hypothesis (37), the distribution of FQ within the body is proportional to the free concentration of unbound FQ in circulating plasma. The reversible binding to blood proteins, such as serum albumin, may have consequences for the delivery of FQ and their metabolites to cells and tissues. If a molecule is highly bound to plasma proteins, the amount of drug that is available to diffuse into the target tissue may be significantly reduced, and the efficacy of the drug may consequently be poor. Here, it was found that TGC decreased the affinities of FQ for HSA. TGC in the blood will affect the transporting ability of serum albumin for FQ, which may improve the free concentrations of unbound FQ and enhance their pharmacological effects. These effects are significant *in vivo*.

CONCLUSION

In this study, the interaction of sparfloxacin and levofloxacin with HSA in the absence and presence of tigecycline was studied by spectroscopic methods, including fluorescence and UV-Vis absorption spectroscopy, under physiological conditions. The results showed that sparfloxacin and levofloxacin had a strong ability to quench the intrinsic fluorescence of HSA by creating the complexes through a static quenching mechanism. The binding constants (K_A) of the HSA-SPF and HSA-LVF systems were 10.75×10^4 and 9.24×10^4, respectively. The results illustrated that there is a strong binding force between HSA and the investigated drugs, and one molecule of drug binds to one molecule of HSA with high affinity. The results confirmed that the binding constant (K_A) values of the HSA-FQ system decreased in the presence of TGC, indicating that TGC can affect the binding ability of FQ for HSA. This may increase the free plasma concentration of unbound FQ and enhance their pharmacological and toxicological effects. Studies on the interaction between plasma proteins and small molecule-drugs are thus an interesting field of future research.

Acknowledgments

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Conflict of Interest

All of the authors declare no conflict of interest.

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CONE-BEAM COMPUTED TOMOGRAPHY STUDY OF THE ROOT CANAL MORPHOLOGY OF MANDIBULAR ANTERIOR TEETH IN SERBIAN POPULATION

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KOMJUTERIZOVANA TOMOGRAFIJA KONUSNOG ZRAKA U ISPITIVANJU MORFOLOGIJE KORENSKOG KANALA PREDNJIH ZUBA DONJE VILICE U SRPSKOJ POPULACIJI

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ABSTRACT

The aim of this study is to describe the morphological characteristics, the number of roots and number of root canals of mandibular central incisors, lateral incisors and canines, and the relationship of these characteristics with the sex and the jaw side where the tooth is located, in the Serbian population using cone-beam computed tomography (CBCT).

CBCT images of a total of 902 mandible front teeth, including 296 central incisors, 294 lateral incisors and 312 canines were analyzed in the database. For assessing the morphology of the root canal, Vertucci method of classification was used.

Central incisors had two canals in 27%, similar as lateral incisor which had two canals in 26.5%. Mandibular canines had two canals in 7.1% and there was a significant difference between genders. The most prevalent root canal configuration type in all mandibular anterior teeth was type I. In the groups of mandibular incisors with two root canals the most common was type III. In the group of mandibular canines with two root canals, type V had the greatest occurrence.

Most mandibular anterior teeth had one root canal. Two root canals were found in 27% of the mandibular incisors. There is a significant difference in root morphology between genders. It is important for dental practitioner to expect different morphological variations when performing endodontic treatment. More studies are needed to further define morphological characteristics of roots of mandibular anterior teeth in Serbian population.

Keywords: *Cone beam computed tomography, mandibular incisors, mandibular canines, root canal configuration, root canal morphology*

SAŽETAK

Cilj ove studije je da koristeći komjuterizovanu tomografiju konusnog zraka (CBCT) opiše morfološke karakteristike, broj korena i korenskih kanala centralnih i lateralnih sekutića i očnjaka donje vilice u srpskoj populaciji, kao i da utvrdi korelaciju ovih karakteristika sa polom i stranom vilice u kojoj je zub lokalizovan.

CBCT snimci 902 prednja zuba donje vilice, uključujući 296 centralnih i 294 lateralnih sekutića i 312 očnjaka su analizirani iz baze podataka. Za procenu mofologije korenskog kanala upotrebljen je Vertučijev metod klasifikacije.

Centralni sekutići su imali dva kanala u 27% slučajeva, slično kao i lateralni sekutići koji su imali dva kanala u 26,5% slučajeva. Očnjaci su imali dva kanala u 7,1% slučajeva, pri čemu je bilo značajnih razlika između polova. Preovlađujući tip konfiguracije korenskog kanala svih prednjih zuba donje vilice je bio tip I. U grupi sekutića koji su imali dva kanala najčešći tip konfiguracije je bio tip III. U grupi očnjaka koji su imali dva kanala preovladavao je tip V.

Većina prednjih zuba donje vilice je imala jedan korenski kanal. Dva korenska kanala su pronađena u 27% slučajeva kod sekutića. Postoji značajna razlika u morfologiji korena između polova. Za stomatologa je važno da očekuje različite morfološke varijacije tokom izvođenja endodonskih zahvata. Potrebno je sprovesti više studija za dalje definisanje morfoloških karakteristika korena prednjih zuba donje vilice u srpskoj populaciji.

Ključne reči: *Komjuterizovana tomografija konusnog zraka, mandibularni sekutići, mandibularni očnjaci, konfiguracija korenskog kanala, morfologija korenskog kanala*

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INTRODUCTION

The knowledge of the internal morphology of the roots of the teeth is an essential prerequisite for the successful implementation of endodontic therapy. One of the causes of failure of endodontic therapy is omission of one or more root canals, whose presence was not observed. Most common reason for the omission of endodontic root canal therapy is the morphological variations of the tooth root canal system (1).

In studies of the morphology of roots, different methods of evaluating the internal structure of teeth in clinical and laboratory conditions can be used (2-4). Standard methods of observing the internal morphology of the teeth, in clinical terms, involves the use of retro alveolar radiological examinations. Main disadvantage of this technique is reduction of a three-dimensional object to two-dimensional image, also, features superimposed roots and roots of teeth with each other and with the adjacent anatomical structures, which can give a false idea of internal morphology of the teeth (2). In clinical practice, in addition to retro alveolar radiological examinations, inspection with loupes or/and operating microscope enhance canals inspection (3). In experimental conditions, various method can be used: colouring teeth (clearing and staining technique) and cutting (cross section) and digital radiological examinations, but these methods requires the use of extracted teeth with no signs of pathological changes (usually extracted for orthodontic reasons), thus obtaining samples of younger respondents which may affect the final results (4). In last decade, for the assessment of the internal morphology of the roots computed tomography with cone beam (Cone Beam Computer Tomography, CBCT) is used, which provides an overview of the object of interest in three dimensions without the superimposition of anatomical structures from different angles and planes (axial, sagittal, and coronal) (5).

Numerous studies have demonstrated that the anterior teeth (central incisors, lateral incisors and canines) in the mandible can significantly vary in the root canal configuration (6-8). There are differences in the root canal morphologies in different populations. *Altunsoi et al*. announced that in the Turkish population the most common finding is a single canal of root canal in the mandibular anterior teeth with a range of 77-95%, and then the two canals with a range of 5.3-18.9%. In the same CBCT study, type I canal configuration of Vertucci is the most widespread in the mandibular anterior teeth 77-95%, and then the configuration type V was observed in the range of 1.8-14.4% (6). In studies performed by using the CBCT, *Liu et al.* reported that the type I canal configuration of the lower central and lateral incisors were 91.1% and 82.5% in the Chinese population (7). *Aminsobhani et al.* reported that the type I configuration canal for central, lateral incisors and canines are 72.7%, 70.6% and 71.8%, in the Iranian population (8).

There are many studies examining the morphological characteristics of the mandibular anterior teeth using CBCT (6-14). In the Serbian population such morphological studies were not conducted.

The aim of this study is to describe the morphological characteristics, the number of roots and number of root canals of mandibular central incisors, lateral incisors and canines, and the relationship of these characteristics with the sex and the jaw side where the tooth is located, in the Serbian population using CBCT.

MATERIAL AND METHODS

The study protocol was approved by the Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac, Serbia and the research was carried out respecting the Declaration of Helsinki and Good Clinical Practice guidelines.

CBCT images of a total of 902 mandible front teeth, including 296 central incisors, 294 lateral incisors and 312 canines were analyzed in the database.

For the purposes of retrospective study we used the CBCT images made in Radiological department, Faculty of Medical Sciences, University of Kragujevac, between September 2014 and October 2016. The scans were obtained using Orthophos XG 3D device (Sirona Dental Systems GmbH, Bensheim, Germany), with three-dimensional settings for recording, VOL1 or VOL1 HD, and a voxel size of 160μm; the layer thickness were 0.16mm and size of observed filed (Field of view, FOV) was 5x8x8 cm. The reasons for CBCT scanning were different (prosthetic, surgical, orthodontic and endodontic).

The main images inclusion criterion was the existence of at least one permanent incisor and canine in the mandible. Other Inclusion criteria were following: 1) tooth is fully visible; 2) have completed root growth; 3) have no radiologically visible periapical lesion; 4) have no radiologically visible external or internal root resorption; 5) are not treated endodontically and 6) have no prosthetic restorations.

CBCT images were analyzed using a software program GALAXIS v1.9.4 (Sirona Dental Systems GmbH, Bensheim, Germany), in the axial, sagittal, and coronal sections (Figure 1.) Observations were conducted at Philips LED monitor size 23-inch image with a resolution of 1920 x 1080 pixels in a room with dim lighting. Brightness and contrast are adjusted using a software program.

On CBCT scans we observed following:

- The root number for each tooth
- The root canals number
- Position of the tooth- left or right side of the mandible
- The root canal configurations of Vertucci classification

For the classification of the morphology of the root canal was a used Vertucci method of classification (15):

Type I: A single canal appears from the pulp chamber to the apex.

Type II: Two separate canals leave the pulp chamber but merge into one to the exit.

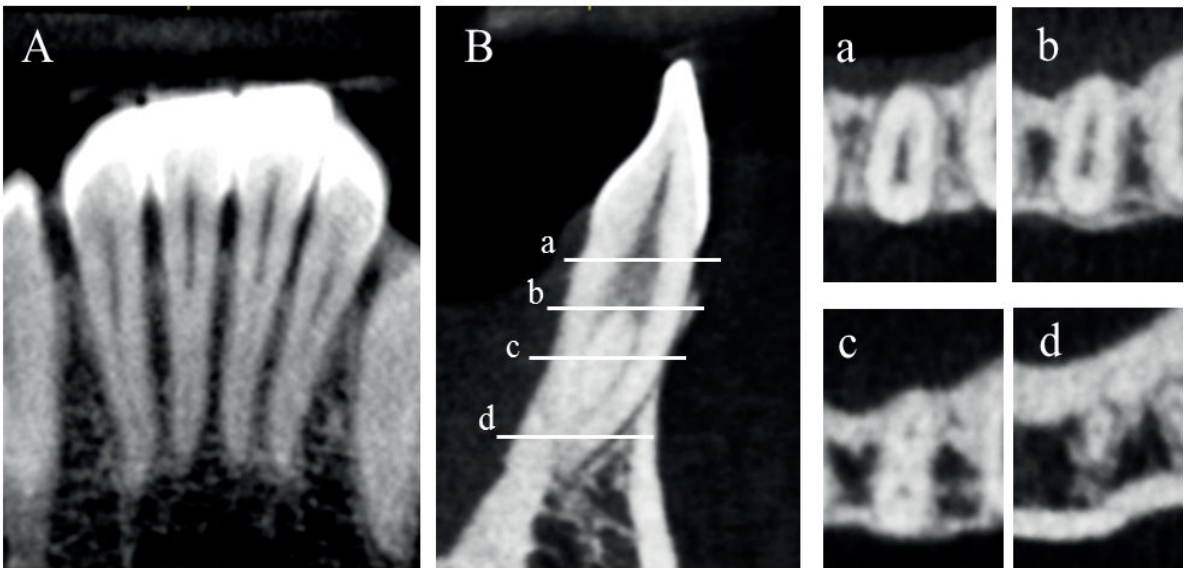

Figure 1. A CBCT image of a mandibular incisor with type III Vertucci root canal configuration. Image A shows a coronal section view of mandibular incisors, image B shows sagittal section view of mandibular central incisor. Different axial section views are shown on images a, b, c, d. Notice how in coronary part of the root, a root canal starts as a single (a), then separates into two canals (b, c) and finishes as one root canal at the apex (d).

Type III: A single canal leaves the pulp chamber, divides into two within the root, and then merges to the exit.
Type IV: Two distinctly separate canals are present from the pulp chamber to the apex.
Type V: A single canal leaves the pulp chamber but divides into two.
Type VI: 2 separate canals leave the pulp chamber, join at the midpoint, and then divide again into two with two separate apical foramina.
Type VII: One canal leaves the pulp chamber, divides and then rejoins within the root, and finally redivides into two separate canals with two separate apical foramina.
Type VIII: Three separate and distinct canals begin from the pulp chamber to the root apex.

Different types of root canal configuration by Vertucci classification is shown in Figure 2.

An additional analysis of statistical data was performed using a commercial software package SPSS v20.0 for Statistics (SPSS Inc., Chicago, IL, USA). The frequency of the number of roots, the canal number and morphology of root canal root are described using measures of descriptive statistics: frequency, percentage, mean (average), median, standard deviation (SD) and scope (range). The frequencies of the different parameters and correlations were analyzed between the sexes and side of the jaw where the tooth is located. Chi-square (χ^2) test was used to compare the frequency (frequency) of categorical variables and to demonstrate statistical significance. All results where the probability of the null hypothesis was less than 5% ($p<0.05$), were considered statistically significant.

RESULTS

A total of 902 mandibular anterior teeth, including 296 central incisors, lateral incisors 294 and 312 canines, were observed.

All of the mandibular incisors had one root (n=590), while the incidence of two roots was 5.8% in the mandibular canine group (n=312). There was a statistically signifi-

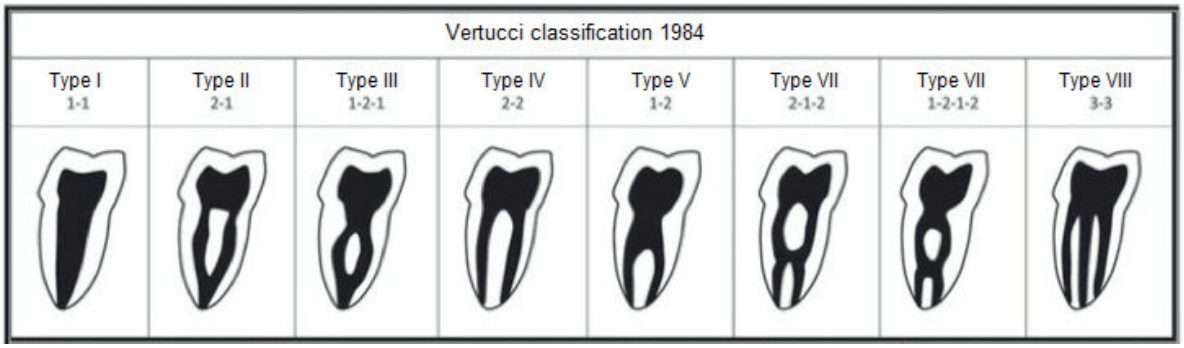

Figure 2. Different types of root canal configuration by Vertucci classification.

Table 1. Number of roots in mandibular anterior teeth

Tooth	Gender	Number of roots		Number of teeth
		One root	Two roots	
Mandibular central incisors	male	166 (100%)	0	166 (100%)
	female	130 (100%)	0	130 (100%)
	total	**290 (100%)**	**0**	**290 (100%)**
Mandibular lateral incisors	male	164 (100%)	0	164 (100%)
	female	130 (100%)	0	130 (100%)
	total	**294 (100%)**	**0**	**294 (100%)**
Mandibular canines*	male	160 (97.6%)	4 (2.4%)	164 (100%)
	female	134 (90.5%)	14 (9.5%)	148 (100%)
	total	**294 (94.2%)**	**18 (5.8%)**	**312 (100%)**

*Statistical significance at the level of p=0.013

cant difference between the number of roots and gender (p<0.05, p=0.013), where two-rooted canines occur more often among females (n=14) than males (n=4) (Table 1).

Most mandibular anterior teeth had one root canal. Central incisors had two canals in 27%, similar as lateral incisor which had two canals in 26.5%. There was no significant differences in distribution of number of root canals of central and lateral incisors between the genders (p>0.05; p=0.066 and p=0.595 respectively), although the number of teeth with two root canals was higher among males (52:28 for central, 46:32 for lateral incisors). Mandibular canines had two canals in 7.1% and there was a significant difference between genders (p<0.05, p=0.015), where two canals were more prevalent among females than males (16 versus 6 teeth respectively) (Table 2).

According to Vertucci classification, the most prevalent root canal configuration was type I in all teeth groups (central incisors 73%, lateral incisors 73.5%, and canines 92.9%). Among the central incisors with two root canals the most common was type III, followed by type II and type IV (21.6%, 4.7% and 0.7% respectively). Other types were not observed. In the group of lateral incisors, after type I, most prevalent configuration type was type III, then type II, type V and type IV (18.4%, 5.4%, 2% and 0.7% respectively). Type VI and type VII were not observed. In the group of two-canaled mandibular canines, the most common was the type V (5.8%), while of the other types only types II and III were observed in 0.6% each. The difference in the frequency of different configuration types and gender was only significant in the group of mandibular canines, where the type V was more frequently seen in females (Table 3). There was no statistically significant difference between any parameter and side of the jaw where the tooth is located.

DISCUSSION

In previous studies of root morphology, different *in vitro* and *in vivo* methods were used to assess the inter-

Table 2. Number of root canals in mandibular anterior teeth

Tooth	Gender	Number of root canals		Number of teeth
		One root canal	Two root canals	
Mandibular central incisors	male	114 (68.7%)	52 (31.3%)	166 (100%)
	female	102 (78.5%)	28 (21.5%)	130 (100%)
	total	**216 (73.0%)**	**80 (27.0%)**	**296 (100%)**
Mandibular lateral incisors	male	118 (72.0%)	46 (28.0%)	164 (100%)
	female	98 (75.4%)	32 (24.6%)	130 (100%)
	total	**216 (73.5%)**	**78 (26.5 %)**	**294 (100%)**
Mandibular canines*	male	158 (26.3 %)	6 (3.7%)	164 (100%)
	female	132 (89.2%)	16 (10.8%)	148 (100%)
	total	**290 (92.9%)**	**22 (7.1%)**	**312 (100%)**

*Statistical significance at the level of p=0.015

Table 3. Distribution of root canal types of mandibular anterior teeth

Tooth	Sex	Vertucci classification					Number of teeth
		Type 1	Type 2	Type 3	Type 4	Type 5	
Mandibular central incisors	male	114 (68.7%)	12 (7.2%)	38 (22.9%)	0 (0%)	2 (1.2%)	166 (100%)
	female	102 (78.5%)	2 (1.5%)	26 (20.0%)	0 (0%)	0 (0%)	130 (100%)
	total	**226 (73.0%)**	**14 (4.7%)**	**64 (21.6%)**	**0 (0%)**	**2 (0.7%)**	**296 (100%)**
Mandibular lateral incisors	male	118 (72.0%)	8 (4.9%)	36 (22.0%)	0 (0%)	2 (1.2%)	164 (100%)
	female	98 (75.4%)	8 (6.2%)	18 (13.8%)	2 (1.5%)	4 (3.1%)	130 (100%)
	total	**216 (73.5%)**	**16 (5.4%)**	**54 (18.4%)**	**2 (0.7%)**	**6 (2.0%)**	**294 (100%)**
Mandibular canines*	male	158 (96.3%)	0 (0%)	2 (1.2%)	0 (0%)	4 (2.4%)	164 (100%)
	female	132 (89.2%)	2 (1.4%)	0 (0%)	0 (0%)	14 (9.5%)	148 (100%)
	total	**290 (92.9%)**	**2 (0.6%)**	**2 (0.6%)**	**0 (0%)**	**18 (5.8%)**	**312 (100%)**

*Statistical significance at the level of p=0.011

nal structure of the tooth roots. *In vitro* methods used in previous studies include the use of clearing and staining techniques, cross sectioning, stereomicroscopy, radiography and micro computed tomography, but their implementation requires tooth extraction (15, 16-21). Dental retro alveolar radiographs were used in clinical examination, but this method is less accurate for assessing internal tooth morphology than others because it shows the complex structure of an object as a two-dimensional image (22). CBCT is a newer, non-invasive radiological methods for observation of orofacial structures, which is used for assessing external and internal morphology of the roots *in vitro* (9) as well as *in vivo* (6, 8, 10-14). CBCT was shown to be as accurate as the gold standard (clearing and staining technique) (23). In this study, data were collected by analysing CBCT images.

Knowing the number of roots of the teeth is important because it is most common for mandibular anterior teeth to have one root (15). In this study, out of 584 mandibular incisors, all had one root, which is equal to the results in the study of *Han et al.*, *Lin et al.*, and *Aminsobhani et al.* who used the same method for analysing the teeth roots (8, 11, 12). By using clearing and staining techniques *Rahimi et al.* also showed one root in all mandibular incisors (16). A smaller number of two-rooted lateral incisors (0.3%) were found by *Zhengyan et al.* (13). The results of the number of roots of mandibular canines in earlier studies vary from 0.8% to 12% (8, 11-14, 16). Our results show the incidence of two roots in a group of mandibular canines to be 5.8% and we also showed more frequent occurrence of two roots in females (p<0.05). The percentage of number of roots in previous studies comparing to present study are shown in Table 4.

Analysis of the number of root canals in mandibular incisors showed the incidence of one canal to be 73% for the central and 73.5% for lateral incisors. In previous studies that used the clearing and staining technique the frequency of one root canal in mandibular incisors ranged from 62% to 75% (15-18). Studies on CBCT showed the frequency of one canal in rage from 61% to 89% (6, 9-13). In this study, the frequency of two canals in the group of the mandibular canines was 7.1%, which is similar to the research of *Rahimi et al.* (8.4%, using clearing and staining) (16) and *Altunsoy et al.* (6.6%, using CBCT) (6). Statistical analysis did not show the difference in the number of root canals between the genders in mandibular incisors

Table 4. Percentage of number of roots found in mandibular anterior teeth in previous studies and present study

	Method	Central incisors		Lateral incisors		Canines	
		One root	Two roots	One root	Two roots	One root	Two roots
Rahimi et al. (16)	Clearing and staining	100	0	100	0	88	12
Lin et al. (11)	CBCT	100	0	100	0	/	/
Han et al (12)	CBCT	100	0	100	0	98.7	1.3
Zhengyan et al. (13)	CBCT	100	0	99.7	0.3	99.2	0.8
Soleymani et al. (14)	CBCT	/	/	/	/	98.7	1.3
Aminsobhani et al. (8)	CBCT	100	0	100	0	96.3	4.7
Present study	CBCT	100	0	100	0	94.2	5.8

Table 5. Percentage of different root canal configuration types in central and lateral mandibular incisors found in previous studies and present study

	Method	Group of incisors	Sample	Vertucci classification (%)					
				Type I	Type II	Type III	Type IV	Type V	Other types
Vertucci (15)	Clearing and staining	Central	100	70	5	22	3	0	/
		Lateral	100	75	5	18	2	0	/
Rahimi et al. (16)	Clearing and staining	Central	186	64.5	18.3	16.7	0.5	0	/
		Lateral	128	61.7	16.4	21.1	0.8	0	/
Leoni et al. (21)	mCT	Central	50	50	0	28	0	0	22
		Lateral	50	62	0	28	0	0	10
Da Silva et al. (10)	CBCT	Central	200	64.5	0	18	0	14.5	3
		Lateral	200	60.5	0.5	25.5	0	12	1.5
Lin et al. (11)	CBCT	Central	706	89.1	2.4	6.2	1.7	0.6	/
		Lateral	706	74.5	3.7	19.3	2.1	0.4	/
Han et al (12)	CBCT	Central	1286	84.3	3.4	6.5	1.2	3.9	0.7
		Lateral	1294	72.6	4	15.5	2.3	5	0.4
Altunsoy et al. (6)	CBCT	Central	1582	84.5	0.3	0.8	4.2	10	/
		Lateral	1603	80.2	1.8	1.7	5.4	12.1	/
Zhengyan et al. (13)	CBCT	Central	3375	96.3	0.1	3.5	0.15	0.8	/
		Lateral	3257	89.4	1.05	7.7	0.3	1.15	/
Aminsobhani et al. (8)	CBCT	Central	632	72.7	11.3	4.7	7.7	3.6	/
		Lateral	614	70.6	7.1	3.7	15.4	3.2	/
Present study	CBCT	Central	296	73	4.7	21.6	0.7	0	/
		Lateral	294	73.5	5.4	18.4	0.7	2	/

although the central incisors with two canals were more prevalent in males (31.3%) than females (21.5%). Greater frequency of two canals of mandibular incisors in males was shown by *Lin et al.* (11). In the group of mandibular canines we showed a higher incidence of two root canals in females which is different from the study by *Soleymani et al.* who showed that two roots are more prevalent in males (14).

We used Vertucci classification into eight types for assessing root canal configuration (15), same as most of the previous studies (6, 8-21). In the group of mandibular central incisor with two root canals, the most common was type III in 21.6%. Among the other types, only type II (4.7%) and Type IV (0.7%) were observed. Study of *Vertucci* shows the frequency of type III in 22% and type II in 5%, while the type IV is shown to be more frequent than in our study (3%) (15). Similar results *Vertucci* showed for lateral incisors (type III 18%, type II 5% and type IV 2%) (15). In this study 18.4% of lateral incisors were type III, 5.4% were type II, 2% were type V and 0.7% were type IV. These results differ from study of *Altunsoy et al.* where the most prevalent were type V and type IV in the group of mandibular incisors (6). Study by *Han et al.* showed that among the incisors with two canals, the most common was type III, but with a lower percentage compared to our study (6.5% of central incisors and 15.5% of lateral incisors) (12). The percentage of different root canal configuration types by Vertucci classification of central and lateral mandibular incisors in previous studies and present study are shown in Table 5.

Vertucci in his study showed that in the group of the mandibular canines most common was type I (78%), followed by type II (14%), type IV (6%) and type III (2%) (15). Our data hasn't shown a greater diversity in the types of root canal configuration in canines. The most frequent was type I (94.2%), while out of other types significantly present was only type V (5.8%). The types II and III were only found in 2 teeth each, out of total 312 canines. The study of *Amardeep et al.* who used CBCT on extracted teeth to analyse root canals, found morphological type I in the majority of teeth (79.6%) (9). In contrast to our study, the next most frequent type was the type III (13.6%), while the type V was present in lower percentage (2%) (9). *Pineda and Kuttler* used radiographic images of canines for analysing root canal morphology and also showed greatest occurrence of type I (81.5%), while the type II (13.5%) was the next most common (20). In a research conducted by *Zhengyan et al.*, the results for type I were mostly consistent with the results of our study (95.8%), but the type III was following by frequency (2.1%) (13). *Soleymani et al.* haven't found a significant difference between morphological types of root canals and gender (14), in contrast to our study, where we have shown that the type V was more common among female. Table 6. shows the occurrence of different root canal configuration types by Vertucci classification of mandibular canines in previous studies and present study.

Table 6. Percentage of different root canal configuration types in mandibular canines found in previous studies and present study

	Method	Sample	Vertucci classification (%)					
			Type I	Type II	Type III	Type IV	Type V	Other types
Vertucci (15)	Clearing and staining	100	78	14	2	6	0	/
Rahimi S. et al. (16)	Clearing and staining	149	91.6	6.1	2.3	0	0	/
Sert et al. (18)	Clearing and staining	200	76	15.5	6.5	1.5	0	/
Vaziri PB. et al (19)	Cross section	100	88	5	7	0	0	/
Pineda et al. (20)	Radiography	187	81.5	13.5	0	5	0	/
Da Silva EJN. et al. (10)	CBCT	200	90.5	1	4	2.5	2	/
Han et al (12)	CBCT	1291	93.7	0.6	3.3	0	0.5	/
Altunsoy M. et al. (6)	CBCT	1604	92.8	2.1	1.2	1.35	2.65	/
Zhengyan et al. (13)	CBCT	3014	95.8	0.7	2.1	0.2	0.4	/
Soleymani et al. (14)	CBCT	300	89.7	3.7	5.7	0	1	/
Aminsobhani et al. (8)	CBCT	608	71.8	10.3	2.8	12.8	2.3	/
Present study	CBCT	312	92.9	0.6	0.6	0	5.8	/

Previous studies have suggested that in considering number of roots and root canal configuration, other than technique used for evaluation, sex and ethnical background of sample may contribute to diversity in root morphology (6, 11, 13, 14, 18). Our study showed that in a group of mandibular incisors, a higher occurrence of two root canals was among males, while in a group of mandibular canines higher prevalence of two root canals was in females.

Root morphology varies in studies conducted in different countries and even in different regions of the same country (6, 8, 9, 11-14, 16-19). The studies carried out in a Turkish population showed that the incidence of single root canal in mandibular incisors can vary in a range from 36% to 85% (6, 18). In Chinese population the occurrence of two root canals of mandibular incisors in different studies was shown to be in range from 4% to 16% for central and from 11% to 27% for lateral incisors (11-13). Studies of mandibular canines in Iranian population showed that the prevalence of type I of root canal configuration can vary from 72% to 92% (8, 14, 16, 19). There aren't any previous studies of number of roots and/or root canal configuration of mandibular anterior teeth carried out on Serbian population.

CONCLUSION

The results of present study showed that around 27% of mandibular incisors have two root canals and that among incisor with two canals the most prevalent is the type III of Vertucci classification. This study also showed that the incidence of mandibular canines with two canals can be as high as 7.1%. These findings emphasize the importance of knowledge of variations in root canal morphology, since excluding the possibility of morphological variation can lead to failure in endodontic therapy. Also, patient's gender and ethnic origin should be considered when performing the preoperative evaluation of endodontic treatment. CBCT was shown to be a clinically useful tool for the detection of different root canal configurations. More studies are needed to further define morphological characteristics of roots of mandibular anterior teeth in Serbian population.

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LIFESTYLE RISK FACTORS IN THE DEVELOPMENT OF KIDNEY CANCER: A RUSSIAN EXPERIENCE

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STIL ŽIVOTA KAO FAKTOR RIZIKA ZA NASTANAK KARCINOMA BUBREGA: ISKUSTVA IZ RUSIJE

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ABSTRACT

The objective of our study was the evaluation of major lifestyle risk factors for the development of renal cell carcinoma (RCC) and the assessment of chances of developing this disease among inhabitants in the region, in the presence of and with combinations of the studied risk factors. Risk factors for developing RCC that are associated with lifestyle (smoking, obesity and hypertension) were observed in 500 patients with RCC aged 30-64 years who were investigated at the Krasnoyarsk Oncology Dispensary (study group) and 858 participants in the Krai (control group). The results of our study showed that smoking increases the risk of developing RCC 2.9 times and arterial hypertension 3.3 times in men; in women, obesity and hypertension increased these risks 2.6 and 3.2 times, respectively. All three risk factors were combined in 13.6% of men and in 8.4% of women with RCC. Our data may be useful for the prevention, development of screening programmes and early diagnosis of RCC.

Keywords: *risk factors, renal cell carcinoma, kidney cancer, smoking, obesity, arterial hypertension*

SAŽETAK

Cilj ovog istraživanja bio je evaluacija najznačajnijih faktora rizika koji potiču od stila života na razvoj karcinoma bubrežnih ćelija (KBĆ) i procena šanse za razvoj bolesti u regionu, u zavisnosti od prisutnosti ispitivanih faktora rizika. Faktori rizika za razvoj karcinoma bubrežnih ćelija koji su povezani sa stilom života (upotreba nikotina, gojaznost i hipertenzija) su posmatrani kod 500 pacijenata obolelih od karcinoma bubrežnih ćelija starosti 30-64 godina u Onkološkoj klinici Krasnojark (eksperimentalna grupa) i 858 ispitanika u kontrolnoj grupi u Krai. Rezultati studije su pokazali da upotreba nikotina 2,9 puta, dok hipertenzija 3,3 puta povećava rizik za razvoj karcinoma bubrežnih ćelija kod muškaraca; sa druge strane kod žena gojaznost i hipertenzija povećavaju ovaj rizik za 2,6 i 3,2 puta. Sva tri faktora rizika su bila prisutna kod 13,6% muškaraca i 8,4% žena sa karcinomom bubrežnih ćelija. Ovi podaci mogu biti od koristi za prevenciju bolesti, razvoj programa za skrining i ranu dijagnozu karcinoma bubrežnih ćelija.

Ključne reči: *faktori rizika, karcinom bubrežnih ćelija, karcinom bubrega, upotreba nikotina, gojaznost i hipertenzija*

INTRODUCTION

Renal cell carcinoma (RCC) is a multifactorial disease. Approximately 100 risk factors for the development of this malignant neoplasm have been described in the literature (1). However, in accordance with principles of evidence-based medicine, only three of these factors have been confirmed in large non-randomized, well-planned controlled trials (level of evidence: 2a): smoking, obesity and arterial hypertension (2). Smoking is a risk factor for development of RCC, as proven by the International Agency for Research on Cancer (3, 4). A large meta-analysis conducted in 24 centres showed that compared with non-smokers, there is a greater risk of developing RCC in ever-smokers. The risk of developing this SNR increases in proportion to the number of cigarettes smoked per day. Compared to those who never smoked, people who smoke more than 20 cigarettes a day increase the risk of developing RCC by 60-100%. The risk of developing RCC decreases only 10 years after quitting smoking (5). On the other hand, regarding excess body weight, the relationship between increase in body mass index (BMI) and risk of RCC development has been proven, and this risk is more pronounced in women than in men and depends on the degree of obesity. Thus, for every 5 unit (kg/m^2) increase in BMI, the risk of developing RCC increases by 24% in men and 34% in women (6, 7). The mechanism of this effect is associated with chronic hypoxia, insulin resistance development in tissues and compensatory hyperinsulinaemia, endocrine status changes with hyperproduction of adipokines, oestrogen,

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growth factors, immune response changes and cholesterol metabolism, increased lipid peroxidation and oxidative stress (8, 9). The association of arterial hypertension (AH) and the means used for its treatment, with the development of RCC, has been established in a number of epidemiological studies (10-12). The biological mechanism of this connection is not fully understood but is most likely associated with chronic renal hypoxia, lipid peroxidation, and formation of free radicals (13, 14). The risk of RCC in patients with AH is increased by 60% according to one author (11), and another group of researchers (12) found a 24% increase in risk in women and 15% in men. To date, the role of antihypertensive drugs in the development of RCC has not been determined, but it has been established that controlled BP reduces the risk of developing RCC (11, 12).

The aim of the study was to examine the major lifestyle risk factors for the development of RCC and to evaluate the chances of developing this disease among inhabitants of the region in the presence and combination of studied risk factors.

MATERIALS AND METHODS

The three major lifestyle-associated risk factors (smoking, obesity and hypertension) for the development of RCC were studied in 500 patients with RCC, age range 30-64 years old, at the Krasnoyarsk oncological dispensary (study group) and in 858 participants of the Krasnoyarsk Territory (control group). Study participants were from similar sex and age groups from a study of the main behavioural risk factors for the development of chronic non-communicable diseases within the framework of the targeted programme 'Prevention and control of socially significant diseases' (15). Evaluation of smoking-related indicators was carried out on the basis of questionnaire, which included questions about the existence of bad habits, quality of tobacco products, smoking experience and the number of cigarettes smoked per day. These questions are used in most major sociological studies on tobacco consumption in our region, Russia, Europe and the United States (5, 16, 17).

Body weight estimation was carried out using body mass index (BMI), developed by Quetelet *et al.,* and calculated by the formula (m=body weight in kg; h=growth in metres):

$$I = \frac{m}{h^2}$$

Interpretation of BMI values was carried out in accordance with WHO recommendations (18).

Arterial hypertension (AH) was observed in patients with RCC and study participants who had a history of hypertension, or a persistent increase in blood pressure values of more than 140/90 mm Hg (19).

Statistical analyses

All data are presented as frequency in percent (%). To assess the risk of developing RCC in the presence of a number of predisposing factors, odds ratios (ORs) were calculated with 95% confidence intervals (95% CI). In each case, confidence intervals were calculated for the OR. The chances were considered statistically significant if the unit did not fall within the confidence interval.

RESULTS

Smoking

Among the study group patients, 245 (49.0%) were identified as smokers, and of these, there were 211 men (73.8%) and 34 women (15.9%). In the control group (regional population), 285 (33.2%) were smokers, including 208 men (49.2%) and 77 women (17.7%). The data obtained indicate a statistically significant ($p<0.05$) prevalence of smoking among patients with RCC. This predominance is formed mainly by male smokers in the study group (smoking rate was 1.5 times higher than in the control group men). For females, there were no significant differences in smoking rates in the compared groups. Age-specific analysis of the prevalence of smoking in the compared groups revealed a significant predominance of smokers among men in the study group. Additionally, compared to the control group, among men with RCC in the age groups 35-39, 45-49, 50-54, 55-59, and 60-64 years and women aged 35-39 years, the prevalence of smoking was significant ($p<0.05$) (Figure 1).

Regarding the quality of tobacco products used by patients with RCC, it was found that most of the patients who used tobacco smoked cigarettes: men 65.4%, women 70.6%; 3.3% of men and 0% of women smoked cigarettes; 2.8% of men and 0% of women smoked cigarettes; other kinds of tobacco products were used by 6.6% of men and 5.9% of women.

Among the control group, the majority of smokers had a smoking history of up to 10 years, and among those in the study group, the smoking history was 20-29 years. In this case, among patients with RCC compared with the control group, the proportion of people who had smoked for less than 10 years was much lower, and those who had smoked for more than 30 years were predominant.

For males, there were significant smoking differences in 2 subgroups: smoking experience of up to 10 years and smoking experience of 30-39 years. In the first subgroup, smoking experience in the study group was significantly lower (3.8 times), and in the second subgroup was 2.8 times higher ($p<0.05$).

Among women, significant differences in smoking experience were found in all subgroups. In the subgroup with smoking experience of up to 10 years, the proportion of women in the control group was 7.3 times higher than that of the study group, and in the subgroup with 10-19 years of experience, there was a similar trend with a difference in indices of 2.2. In subgroups with smoking experience of 20-29 years, 30-39 years, and 40 years or more, the propor-

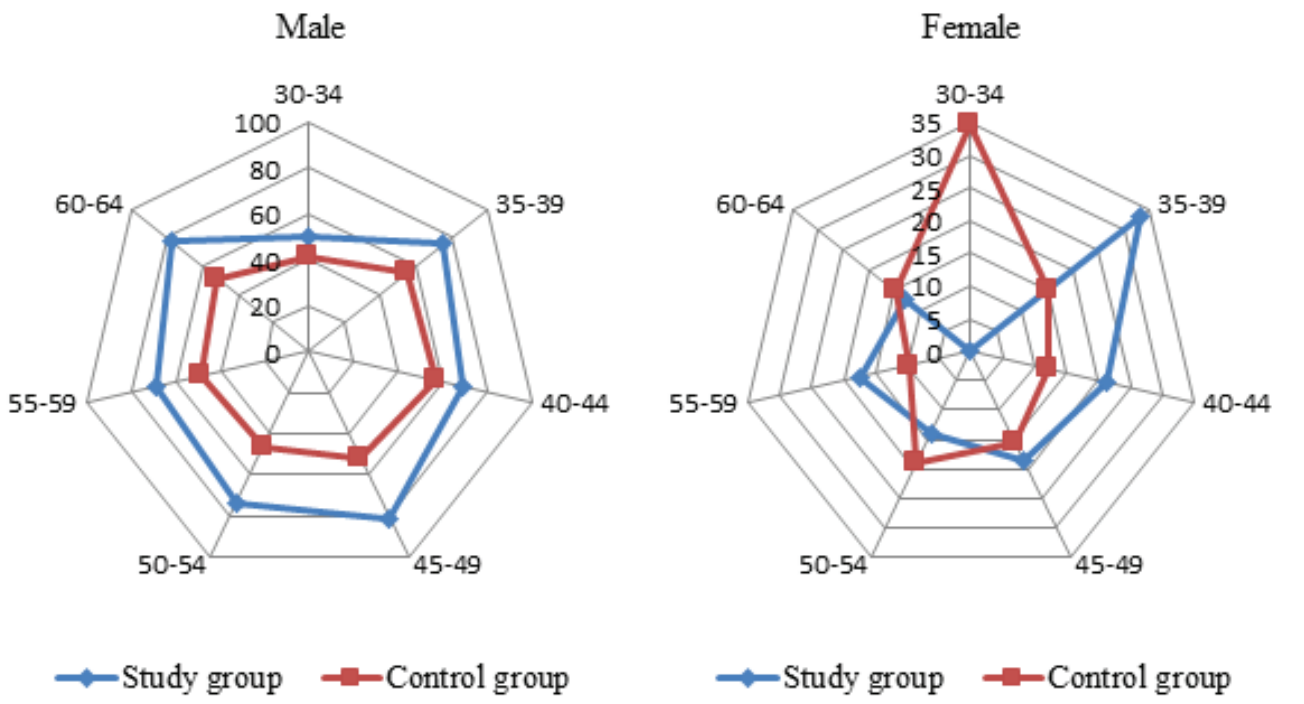

Figure 1. Prevalence of smoking in men and women in the compared groups.

tion of women in the study group was much higher; 1.8, 2.9, and 7.9 times, respectively, than a similar indicator in the control group.

Furthermore, 47.3% of patients with RCC smoked 10 to 20 cigarettes a day. The same number of cigarettes smoked was smoked by men with RCC, and among women surveyed, the prevalence of smoking was up to 10 cigarettes a day (50.0%). Comparative analysis of the number of smoked cigarettes did not reveal significant differences for this indicator for the groups compared.

Obesity

Compared with the control group, we found two statistically significant trends in our age-specific analysis of BMI: the prevalence of men with normal body weight (BMI 18.5-25) and the reduced in overweight (BMI 25-30) among RCC patients aged 35-39, 40-44 and 50-54 years (Figure 2).

Age-specific analysis of BMI in the compared groups of women revealed a 4.2 times predominance of persons with excess weight in the 30-34 years age group (BMI 25-30). In

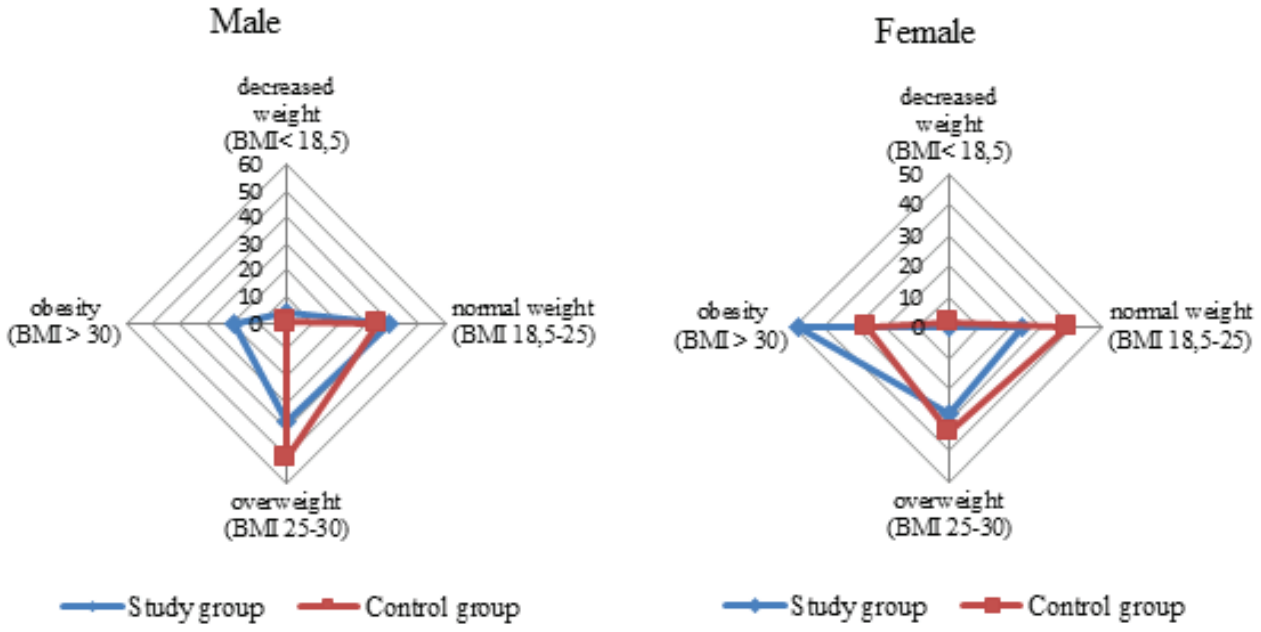

Figure 2. BMI distribution of men and women in the compared groups.

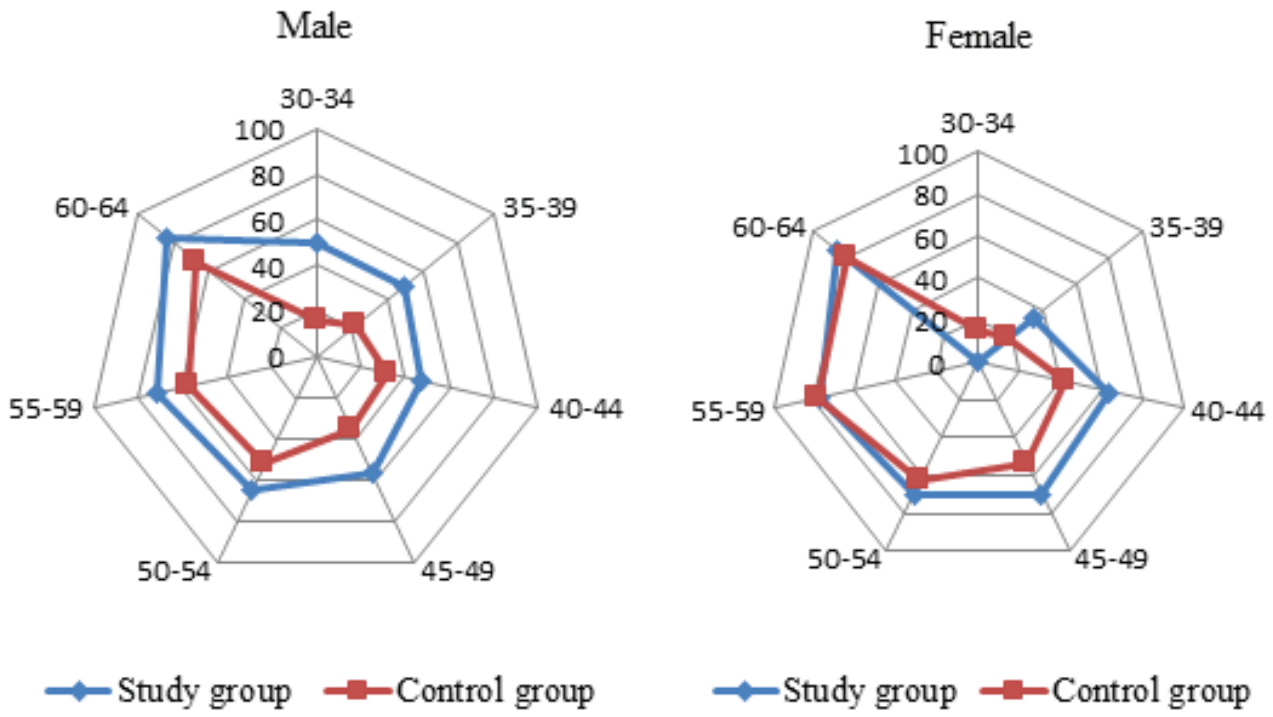

Figure 3. Prevalence of AH in men and women in the compared groups.

the 35-39 years age of the study group, there were 31.6% more people with a body mass deficit (BMI<18.5), 21.7% fewer women with normal body weight (BMI 18.5-25) and 18.3% more obese people (BMI>30) than in the control group. In the 40-44 years age in the study group, the number of persons with normal body weight was reduced by 43.8%, and there were 39.7% more women with obesity than in the control group. In the 60-64 years age group, there were 18.4% fewer overweight women in the study group and an 18.9% increase in the number of obese people compared to the control group. Despite the multidirectional character of age-specific trends, the final data show that in comparison with the control group, there was a predominance of obese women in the study group and a decrease in the proportion of people with normal body weight (Figure 2).

Arterial hypertension

In the study group, the average prevalence of arterial hypertension (AH) was 66.1% and 75.7% among men and women, respectively. In general, the prevalence of hypertension in patients of both sexes with RCC was 70.2%, which was significantly higher than the 43.4% in the control group ($p<0.05$). The prevalence of hypertension tended to increase with age among both men and women.

Age-specific analysis of the prevalence of hypertension in men and women in the compared groups is shown in Fig. 3. For both sexes, there was a statistically significant prevalence ($p<0.05$) of hypertension among patients with RCC in age groups 35-39, 40-44, and 45-49 and in men with RP at ages 30-34 and 60-64.

Combination of risk factors

The combination of all three risk factors for RCC was identified in 11.4% (13.6% of men, 8.4% of women), smoking and obesity in 14.4% (16.1% of men, 12.1% of women), smoking and hypertension in 34.8% (58.3% of men, 9.3% of women), and obesity and hypertension in 29.0% (17.8% of men, 43.9% of women).

Risk assessment of RCC development

In assessing the impact of risk factors on the development of RCC in men, it was found that the likelihood of developing disease was 2.9 times higher among smokers and 3.3 times higher among patients with AH. In women, the incidence of RCC increased by a factor of 2.6 in the presence of obesity and by 3.2 times with elevated BP fig-

Table 1. The ratio of the chances of developing RCC among participants of the region, depending on risk factor

Risk factor	Sex	*OR*	95% CI	
			Lower bound	Upper bound
Smoking	Male	2.894*	2.091	4.007
	Female	0.881	0.566	1.369
Obesity	Male	1.494	0.998	2.238
	Female	2.621*	1.860	3.693
Arterial hypertension	Male	3.256*	2.378	4.458
	Female	3.232*	2.245	4.653

Note: * - significant odds ratios.

ures. There was no significant difference in the likelihood of developing RCC in men with obesity and in women who smoked (Table 1).

Among men with smoking as a risk factor, the risk of developing RCC was confirmed for 4 age groups: 45-49 (OR 5.313 (95% CI 2.430-11.612)), 50-54 (OR 3.180 (95% CI 1.606-6.297)), 55-59 OR 2.257 (95% CI 1.077-4.731)) and 60-64 years (OR 3.135 (95% CI 1.217-8.073)). For hypertension, a significant odds ratio of 2.4 times (95% CI 1.2-5.0) was found only for ages 45-49 years. Among female patients, significant chances of developing RCC were found in obese patients in 2 age groups: 40-44 (OR 6.267 (95% CI, 1.778-22.081)) and 60-64 years (OR 2.182 (95% CI 1.044-4.559)).

DISCUSSION

From a medical and demographic point of view, Krasnoyarsk Territory is the largest part of the Russian Federation with a regressive type of population and gender disproportion (the prevalence of women is 14.5%), in addition to high mortality among able-bodied people (30.6%), most of whom are men (78.2%). Oncological diseases make a significant contribution to the mortality of the working-age population in our region as the third-most common cause of death (20). The mortality from RCC is significant to the region's medical, demographic and economic losses for a number of reasons: against a background of high mortality (JV 4.54, in the Russian Federation, 3.43 per 100,000 population), low, active detection rates were 7.5% (RF 11.1%), and early diagnosis of the disease was 46.2% (RF 55.8%).

Using the analysis of major lifestyle risk factors for developing RCC, we demonstrated the prevalence of smoking in men in virtually all analysed age groups and for women with RCC at ages 35-39 years. According to the quality of tobacco products used by patients with RCC, our data are comparable with the results of regional and Russian (15, 16) sociological polls of the population: 59.8% of men smoke, and 81.5% of women use factory-produced cigarettes in Russia (in our study 65.4% and 70.6%, respectively); 2.2% of men and 0.7% of women smoked cigarettes (our study; 3.3% and 0.0%); 1.8% of men and 1.4% of women smoked cigarettes (our study; 2.8% and 0%); and other kinds of tobacco products are used by 8.3% of men and 3.8% of women (our study 6.6% and 5.9%). Compared to the regional population (control group), smoking experience was significantly higher among patients with RCC, which was confirmed by the prevalence in this group of people who smoked for more than 20 years and the proportion of people who smoked for less than 10 years.

Overall, 31.6% of patients with RCC were obese; women, 65.2% and men, 34.8%. In the control group, the proportion of obese individuals was 20.0% (women, 66.3%; men, 33.7%). The most interesting and significant trend was revealed in the analysis of BMI, where compared with the control group, there was a predominance of obese females in the study group. No less important is the fact that this trend was formed mainly at the expense of age groups 40-44 and 60-64.

There was a prevalence of AH among men with RCC in almost all age groups and in women at younger ages (35-39, 40-44, 45-49 years).

Still, the importance and actuality of combined risk factors for the development of RCC is present in the literature. For example, when smoking and obesity are combined, the gender significance of the factors increases; in men, the risk of developing the disease increases by 47.0%, and in women by 33.9% (1). Krasnoyarsk residents have found that for men, risk factors such as smoking and hypertension (increased risk of developing RCC is 2.9 and 3.3 times, respectively) are more significant, and for women, obesity and hypertension (in 2.6 and 3.2 times respectively). All three risk factors are combined in 13.6% of men and in 8.4% of women with RCC.

CONCLUSION

A comprehensive analysis of the given risk factors for the development of RCC indicates that most attention should be paid to lifestyle-related factors: smoking, obesity and hypertension. On the other hand, these factors are preventable through primary and secondary prevention procedures, and they may be a useful tool for the screening and early diagnosis of RCC.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding this manuscript.

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EATING HABITS AND STANDARD BODY PARAMETERS AMONG STUDENTS AT UNIVERSITY OF BANJA LUKA

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NAVIKE U ISHRANI I OSNOVNI TELESNI PARAMETRI KOD STUDENATA UNIVERZITETA U BANJA LUCI

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ABSTRACT

Poor dietary habits have become one of the most important concerns among public health policy makers in recent years, due to the impact they have on both economic and health systems of a country. The transitional period toward young adulthood, marked with high school graduation and the beginning of college years, has been identified as critical in terms of its influence on young people's bad eating habits. The aim of this study was to assess whether the results obtained through **Food Frequency Questionnaire** *significantly correlate with standard body parameters. Participants included 210 students from the University of Banja Luka, with the mean age of 21.94 ± 2.73 years. Factorization of* **Food Frequency Questionnaire Instrument** *extracted seven factors which were subjected to multiple regression analysis as independent variables, and correlated to dependent variables - anthropological measurements. This study shows that the factors labeled as consumption of bread, consumption of healthy food, and intake of carbohydrates, are significantly related to Body Fat Percentage, whereas factors labeled as intake of food of animal origin, and intake of fruits and vegetables, are statistically significant in terms of their relation to Waist-to-Hip Ratio. Only one factor, labeled as intake of unhealthy food, is significantly related to Body Mass Index; this is to suggest that Body Mass Index has again showed many limitations with regard to its research relevance. This research has also found that students of the University of Banja Luka typically consume white bread, known to have a direct link with overweight and obesity.*

Keywords: *Body Mass Index, Eating Habits, Food Frequency Questionnaires, Body Fat Percentage, Waist-to-Hip Ratio.*

SAŽETAK

Loše navike u ishrani su postale jedna od najznačajnijih briga u politici javnog zdravlja poslednjih godina, jer one imaju uticaj kako na ekonomski tako i na zdravstveni sistem zemlje. Najkritičniji period u pogledu uticaja na stvaranje loših navika u ishrani je period odrastanja, odnosno period kraja srednje škole i početak fakulteta. Cilj ovog istraživanja bio je da se proceni da li rezultati dobijeni korišćenjem upitnika Food Frequency značajno koreliraju sa osnovnim telesnim parametrima. U istraživanju je učestvovalo 210 studenata sa Univerziteta u Banja Luci, prosečne starosti 21,94±2,73 godina. Korišćeni upitnik je imao sedam faktora koji su podvrgnuti višestrukoj regresionoj analizi kao nezavisne varijable, a potom korelirani sa antropometrijskim merenjima koji predstavljaju zavisne varijable. Ova studija je pokazala da faktori označeni kao konzumiranje hleba, konzumiranje zdrave hrane i unos ugljenih hidrata su značajno povezani sa procentom masti u telu, dok su faktori označeni kao unos hrane animalnog porekla, unos voća i povrća značajno povezani sa odnosom obima struk/kuk. Samo jedan faktor, označen kao unos nezdrave hrane, je značajno povezan sa indeksom telesne mase, što još jednom ukazuje na činjenicu da indeks telesne mase ima dosta ograničenja u pogledu njegove relevantnosti za istraživanje. Takođe je ovim istraživanjem utvrđeno da studenti Univerziteta u Banja Luci tipično koriste beli hleb iako je poznatno da on dovodi do gojaznosti i nastanka prekomerne telesne mase.

Ključne reči: *Indeks telesne mase, navike u ishrani, upitnik Food Frequency, procenat telesnih masti, odnos obima struk/kuk*

ABBREVIATIONS

BMI - Body Mass Index
FE - Faculty of Economics
FFQ - Food Frequency Questionnaire
FM - Faculty of Medicine
FPES - Faculty of Physical Education and Sport
BF% - Body Fat Percentage
WHO - World Health Organization
WHR - Waist-to-Hip Ratio

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INTRODUCTION

Overweight and obesity prevalence across the globe (1) has witnessed a rising trend in the recent years, so that is almost possible to define it as a global pandemic (2), whereas its link to various diseases (3) puts it in the focus of public health debates (4). Overweight and obesity epidemic is equally found in developed and developing countries (5), and it is rapidly growing in the Third World Countries (6). It is recorded in all age groups including children, adolescents (4), and university students (7, 8).

Late adolescence and early adulthood period has been reported as indicative in relation to weight changes and adoption of poor dietary and exercise patterns (9); numerous studies from US have confirmed that freshman years among university students are critical in weight gain and behavioral patterns that may contribute to overweight and obesity (10-12). Weight gain in freshman college students and perceived health among European undergraduate has proven to follow the same pattern, but with a more pronounced perception of overweight and obesity among female students (8), even within other age groups (13, 14) (gender-sensitive dimorphism). However, a study carried out at the University of Banja Luka reported high prevalence of overweight and obesity 24.80%, but with different results in comparison to other European countries, where male students were recorded to be more overweight/obese (17). Analyses of overweight and obesity prevalence conducted in the region of Southeastern Europe have come up with similar results (18-20).

World Health Organization defines overweight and obesity as abnormal or excessive fat accumulation that may impair health (21). This is not to suggest that there is a single individual according to whose body measures we can compare overweigh/obesity values, but the point is that the fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended (22). Dysfunction of feedback mechanism of satiety, hyperinsulinemia, insulin resistance and genetic predisposition, are seen as one the main causes of overweight and obesity (23). Some authors even define this term as obesogenic environment - an environment that promotes weight gain and offers a wide array of foods loaded with fat and sugar - nutrition known to have a jeopardizing effect on human metabolism (24). On the other hand, there are authors who link overweight and obesity with socioeconomic status of an individual, sedentary (urban) lifestyle, size of family, physical inactivity, educational status, cultural factors and bad eating habits (25).

Freshman adaptation to university life is highly demanding for young adults (26, 27), so if they fail to adequately adapt to this new environment, the poor dietary patterns and weight gain are likely to ensue (27). The reason probably lies in the fact that students experience certain number of transitional challenges when they transfer from secondary school to college environment, all of which may perpetuate in poor dietary and exercise patterns (28). Physical activity and proper eating habits are seen as key factors in the prevention of overweight-related diseases (29), whereas sedentary lifestyle and excessive calorie intake are labeled as two factors which contribute most to overweight and obesity. Changes in physical activity (30) during early college years (young adulthood) may contribute to overweight and obesity, as this is when adolescents change their behavioral and dietary patters (31). Moreover, academic burden students experience on the daily basis is discouraging with regard to adoption of healthy eating and exercise habits (32). Changes in weight, composition and shape of body are reported to be significant and incremental, and they may result in rather unfavorable lifelong health issues (33, 34).

Prevalence of underweight, alongside with overweight and obesity, has been reported in university students from the region of Murcia (35). Olearo et al. (36) claim that 12.5% of university students are underweight, while a study carried out by Pelzner et al. (7), as well as other similar researches (37), found underweight prevalence more common among female students. Raseta et al. (17), and other authors from our region (18-20), came up with similar findings. This is to say that the issue of underweight should receive an adequate attention as well (38).

The aim of this study was to investigate whether the results obtained through self-reported *Food Frequency Questionnaire [FFQ]* correlate with values measured by *Body Mass Index [BMI], Body Fat Percentage [BF%] and Waist-to-Hip Ratio [WHR]* in students from the University of Banja Luka.

MATERIALS AND METHODS

This study was conducted as the cross-sectional one, with 210 participants from the University of Banja Luka – the mean age 21.94 ± 2.73 years. Out of the total number of participating students, 30 per year of study (first- and third-year students) were from the Faculty of Physical Education and Sport [FPES], and the Faculty of Economics [FE] – total of 120 students; and 90 were students from the Faculty of Medicine [FM] – 30 students per year of study (first, third, fifth). Male to female ratio was imbalanced (107 male or 50.95%, and 103 female or 49.05%) due to the general gender structure at the Faculty of Physical Education and Sport, and Faculty of Economics. Students were recruited on a voluntary basis, and an informed consent from the students was obtained prior to their participation. This study was carried out in accordance with the highest ethical principles set out in the Declaration of Helsinki (DoH), and it was compliant with the similar legislation concerning human experimentation in Bosnia and Herzegovina.

Anthropometric measurements were performed in accordance with the recommendations from the International Biological Program (IBP) and World Health Orga-

nization (WHO). The measurements were performed at the Institute of Sport of the Faculty of Physical Education and Sport, during the months of May and June 2015. The students were measured according to the following parameters: Body Mass (measured in kilograms using medical weighing scales having precision of 0.1 kg), Body Height (the height was taken barefoot in centimeters by using the Martin anthropometer, and it was recorded with the precision of 0.1 cm), Waist Circumference (measured at the midpoint between the lowest point of the rib cage and the highest point of femoral crest of the pelvic bone), Hip Circumference (measured at the widest point while being at level with the trochanter), Biceps Skinfold (measured on the anterior side of the middle of forearm just above m. biceps), Triceps Skinfold (measured on the dorsal side of the middle of forearm just above m. biceps), Subscapular Skinfold (measured below the lower edge of the scapula), and Suprailiac Skinfold (measured 1 cm above and 2 cm medially from anterior superior iliac spine (ASIS) - *spina iliaca anterior superior*). Circumferences were recorded by using measuring tape having precision of 0.1 cm, while skinfold measurements were obtained using a John Bull Calipers with 0.2 mm precision and standard pressure of 0.01 Pa on 1 mm^2 of skin.

According to WHO's overweight and obesity factsheet (39), BMI values were determined as follows: ≤18.5 underweight, between 18.5 and 24.9 normal (healthy) weight, 25.0 - 29.9 overweight, and ≥30 obese. WHR values were classified according to WHO's recommendations (40), and with respect to gender dimorphism – females: <0.75 excellent, 0.75-0.79 good, 0.80-0.86 average and >0.86 at risk, and males: <0.85 excellent, 0.85-0.89 good, 0.90-0.95 average, and >0.95 at risk. The calculation of Body Fat Percentage was based on the formula developed by Durin and Womersley (41), with predicted values for 17-29 years old people expressed as logs of the total four skinfold types. The classification of the obtained values was performed in accordance with Bray (42), again with the respect of the gender dimorphism – females: 20-30% physiological (normal) values, 30-33% overweight, >33 obesity; males: 12-20% physiological (normal) values, 20-25% overweight, >25% obesity.

As an index of weight-for-height, BMI has been regarded as simple but at the same time the method with serious limitations (43) when it comes to body composition analysis within an entire population. BMI and BF% tend to be misinterpreted, particularly when observed across different age groups. BMI is comprised of both fat and muscle mass, so it sometimes can be misleading in terms of evaluating somebody's overweight and obesity status, especially in people with normal or relatively low values of BF% (44) - professional athletes for example (18). Although BMI values in middle-aged people (45) can be relevant, in younger population, however, BMI is seen as the least reliable predictor of overweight and obesity. Though it has been used much less than BMI, WHR provides more accurate results with respect to body composition (45).

In order to track down the history of dietary habits in university students, we designed *FFQ instrument* (46), with an intention to account for how often and how much certain amount of food was consumed during the reference period (47). Calibration was performed by factor analysis (Brattlett Test of Sphericity was significant at $p < 0.01$, and Kaiser-Meyer-Olkin Measure of Sampling Adequacy showed that indicators of sampling adequacy were higher than 0.40 - *KMO* = 0.68), and the number of extracted factors was seven, out of which six had Cronbach's Alpha quotient at above 0.50: (a) intake of food of animal origin (α = 0.72); (b) consumption of white bread (α = 0.92); (c) consumption of healthy food (α = 0.63); (d) intake of unhealthy food (α = 0.63); (e) intake of carbohydrates (α = 0.43); (f) consumption of milk and dairy products (α = 0.71); (g) consumption of fruits and vegetables (α = 0.77). The respondents were asked to provide answers on a Likert-type scale ranging from 1 to 9: 1 = never, 2 = once a month, 3 = twice a month; 4 = once a week; 5 = twice a week; 6 = 3-4 times a week; 7 = 5–6 times a week; 8 = once a day; and 9 = twice a day. One of the items relating to the intake of food of animal origin read: *How often do you eat meat?* The following items exemplify the nature of the other remaining factors: consumption of healthy food – *How often do you eat fish?;* intake of unhealthy food – *How often do you eat sweets?;* consumption of milk and dairy products – *How often do you eat dairy products?;* etc.

The data were coded and entered into a database. We used simple descriptive statistics to provide basic information about the overall characteristics of the sample - One-tailed Bivariate Correlation, factor analysis with Varimax rotation (characteristic values over 1.00), Chronbach's Alpha quotient, and multiple regression analysis. Statistical analyses were carried out with IBM SPSS Statistics 21.0.

RESULTS

The overall view on the obtained results points to the following means of the observed parameters: male students - WHR (*M* = 0.84, *SD* = 0.52), BF% (*M* = 15.47, *SD* = 3.50), and BMI (*M* = 24.36, *SD* = 2.59); female students - WHR (*M* = 0.72, *SD* = 0.04), BF% (*M* = 25.14, *SD* = 3.59) and BMI (*M* = 21.54, *SD* = 2.46). Mean values by fields of study (faculty attended) and gender are shown in Figure 1, with WHR results presented in Graph 1, BF% results in Graph 2, and BMI in Graph 3. The first indicators in all the three graphs give mean values for the observed parameter – for the students of the Faculty of Physical Education and Sport, the Faculty of Economics and the Faculty of Medicine, respectively.

With respect to *FFQ Instrument,* Figure 2 gives clear evidence that the curve of mean values across items is almost identical. Tested students reported the highest value answers (i.e. 5-6 times a week) for item *How often do you eat bread?* (*M* = 7.27, *SD* = 0.16). Consumption of food

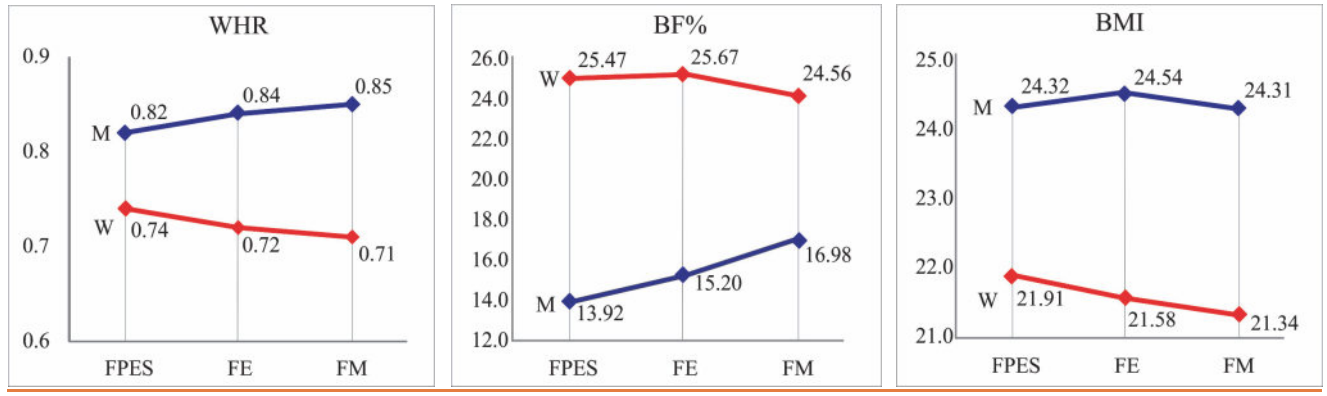
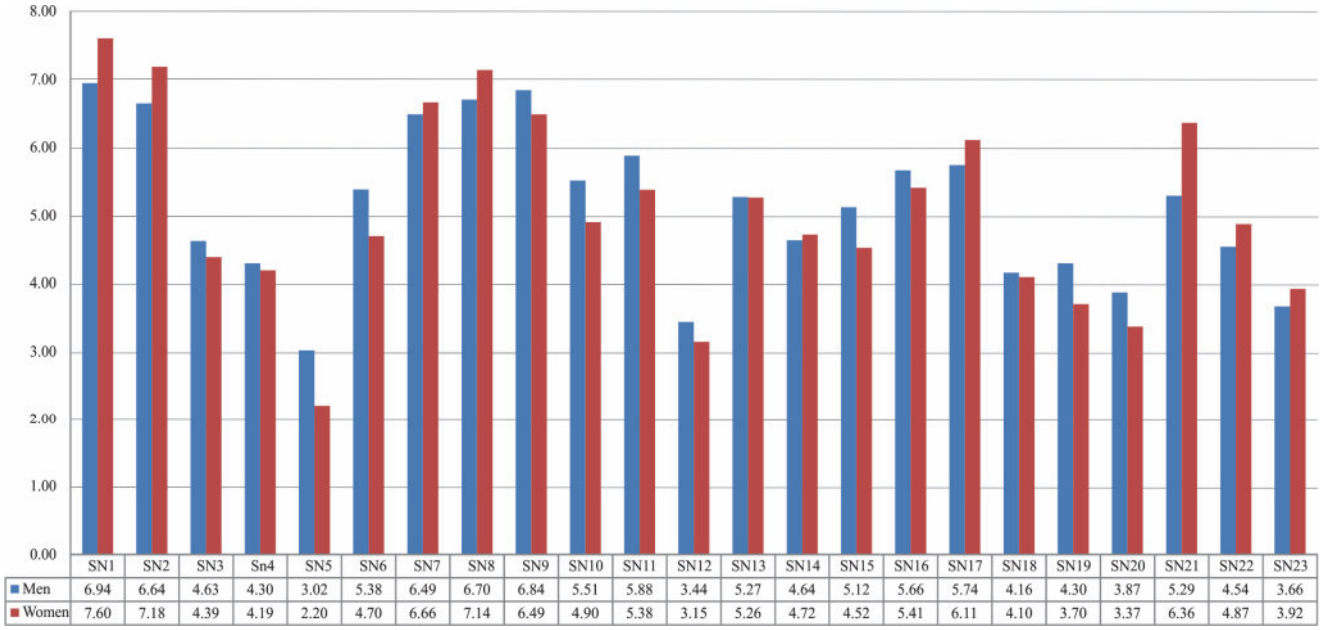


Figure 1
Legend: WHR – Waist-to-Hip Ratio; BF% – Body Fat Percentage; BMI – Body Mass Index; FPES – Faculty of Physical Education and Sport; FE – Faculty of Economics; FM – Faculty of Medicine; M – Men; W – Women.

3-4 times a week was recorded for the items *How often do you eat white bread?* (M = 6.91, SD = 0.18), *How often do you eat fruits?* (M = 6.91, SD = 0.11), *How often do you eat meat?* (M = 6.67, SD = 0.11) and *How often do you eat vegetables?* (M = 6.57, SD = 0.12). The lowest value (i.e. twice a month) was recorded for the item *How often do you eat whole wheat pasta?* (M = 2.62, SD = 0.12), whereas on the following items: *How often do you eat fish?* (M = 3.30, SD = 0.11), *How often do you drink skimmed milk?* (M = 3.62, SD = 0.17), and *Do you eat margarine or butter?* (M = 3.79, SD = 0.15) students responded with *once a week* answers.

Triangular correlation matrix between the extracted factors following the factorization of *FFQ instrument* and the assessment results for variables WHR, BF% and BMI shows that BF% is partially correlated at $p < 0.05$ level with the following four extracted factors: positively with *consumption of bread* and *intake of unhealthy food*, and negatively with *consumption of healthy food* and *intake of carbohydrate;* WHR correlates with three factors: positively with *intake of food of animal origin* and *intake of carbohydrates,* and negatively with *consumption of fruits and vegetable;* BMI has only one negative correlation – namely with *intake of unhealthy food.*



	SN1	SN2	SN3	Sn4	SN5	SN6	SN7	SN8	SN9	SN10	SN11	SN12	SN13	SN14	SN15	SN16	SN17	SN18	SN19	SN20	SN21	SN22	SN23
Men	6.94	6.64	4.63	4.30	3.02	5.38	6.49	6.70	6.84	5.51	5.88	3.44	5.27	4.64	5.12	5.66	5.74	4.16	4.30	3.87	5.29	4.54	3.66
Women	7.60	7.18	4.39	4.19	2.20	4.70	6.66	7.14	6.49	4.90	5.38	3.15	5.26	4.72	4.52	5.41	6.11	4.10	3.70	3.37	6.36	4.87	3.92

Figure 2
Legend: SN1 – How often do you eat bread?; SN2 – How often do you eat white bread?; SN3 – How often do you eat cereals (cornflakes, etc.)?; SN4 – How often do you eat white flour pasta (spaghetti, macaroni)?; SN5 – How often do you eat whole wheat pasta?; SN6 – How often do you eat potatoes?; SN7 – How often do you eat vegetables?; SN8 – How often do you eat fruits?; SN9 – How often do you eat meat?; SN10 – How often do you eat red meat?; SN11 – How often do you eat chicken and lean meat?; SN12 –How often do you eat fish?; SN13 – How often do you eat eggs?; SN14 – How often do you eat delicatessen food?; SN15 – How often do you eat legumes (beans, peas, pulses)?; SN16 – How often do you drink milk?; SN17 – How often do you eat dairy products?; SN18 – How often do you consume whole milk dairy products?; SN19 – How often do you consume partly skimmed milk?; SN20 – How often do you consume skimmed milk?; SN21 – How often do you eat sweets?; SN22 – How often do you eat snacks?; SN23 – Do you consume margarine or butter?

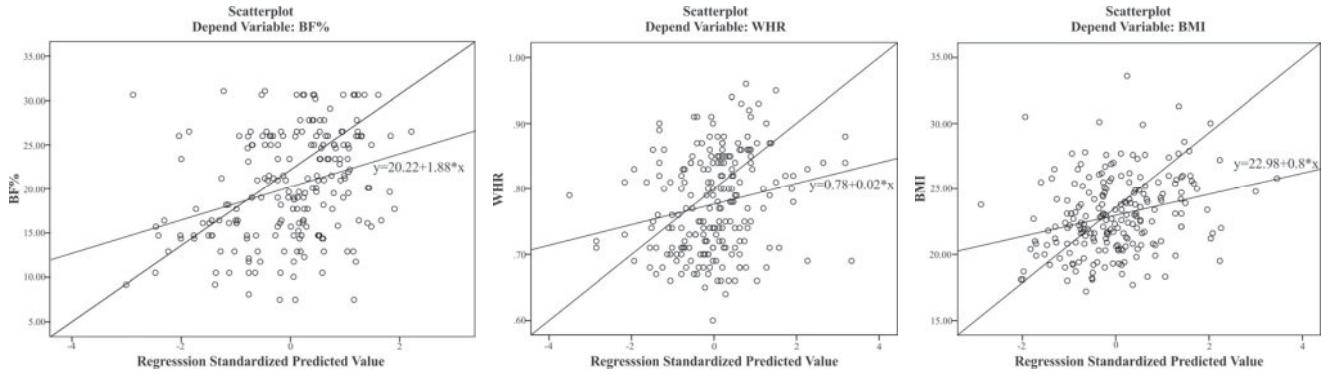


Figure 3

The dependent variable BF% shows statistically significant correlation ($F_{(3, 206)} = 7.48$; $p = 0.00$) in the third iteration of Stepwise Regression Model, with following three factors extracted from the *FFQ instrument*: *consumption of bread* ($p = 0.00$), *intake of carbohydrates* ($p = 0.01$) *and consumption of healthy food* ($p = 0.05$).

The dependent variable WHR shows statistically significant correlation, ($F_{(2, 207)} = 4.71$; $p = 0.01$) in second iteration, with two factors: *consumption of fruit and vegetables* ($p = 0.01$) and *intake of food of animal origin* ($p = 0.03$).

Regression analysis for BMI is not here provided due to the fact that triangular matrix of partial correlations shows that BMI only correlates with one out of seven factors, namely *intake of unhealthy food,* at $p < 0.05$ level.

Diagrams of dispersion of residues indicate that there is a clear dependence between residues and predicted values for all three dependent variables, which is consistent with the linearity assumption (Figure 3).

DISCUSSION

Raseta et al. (17) showed that, according to WHR, 98.10% of male and 87.90% of female students fell within limits marked as excellent and good. Only 1.90% of male students had average (within borderlines) results, while female students fared a bit worse (11.20% were average, and 1.00% were at risk of overweight). In terms of percentage distribution, BF% variable had 74.80% of male and 72.00% of female students within physiological (normal) values, 15.50% male students and 11.20% female students fell within overweight category, whereas 9.70% males and 16.80% females were underweight. The observed group of undergraduate students recorded no cases of obesity. Percentage distribution of BMI variable classified 70.90% students within normal values, 22.40% overweight, 2.40% obesity, and 4.30% underweight. Raseta et al. (17) appear to have substantiated the widespread claims that student population is witnessing an increase in overweight and obesity prevalence regardless of the part of the world it occurs (7), with the region of the Southeastern Europe being no exception (48). If these results are set against similar studies from the European context (8), we cannot help but to conclude that they are inversely proportional to the results presented by our European fellow researchers. In a research that included participants from 16 European countries, female students were reported to have larger proportion in overweight and obesity category as compared to male students. Some other studies from our region (18-20, 49, 50), as well as from other Eastern European countries (51), have confirmed the same trend of overweigh and obesity among university students. Davar (5) concludes that almost all countries are facing obesity endemic, although great variations exist between and within countries. More interestingly, underweight prevalence is on the rise among students, especially among female students (38), which has been corroborated by the study carried out on the population of university students in Banja Luka (17). This phenomenon might be interpreted as female students' concern about their appearance, which is hugely influenced by women celebrities. Their primary motivation to stay slim could be driven by the desire to attract the attention of the opposite sex, peer support, and increase of self-confidence (52).

Bad eating habits, physical inactivity, and sedentary lifestyle can all predispose university students to face weight issues (13), i.e. reduced physical activity and high caloric diet are almost certain risk factors of overweight and obesity in student population (31). As noted before, the transition between adolescence and adulthood, a common age for university attendance, is accompanied by weight gain, so it is obvious that there is a need to initiate lifestyle changing strategies at this particular period of life if we want to prevent overweight and obesity, and contribute to improve one's exercise and diet habits in the long run (53).

Regression analysis of *FFQ Instrument* in third iteration extracted three factors that correlate with BF% variable with statistical significance. The first factor was labeled *consumption of bread* and it included two items from *FFQ Instrument*: (a) *How often do you eat bread?* – students responded 5-6 times a week, and (b) *How often do you eat white bread?* – students responded 3-4 times a week. The second factor, labeled as *intake of carbohydrate*,

included three items from *FFQ Instrument:* (a) *How often do you eat white flour past (macaroni, spaghetti)?* - the response was once a week; (b) *How often do you eat potatoes?* – twice week, and (c) *How often do you eat legumes (beans, peas, pulses)?*– once a week; the third factor was labeled as consumption of healthy food and it was consisted of five *FFQ Instrument* items: (a) *How often do you eat cereals (cornflakes, etc.)?* – twice a week; (b) *How often do you eat whole wheat pasta?* –once a month; (c) *How often do you eat fish?* – twice a month; (d) *How often do you consume partly skimmed milk*? – once a week, and (e) *How often do you consume skimmed milk?* – once a week. The obtained results clearly indicate that white bread consumption, which is associated with fast food, is strongly correlated with weight gain and occurrence of overweight and obesity. On the other hand, intake of healthy food recorded very poor results in terms of its frequency among students from the University of Banja Luka. There are numerous studies which provide plentiful evidence with regard to bad dietary habits in student population (54). Some researchers (48, 55) from our region even go further to conclude that 60-70% of students have bad eating habits. Such dietary pattern is closely linked with weight gain in this population. Food preference and widespread availability of fast food are also contributing to negative eating environment surrounding young students (56). Various reasons are seen to be underlying in this respect: lack of available information on healthy food choices (57), financial side of the food issue – unhealthy food is readily available for less money than healthy food (58), students' financial status (59), as well as exposure to high level of stress among university students, particularly before exams (60). Some US studies have also found that reduced intake of healthy food and bread is a trade-off for increased consumption of fat-loaded food and alcohol (61).

Two factors exhibited statistically significant correlation with variable WHR (in second iteration). The first factor was labeled as consumption of fruits and vegetables and it included two items from *FFQ Instrument: How often do you eat vegetables?* and *How often do you eat fruits?* – students came up with answers 3-4 times a week. The second factor was labeled as intake of food of animal origin and it included the following four items: (a) *How often do you eat meat?* students responded 3-4 times a week; for questions (b) *How often do you eat red meat?*; (c) *How often do you eat chicken and lean meat*; and (d) *How often do you eat eggs?* students gave the same answers – twice a week. Romaguera et al. (62) found that diet with plenty of fruits and dairy products, and little white bread, processed meat, margarine and soft drinks (non-alcoholic beverages), can prevent gain of visceral fat. Students who show tendency towards eating less of fruits and vegetables than recommended on the daily basis, are inclined to drinking alcohol, overeating fast food, and succumbing to excessive calorie intake (14), all of which predispose them to overweight and obesity (13, 54). The present study shows that, in comparison to other types of food, the tested students exhibited preferences towards fruits and vegetables – the similar findings have been presented by Yahia et al. (63). Contrary to the popular belief though, some authors found no correlation between consumption of fruits and vegetables accompanied by physical activity, and prevention of overweight and obesity (64).

BMI was correlated with only one factor, labeled as intake of unhealthy food, which included the following four items: (a) *How often do you eat sweets?* – twice a week was students' response, (b) *Do you consume margarine or butter?* – twice a month, for (c) *How often do you eat delicatessen food?*, and (d) *How often do you eat snacks?*, students responded once a week. Excessive intake of sweets among college students has been in the focus of different studies (53); an increased consumption of snacks before exams in student population was also recorded (48). Increased BMI values have been reported as being in close relation to excessive sweets intake in younger population (65). However, BMI has repeatedly been found insufficient of providing apt classification with regard to weight (nutritional) status of university students. Similar findings appear to have been substantiated by the present study as well - which provides yet another insight into the limitations of BMI's reliability (5, 17, 45).

There are of course certain limitations to this study. First of all, classification of students into the two following categories - (a) those who continue to live at their parents' home during their studies, and (b) those who move away from their parents – would be beneficial as there are numerous researches supporting claims that students who change their permanent residence tend to be more susceptible to dietary changes (66). Furthermore, the number of participating students and faculties is surely one of the most serious limitation. What is more, students often showed little or no motivation to participate in studies of this kind.

It is opined that further research into the issue of eating habits and their impact of body and weight status among student population, should be carried out in the form of longitudinal study with two dependent variables, namely BF% and WHR. That would also call for adjustments in *FFQ Instrument,* in order to eliminate drawbacks noticed during the present study. Finally, the number of participants in any future research should be increased.

CONCLUSION

The vast amount of research findings provided by this paper conclusively correlates Body Fat Percentage, Waist-to-Hip Ratio and dietary habits of the participating students. Bread consumption has been found to stand in the highest correlation to Body Fat Percentage. This implicitly assumes that the students from the University of Banja Luka tend to consume too much fast food and thus account for an increased number of student population in overweight category.

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OLDER HYPERTENSIVE PATIENTS' ADHERENCE TO HEALTHY LIFESTYLE BEHAVIORS

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PRIDRŽAVANJE STARIH HIPERTENZIVNIH PACIJENATA ZDRAVIM ŽIVOTNIM NAVIKAMA

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ABSTRACT

Non-pharmacological treatment including diet, body weight reduction, smoking cessation and physical activity, is very important part of hypertension treatment. The objective of this study was to investigate the adherence to healthy lifestyle behavior in the representative sample of the older hypertensive patients, and to investigate factors associated with adherence in the studied older population. The study was conducted on random sample of 362 long term hypertensive (> five years) patients older than 65 years of age, at Health Care Center of Kragujevac. Adherence was assessed using the structured questionnaire for the analysis of the implementation of both hypertension and diabetes guidelines in the primary care. Both bivariate and multivariate analyses were conducted. Nearly 35% of examined patients were highly adherent; they exercised regularly, avoided smoking for at least five years and consumed special healthy diet prescribed for hypertension. Another 35.6% of the cases reported exercising regularly, 39.5% followed the recommended diet for the hypertension, while 23.4% of the patients have still consumed cigarettes. Multivariate logistic regression demonstrated that received counseling on healthy lifestyle behaviors by physicians and lack of education predicted high adherence to healthy lifestyle behavior. In order to improve adherence of elderly hypertensive patients to healthy lifestyle, strengthening patient-physician relationships through efforts to enhance communication may be a promising strategy to enhance patients' engagement in healthy lifestyle behaviors for hypertension. Such an improvement could be achieved through the education of both the physicians and patients.

Key words: *hypertension, older patients, lifestyle adherence*

SAŽETAK

Nefarmakološki tretman koji uključuje dijetu, smanjenje telesne mase, prestanak pušenja i fizičku aktivnost je veoma važan u lečenju hipertenzije. Cilj ovog istraživanja je bio ispitati na reprezentativnom uzorku starijih pacijenata obolelih od hipertenzije u kojoj meri se oni pridržavaju zdravih životnih navika kao i koji faktori utiču na to. Ispitivanu grupu je činio uzorak od 362 slučajno odabrana pacijenta Doma zdravlja Kragujevac starijih od 65 godina koji su imali hipertenziju duže od pet godina. Komplijansa je procenjivana pomoću strukturiranog upitnika za analizu primene vodiča za hipertenziju i dijabetes u primarnoj zdravstvenoj zaštiti. Za obradu podataka korišćene su bivarijantna i multivarijantna analiza. Približno 35% ispitanih pacijenata je pokazalo dobru komplijansu: redovno je vežbalo, nisu bili pušači najmanje pet godina i pridržavali su se dijete za hipertenziju. 35,6% pacijenata je redovno vežbalo, 39,5% se pridržavalo dijete za hipertenzivne pacijente, dok je 23,4% pacijenata koristilo cigarete. Multivarijantnom logističkom regresijom je pokazano da su savetovanje o zdravim životnim navikama od strane lekara i niži obrazovni status pacijenata prediktori dobre komplijanse kada su u pitanju zdrave životne navike. U cilju većeg pridržavanja zdravim životnim navikama starijih pacijenata obolelih od hipertenzije potrebno je jačanje odnosa pacijent-lekar putem napora da se unapredi komunikacija što može biti strategija koja dovodi do većeg angažovanja pacijenata na polju zdravih životnih navika Taj napredak bi mogao biti postignut edukovanjem kako lekara tako i pacijenata.

Ključne reči: *hipertenzija, stariji pacijenti, pridržavanje zdravim životnim navikama*

ABBREVIATIONS

BMI – body mass index **BP** – blood pressure

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INTRODUCTION

Hypertension is one of the most common illnesses among adults. The national health survey conducted by Institute of Public Health of Serbia „Dr Milan Jovanovic Batut", in 2006th, estimated that 77.2% of population aged over 65 years and 75.7% over 75 years of age had increased systolic blood pressure (1). The non-pharmacological treatment such as life style modifications that include diet, body weight reduction, smoking cessation and regular physical activity in combination with medical therapy are very effective measures for optimum treatment of hypertension. According to the latest Guidelines for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC) lifestyle behaviors that reduce blood pressure are: salt restriction, high consumption of fruits and vegetables, low-fat diet, regular physical exercise and weight reduction (2). Moreover, according to behavioral model, even the most effective therapy will not ensure acceptable results of treatment if a patient is not motivated to adopt physician-recommended healthy lifestyle modifications and medication adherence for hypertension control (3). However, patients' compliance with both part of the treatment is usually low, and that could be a main reason for only one third of patients having their blood pressure controlled (4).

The elderly patients with hypertension are highly specific in regard to their behavior and adherence to antihypertensive treatment. A far more disturbing finding is that the control of hypertension and compliance to the healthy lifestyles in older patients is far worse than that achieved in the hypertensive patients of other age groups. Elderly hypertensive patients require better life quality control even more than the other patients since they retained harmful lifestyles behaviors such as physical inactivity and non-antihypertensive diet, while smoking cessation was accepted (5). On the other hand, the age is not significant predictor of adherence to physical exercise, while the exercise duration correlates well with the percentage of adherence (6). In addition, self-care ability of hypertensive patients is important factor in the control of hypertension that is most often impaired in the elderly (7). The adequate knowledge of a healthy life styles and proper skills of patients are also necessary for positive influence of the adherence to good quality of life (8, 9). However, the other potential factors that may influence the adherence to healthy lifestyle in elderly have not yet been investigated.

The aim of this study was to investigate the adherence to healthy life style behaviors in older hypertensive patients and the factors associated with adherence, such as those that are patient's related including demographic factors, general behavior and beliefs and physician's related including counseling by physicians about healthy lifestyle and self – management of hypertension.

PATIENTS AND METHODS

The study was conducted at Health Care Center of Kragujevac, in November 2015. year and it included only the urban population of hypertensive patients. The study was approved by the Ethics Committee of Health Center Kragujevac and was done and in accordance with the Helsinki Declaration.

The inclusion criteria of patients were as followed: above 65 years of age and diagnosed with hypertension 5 years ago or more, while the exclusion criteria were: the patient's refusing to participate in the study, the patient's acute condition visiting the Health Care Center at the moment of the investigation performed and the patient's dementia (as diagnosed by psychiatrist or neurologist).

Those patients that fulfilled the inclusion criteria were enlisted with for the encounter with the physician every day with the assigned number starting from number one to the maximum count of patients for that day. Then, applying the random number generation function from Microsoft Excel computer program, a sample of 1 out of 10 of the number of patients for that day was randomly selected for the participation in the study**.** These patients were provided with the structured questionnaire that included questions about their sociodemographic and medical characteristics, lifestyle behaviors, knowledge and beliefs about hypertension and its management, and about received counseling regarding their lifestyle and self-management (with questions shown in Table 1-3). The same questionnaire was already used in previous study regarding the analysis of the implementation of the hypertension and diabetes guidelines in the primary care (10). For the illiterate patients, the questionnaire was filled by an investigator, on the basis of oral responses received by the patients.

The study's outcome variable was the patient's reported adherence to the healthy lifestyle behavior. The patients were asked about implementing the following elements of the healthy lifestyle**:** the hypertension diet (limited salt consumption, calorie limitation), the physical activity (at least half an hour daily exercise), and the smoking cessation (at least for the past 5 years if they were previously smokers). The patients who reported the adherence to all of the elements of the healthy lifestyle were designated as the "highly adherent", and the rest of the patients were designated as the "low adherent".

The independent variables of the study were: the demographic characteristics (age, gender, education, family status), the body mass index, the blood pressure level-measured by a health worker during the actual visit, the blood pressure control (i.e. for the question 'is your BP controlled' from the questionnaire –the 'yes' response was assigned to the patients whose BP was mostly below 140 mmHg for systolic and 90 mmHg for diastolic, and never exceeded BP of 160/95 mmHg; the response 'sometimes' was assigned to those with BP that exceeded 160/95 mmHg, 1-2 times monthly, and the response 'no' was assigned to the patients with BP higher than 140/90 mmHg generally), regularity

Table 1. Sociodemographic and medical characteristics of patients

characteristic	number of patients
	362 (100)*
Gender	
Male	156 (43)
Female	206 (57)
Family status	
Married/living with partner	231 (63.7)
Divorced/separated	6 (1.4)
Widowed	120 (33.2)
Single	5 (1.7)
Education (years of education)	
without education	34 (9.4)
up to 4 yrs	43 (11.9)
5-8 yrs	112 (30.9)
9-12 yrs	130 (35.9)
13+	43 (11.9)
BMI	
Normal	143 (39.5)
Overweight	160 (44.2)
Obese	59 (16.3)
Systolic and diastolic blood pressure when last checked	
Healthy, below 140/90	178 (49.2)
140/90-159/99	131 (36.1)
160/99-179/109	30 (8.2)
Over 180/110	13 (3.5)
Is your blood pressure controled?	
Yes	208 (57.5)
Sometimes	75 (20.7)
No	79 (21.8)

* the number in parenthesis is the percentage of respodents with valid answer for each field

of medication (whether the patient take all the prescribed medications regularly), the received counseling regarding healthy lifestyle and self-management (the content of this variable is shown in Table 3), the knowledge about hypertension, beliefs about hypertension management, and perceived responsibility for the hypertension management (the contents of the last three variables are shown in Table 2). If the patient answered *yes* on all questions regarding lifestyle and self-management counseling by physician, than patient's received counseling about lifestyle and self-care management was mark as high. The knowledge of those who answered correctly on all the statements about hypertension (section *Knowledge* in Table 2) was marked as high and the others' as low. If all patient's beliefs about the hypertension management were constructive (section *Beliefs* in Table 2) than those beliefs were marked as high, and if that was not the case – beliefs were marked as low. The

Table 2. Patients reported health behaviors, knowledge and beliefs about hypertension and its management

Lifestyle behavior		
Do you exercise regularly (at least half an hour daily)?		
Yes	129	(35.6)*
No	233	(64.4)
Do you smoke and have you ever in the past?		
I smoke	85	(23.4)
I used to smoke	118	(32.6)
I have never smoked	159	(44.0)
Do you follow a special diet for your hypertension (low calorie, low fat, salt-free)?		
Yes	143	(39.5)
Sometimes	55	(15.2)
No	164	(45.3)
Level of adherence to healthy lifestyle (regurarly exercise, do not smoke and follow diet)		
High	126	(34.8)
Low	236	(65.2)
Knowledge		
Unbalanced blood pressure can damage bloood vesels and lead to heart attaccks and strokes		
true	320	(88.4)
false	6	(1.6)
Do not know	36	(10.0)
Being overweight affect blood pressure		
true	280	(77.4)
false	31	(8.5)
Do not know	51	(14.1)
Salt consumption raises blood pressure		
true	322	(88.9)
false	4	(1.1)
Do not know	36	(10.0)
Physical exercise helps reduce blood pressure		
true	226	(62.4)
false	25	(6.9)
Do not know	111	(30.7)
Medication is all that is needed to treat hypertension		
true	106	(29.3)
false	172	(47.5)
Do not know	84	(23.2)
Beliefs		
I believe that medication to reduce hypertension will help me feel better		
Agree	307	(84.0)
Do not entirely agree	27	(7.4)
Disagree	9	(2.4)
Do not know	19	(5.2)
I believe that a diet to reduce hypertension will help me feel better		
Agree	247	(68.2)
Do not entirely agree	11	(3.0)
Disagree	39	(10.8)
Do not know	65	(18.0)
A hypertension patient has to be treated constantly, whether or not his/her health improves		
Agree	223	(61.6)
Do not entirely agree	22	(6.1)
Disagree	64	(17.7)
Do not know	53	(14.6)
I believe that is possible to control my blood pressure		
Agree	291	(80.4)
Do not entirely agree	31	(8.6)
Disagree	12	(3.3)
Do not know	28	(7.7)
Who is responsible for ensuring your blood pressure is balanced?		
Full/main responsibility is with the doctor and/or nurse	121	(33.5)
Full/main responsibility is with the patient	241	(66.5)

* the number in parenthesis is the percentage of respodents with valid answer for each field

Table 3. Patients' reports on received counseling regarding lifestyle and self-management

Counseling by medical staff	number of patients	
Lifestyle counseling		
Current physician recommended physical activity		
Yes	105	(29.0)*
Current physician discussed smoking cessation (current smokers only)		
Yes	35	(41.2)
Current physician discussed the need for suitable diet-what you may and may not eat		
Yes	198	(54.7)
Current physician discussed your desirable weight		
Yes	173	(47.8)
Self-management counseling		
Current physician explained the risks and complications of high blood pressure		
Yes	183	(50.5)
Current physician explained how to measure blood pressure by yourself		
Yes	177	(48.9)
Current physician explained about signs for deterioration		
Yes	104	(28.7)

* the number in parenthesis is the percentage of respondents with valid answer for each field

normal range for BMI was 18.50 - 24.99, for the overweight 25.00 - 29.99 and for the obese BMI were 30 or more.

The raw data were processed by the descriptive statistics, with the percentages of patients having certain value of the variables. The influence of the following variables of the study including gender, education, family status, perceived responsibility on hypertension management, beliefs about hypertension management, knowledge about hypertension and received counseling by medical team on the study outcome (adherence or non-adherence) was tested by the univariate logistic regression, and the crude odds ratios were obtained. Furthermore, all the variables tested with univariate analysis were entered into the multivariate logistic regression, and the adjusted odds ratios were obtained.

The level of significance for the acceptance of a null hypothesis was $p< 0.05$. All calculations were performed by the statistical software SPSS Version 18.0 (SPSS, Chicago, IL).

RESULTS

This study involved the random sample of 362 outpatients of a total cohort of 3470 elderly hypertensive patients. Twenty two patients refused to participate in the study. The mean age of the studied population was 71.9 years. Demographic characteristics of the study group are presented in Table 1. The facts related to patients' general lifestyle behavior, their knowledge about hypertension and beliefs about hypertension and its management are summarized in Table 2. The information concerning the received counseling about lifestyle and self-management by medical staff is shown in Table 3.

The variables such as lack of education and high score in reported counseling by the primary care physician on healthy lifestyle behavior predicted high scores of adherence to healthy lifestyle behavior (odds ratio 0.31, $p<0.05$; 29.58, $p<0.05$, respectively). The other variables (gender, family status, perceived responsibility on hypertension management, and beliefs and knowledge about hypertension) did not have statistically significant impact on the adherence to healthy lifestyle (Table 4).

DISCUSSION

The results of our study confirmed the well-known fact that adherence of hypertensive patients to healthy lifestyle in is far from optimal (10). According to the data obtained by "The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure" suggesting that only one third of hypertensive patients had well controlled blood pressure (5), at least a part of this problem could have been assigned to the low adherence to healthy lifestyle. Study conducted in Poland also proved that implementation of non-pharmacological treatment among hypertensive patients is at very

Table 4. Multivariate logistic regression: predictors of healthy lifestyle behavior

	OR(crude)	CI 95%	OR adjusted	CI 95%
Gender (male)	0.58*	0.37-0.90	0.62	0.35-1.08
Education (up to 8 years)	1.349	0.87-2.08	0.23*	0.10-0.52
Family status (married)	0.97	0.62-1.53	0.57	0.31-1.04
Perceived responsibility (of medical team)	0.71	0.44-1.13	0.73	0.38-1.42
Constructive beliefs about HTA (high)	2.38*	1.34-4.22	1.70	0.82-3.50
Knowledge about HTA (high)	1.22	0.67-2.20	1.09	0.46-2.57
Counseling (high)	16.74*	9.64-29.01	34.89*	16.06-75.82

p<0.05*
Hosmer and Lemshow test chi-square 2.92, p=0.94

low level (11). Research on Korean hypertensive patients demonstrated that awareness of hypertension is increasing, but that did not lead to better adherence to healthy lifestyle (12).

Recent study showed that the intensive antihypertensive medical treatment combined with the lifestyle modification led to the better blood pressure control of hypertensive patients including the elderly (10). Apart from the factors linked to the patient itself that may influence adherence to either medication or healthy lifestyle (11) including depression, low social support and low quality of life, knowledge, beliefs and attitudes towards the hypertension, the patients counseling by their physicians also plays a key role in managing the hypertension. The results of our study demonstrated that only a third to no less than a half of hypertensive patients has been advised by their physician about the appropriate diet, physical activity and smoking cessation that were an obligatory part of a treatment. In addition, the counseling by the other health workers including pharmacists also plays an important role in medical adherence as well as in the adherence to lifestyle behavior (12, 13). Among the reasons for the low counseling rates by physicians could be assigned to their poor and/or inadequate knowledge regarding the hypertension and hypertension guidelines (14) and the short period of time allowed for the physician-patient encounter since the proper physicians counseling required more time (15, 16). Therefore, in order to improve their own counseling skills, the physicians need to be properly trained and to be more persistent and convincing in advising the hypertensive patients about the necessity of a lifestyle modification, since only a few hypertensive patients remembered being advised (17). Concerning the older hypertensive patients, there is the clinical inertia among the physicians which contributes to the inappropriate treatment of the elderly (18). In addition, the self-care ability which is more often reduced in some of the older patients impairs their compliance with the healthy lifestyle behavior, and makes their blood pressure being harder to control (6).

The older patients are generally less educated compared to the rest of the population, and therefore with lower health literacy. It has already been shown that the less educated patients were less aware of the effects of a lifestyle on health (19). Such patients have reduced ability to remember instructions received from the physician and also difficulty to recall information (20). The health care workers, both physicians and nurses should try to communicate more effectively with the patients using simple language and providing them with the information that would be understood by everyone around them. However, paradoxically, in our study the less educated elderly patients were more adherent to the healthy lifestyle than those with more education suggesting that the one of the reasons for the better adherence could be a higher submissiveness of less educated patients to the "orders" received from the physicians in a country with the authoritarian social structure (21, 22).

Considering that the received lifestyle counseling could be a good predictor of a healthy lifestyle behavior that was demonstrated in our and previous study (9), it could be concluded that the improving the communication skills between the physician and the patients is required to achieve the improvement in patient's adherence to the recommended lifestyle as well as to the prescribed medication.

It is important to note that this study was limited by several factors. Firstly, it was based on the patients self-report about their lifestyle behavior and received counseling by physician; therefore the patient's tendency to be "socially desirable" could be one of the reasons for the incorrect (untruthful) answers on some of the questions. On the other hand, only the patients are able to provide the information regarding their lifestyle behaviors and physician's lifestyle counseling. Secondly, making the conclusion about the cause and the effects is impossible due to the cross-sectional nature of the study design. Our results could only reveal the possible relationship between the lifestyle behaviors and the independent variables in the multivariate regression model. In addition, another limitation factor of this study is the fact that the depression was not considered as a possible factor affecting the adherence to the patient's life-style modification.

CONCLUSIONS

Our study showed that counseling of the older hypertensive patients about a healthy lifestyle and self-care by their physician helped them modifying and keeping a healthy lifestyle. Furthermore, this study demonstrated that the counseling rates by physicians were low, suggesting the requirements of the improvement of the communication skills between patients and physicians. Following the appropriate communication training skills, the physicians would be able to counsel their patients more effectively and their efforts would result in the improvement of patients' adherence to healthy lifestyle. Moreover, it is also very important to involve patient's relatives in his or her everyday activities, especially for the patients who suffer from dementia and depression since they require the everyday family support regarding taking their medication, proper diet and physical exercise. Last, but not the least, the role of the nursesis substantial through the different supporting programs such as patients' clubs. Local media, in cooperation with medical authorities, could also take responsibility for the education of the patients and contribute to their healthy lifestyle.

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A QUESTIONNAIRE FOR ASSESSING FEAR OF RADIOTHERAPY IN ONCOLOGY PATIENTS

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UPITNIK ZA PROCENU NIVOA STRAHA OD RADIOTERAPIJE KOD ONKOLOŠKIH PACIJENATA

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The study was conducted in Clinical Centre Kragujevac in the Centre for Oncology and Radiology, Department of radiotherapy.

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ABSTRACT

Radiotherapy is a frequently prescribed and highly effective form of treatment of oncology patients. However, many patients feel rational or irrational fear of the application of radiotherapy, which may provoke mental and physical stress, anxiety, growing anger and hostility, thus reducing quality of life. The aim of this study was to develop, reliability test and validate a questionnaire for assessing the level of fear of radiotherapy in oncology patients.

We performed a prospective qualitative study based on the development, validation and reliability testing of the questionnaire developed for assessing radiotherapy-caused fear in oncology patients treated in the Centre for Oncology and Radiology, Department of Radiotherapy, Clinical Centre Kragujevac.

The study included 154 patients and the final version of the questionnaire integrated 15 questions. After the elimination of inappropriate questions the Cronbach coefficient α was 0.946. The questionnaire consists of two factors which represent 57.423% and 6.925%, making a total of 64.348% of the variance of the questionnaire.

The results of our study show that the questionnaire used is a unique, reliable and valid instrument for assessing the level of fear of radiotherapy in oncology patients the application of which will allow us to identify patients with elevated levels of fear of radiotherapy.

Keywords: *radiotherapy, fear, oncology patients*

SAŽETAK

Radioterapija je često propisivan i veoma efikasan vid lečenja onkoloških pacijenata. Međutim, mnogi pacijenti osećaju kako racionalan, tako i iracionalan strah od primene radioterapije, što može potencirati psihički i fizički stres, anksioznost, razvoj ljutnje, neprijateljski stav, i na taj način dodatno smanjiti kvalitet života. Cilj ovog istraživanja baziran se na izradi, ispitivanju pouzdanosti i validaciji upitnika za procenu nivoa straha od radioterapije kod onkoloških pacijenata.

Sprovedena je prospektivna, kvalitativna studija utemeljena na izradi, proveri pouzdanosti i validaciji upitnika za procenu nivoa straha od radioterapije kod onkoloških pacijenata lečenih na Odeljenju radioterapije Centra za onkologiju i radiologiju Kliničkog centra Kragujevac.

U istraživanju je učestvovalo 154 ispitanika, a finalna verzija upitnika obuhvatala je ukupno 15 pitanja. Cronbachov koeficijent α, nakon eliminacije neadekvatnih pitanja, iznosio je 0,946. Upitnik je integrisao dva faktora koji su predstavljali 57,423% i 6,925%, čineći ukupno 64,348% varijanse upitnika.

Rezultati našeg istraživanja su pokazali da se korišćeni upitnik može smatrati jedinstvenim, pouzdanim i validnim instrumentom za merenje nivoa straha od radioterapije kod onkoloških pacijenata, i da se njegovom primenom na relevantan način mogu identifikovati pacijenti sa prisutnim povišenim stepenom straha od radioterapije.

Ključne reči: *radioterapija, strah, onkološki pacijenti*

ABBREVIATIONS

OP- oncology patients
RT- radiotherapy
QAFRT- questionnaire for assessment fear of radiotherapy
VAS- visual analogue scale

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INTRODUCTION

Radiotherapy (RT) is a frequently prescribed and highly effective form of treatment of oncology patients (OPs). It may be curative or palliative. The aim of implementation of RT is based on the local effect on malignant cells, with the maximum preservation of the surrounding normal tissue. Although the aforementioned type of therapy is associated with a number of useful aspects of implementation and positive impacts on the health, many patients feel rational or irrational fear of the application of RT. Bad and unpleasant experiences associated with other oncology therapeutic options, such as chemotherapy and surgery, often lead to refusals to start or cancellations of RT treatment (1).

Radiotherapy may initiate mental and physical stress, anxiety, growing anger and hostility, and reduce quality of life (1-5). Moreover, changes in mental state can affect the course of the disease, and mental and physical status (1, 4-8). Oncology patients are characterized by a higher level of anxiety than patients with other diseases (1). Anxiety represents a specific mental state of increased vigilance and prudence in anticipation of unpleasant events without the presence of real danger (9). Some OPs, treated with RT, however, experience serious psychological reactions that may manifest as fear of RT, and which may significantly affect compliance. Fear is a response to real dangerous events and can be manifested by defensive reactions: fight, flight or "freezing" (9).

Many patients think that their pathology is characterized by a poor prognosis, because RT is indicated, and very often take the attitude that RT treatment is vain in their case (2). Although the effects of radiation during treatment cannot be felt, most patients have somatization symptoms of psychological discomfort caused by fear. It is interesting to note that those patients who showed a lower level of anxiety before exposure to RT are characterized by greater anxiety during and/or after RT, and vice versa (1). Patients are mostly concerned about the potential impact of RT on family life and work obligations, the occurrence of new cancers, reducing libido, sterility, the occurrence of burns, pain and scarring, etc. (10). Increases in the level of anxiety, discomfort and an intensification of thinking about the disease may be brought about by the treatment; the closed steel door in the room for radiation, the lack of windows, isolation during the radiation procedure and the size of the RT device (1, 2, 11, 12). It can be assumed that the anxiety and fear before treatment are uncertainty conditioned, while anxiety and fear during and after RT treatment are associated with concern about developing side-effects of the treatment and the possibility that they have exhausted all treatment options. Radiotherapy is easier to accept and tolerate for patients who regularly visit radiotherapists, those who possess a higher level of knowledge about RT, than patients who receive palliative therapy and those with a better "performance" status (6, 7, 13-15).

A relatively small number of studies have addressed this issue. Analysis of the level of RT-caused anxiety in OPs is offered in earlier studies based on structured interviews with a psychiatrist, using the Mental Component Summary Scale, the Center for Epidemiologic Studies Depression Scale, the Spielberger State-Trait Anxiety Inventory, the Anxiety subscale of the Hospital Anxiety and Depression scale and other relevant scales dealing with quality of life assessment among OPs facing RT (1-8, 11, 13-16). A detailed analysis showed that only one study examined the level of fear of RT based on interviews that processes fear of RT and how to deal with the fear of RT (15).

Creating an adequate questionnaire to assess the level of fear of RT would be of great practical importance both in terms of early identification of patients with high levels of fear of RT, and in the strategic planning and timely application of preventive measures in the field of professional psychological support before starting RT with the aim of reducing the fear of RT in OPs. The aim of this study was development, reliability testing and validation of a questionnaire for assessing the level of fear of RT among OPs.

MATERIALS AND METHODS

We performed a prospective qualitative study based on the development, validation and reliability testing of the questionnaire for assessing RT-caused fear (QAFRT) in OPs. The study lasted 4 months, or until there was an adequate number of respondents. The research was conducted at the Centre for Oncology and Radiology, Clinical Centre Kragujevac, Department of Radiotherapy with the prior approval of the Ethics Committee Clinical Centre Kragujevac.

Population

The study included all OPs with histopathologically confirmed cancer of any localization and staging of the disease, for whom RT was indicated for the first time and who had been notified and signed an informed consent to participation.

Criteria for inclusion in the study were: patients aged from 18 to 65 years, patients from Serbian-speaking areas, any stage or localization of histologically verified carcinoma, the first time determined treatment of RT. The study included all patients who had voluntarily agreed to participate in the study, who had started treatment RT or applied RT, while radiation therapy was in progress and had completed at least one therapy session, whether as an out-patient or during hospitalization, regardless of whether the radiotherapy was curative or palliative. The criteria for exclusion from the study were: minors and mentally ill patients who were unable to understand the content of the questionnaire adequately.

The variables that were monitored in the study were gender, age, marital status, with whom the patient lives, residence, education, and religiosity. Data related to the disease were obtained by examining the medical records: type of tumor, applied modalities of treatment, comor-

bidities, type of radiotherapy (curative or palliative), radiotherapy techniques (transcutaneous or intracavitary brachytherapy), localization of the radiation field, the number of radiation fields, the radiation dose, the number of fractions, whether a mask for the head or neck were used, whether there was an explanation of the procedure of radiation, and verbal or written information provided by the radiotherapists.

Power of the study

It was envisaged that the power of the study would be 80%, based on the probability that there would be a statistical error of type 1 (α) of 0.05. In accordance with the formula for calculating the sample size when searching the mean value of a continuous variable in the population, with relevant, literature-based standard deviation measurements ± SD = 0.94 and confidence interval width d= ± 0.3, it was determined that the research would require a minimum of 150 patients (17).

Test procedure

The decision for treatment with RT was made exclusively by the Council of Oncology. The questionnaire for assessing the level of fear of radiotherapy in OPs and the visual analogue scale (VAS 0-100) were completed independently by the patients after the first session, in the presence of the researcher, and after the fifth session of RT they were filled out by the researcher who asked the patient questions. The process of completing the questionnaire had previously been explained to all the patients.

Creating the questionnaire

The questionnaire for assessment of fear of radiation therapy in OPs consists of three parts. The first part contains questions related to socio-demographic characteristics. The second part contains information related to the disease. The third part of the questionnaire is related to the patient's fear of RT. Every question, from the last part, has five possible answers according to the Likert scale. The questions were designed based on a review of available literature dealing with this issue and after consultation with the two psychiatrists, seven radiotherapists, two clinical pharmacologists and five senior radiology technicians employed at the Clinical Centre in Kragujevac. The final version of the questions was submitted for consideration to Professor Goran Mihajlovic, a psychiatrist employed at the Clinical Centre in Kragujevac. After obtaining his expert opinion, the final corrections to the questions were made. The questions were listed on the basis of random selection. The last two were trick socially desirable questions. The questionnaire was designed to measure the level of fear in patients after the application of RT. The questionnaire was intended to measure fear of radiation therapy in general. The questions included various aspects of fear of radiation, such as fear of potential side effects after RT exposure, fear of the procedure itself, as well as the possible implications of the type of treatment on the life of the patient after completion of the RT and the course of the disease. The questionnaire is intended for use as a valid instrument for the identification of patients with a high level of fear of RT. Given that in this category of patients fear can be further provoked by other conditions, diseases and treatment options, the results obtained should be evaluated taking into consideration the overall health status of the OP. Patients who show a high level of fear of RT should be referred to a psychologist or psychiatrist, to determine the origin of the fear, prevent serious health consequences and improve the relationship with the patient.

Reliability and validity of the questionnaire

The internal and external reliability of the questionnaire were tested. The internal consistency all the offered questions was tested and Cronbach α QAFRT's in OPs. Cronbach α may range from 0 to 1 and indicates which answers are consistent to the questions. Account was taken only of Cronbach α values above 0.7.

Factor analysis was conducted to assess the validity of the content of the questionnaire. The validity of the structure and criterion validity were investigated by testing of the relationship QAFRT and VAS scale. Temporal stability was obtained by comparing answers to the questions of QA-FRT after the first and fifth sessions of RT, or after seven days.

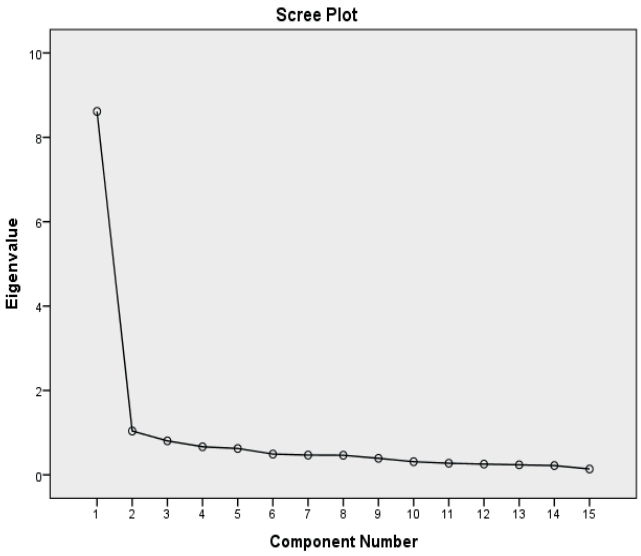
Statistics

Methods of descriptive statistics were used for sociodemographic and medical records (mean and a standard deviation). Data with normal distribution were analyzed by the parameter Student's T test, while for the data without a normal distribution we used the nonparametric Man-Whitney U test. To investigate the frequency of qualitative variables the χ^2 test was used. Spearman's coefficient correlations were used to determine the reliability of the questionnaire and its temporal stability. For statistical analysis we used SPSS software, version 18 (Chicago, IL, USA). The difference was considered significant if the probability of the null hypothesis was below 0.05.

RESULTS

The study included 154 patients, of which 50% were men and 50% women. Thirty-two subjects were excluded from the study pursuant to study protocol violations. The average age of respondents was 62.85 ± 11.26 years and the average duration of education 10.05 ± 3.84 years. About 55% of patients had experienced chemotherapy, while 68.2% of patients had undergone surgery. Curative radiotherapy was conducted in 79.9% patients while for 20.1% of patients the radiotherapy was palliative. Only 30.5% of patients received an explanation from radiotherapists about the therapeutic procedure.

The questionnaire for evaluation of levels of fear of RT initially consisted of 31 items. The answers were coded from 0 to 4. The final version of the questionnaire has integrated 15 questions. The total score of answers on questions ranging from 0-60.



Grafic 1. Scree plot

A correlation matrix was created to determine an adequate connection between the questions. The candidates for elimination were questions whose value correlation coefficient was below 0.2. It was found that two questions correlated poorly with other matters and were eliminated. The remaining questions correlated appropriately.

The majority of answers to the questionnaire mean value ranged from almost 1 to 1.7 (Table 1). Questions with a low standard deviation, and questions that correlated poorly with the rest of those with similar content, were eliminated from the questionnaire.

Table 1. Mean, standard deviation, skewness, kurtosis and Pearson correlation the questions that make up the final version of the questionnaire

Number of issues	Mean	Standard deviation	Skewness	Kurtosis	Pearson correlation
1.	1.45	1.097	0.390	-0.563	0.733
2.	1.62	1.198	0.457	-0.654	0.535
3.	1.21	1.226	0.790	-0.360	0.635
4.	1.74	1.204	0.423	-0.749	0.695
5.	1.46	1.138	0.555	-0.457	0.693
6.	1.28	1.152	0.657	-0.427	0.648
7.	1.47	1.216	0.569	-0.630	0.612
8.	1.12	1.268	0.869	-0.453	0.468
9.	1.50	1.222	0.553	-0.559	0.773
10.	0.97	1.207	1.080	0.115	0.439
11.	1.62	1.126	0.480	-0.528	0.623
12.	1.23	1.158	0.697	-0.469	0.536
13.	1.63	1.242	0.425	-0.755	0.714
14.	1.10	1.274	0.860	-0.422	0.494
15.	1.14	1.253	0.830	-0.450	0.561

The most important factor for assessing the reliability of the questionnaire as a whole, the coefficient Cronbach α stood at 0.955. After the elimination of inappropriate questions this was 0.946.

In order to calculate external reliability, the questionnaire was randomly divided into two parts following the Split-Half models. The value of the Cronbach coefficient for the first part was 0.910 and 0.884 for the second. The coefficients of both parts of the questionnaire amounted to over 0.7 and have a good overall correlation, which contributes to the reliability and integrity of the questionnaire. Predictor reliability, based on the value of the Cronbach coefficient for both parts of the questionnaire, obtained with the help of Spear-man Brown formula, was 0.966.

The correlation of each question with others, was analyzed with the Spearman's coefficient correlation. We set aside two questions that have a value of less than 0.3.

We conducted a factor analysis to determine how many factors were included in the new questionnaire. The Kaiser-Meyer-Olkin test and Bartlett's test of sphericity were determined to be the most appropriate for factor analysis. The values were above 0.5 or 0.932. It was found that the factor analysis can explore the structure of the questionnaire. The value of χ^2 test amounted to 1584.386 (r=0.000).

Given that the eigenvalue was above 1, the questionnaire consists of two factors which represented 57.423% and 6.925%, making a total of 64.348% of the variance of the questionnaire. The Graphic 1 - Scree plot shows a clear point of fracture after the second factor. For the first eigenvalue, the factor value was 5.390, the percentage of variance 35.931%, and the cumulative percentage of variance 35.931% after Varimax rotation. While the other factor eigenvalue value was 4.263 and the percentage of variance 28.417%, the cumulative percentage of variance amounts to 64.349%.

Table 2. Rotated Component Matrix

Component		
Number of question	1	2
P1	**0.693**	0.480
P2	0.180	**0.830**
P3	**0.770**	0.340
P4	0.275	**0.801**
P5	**0.708**	0.461
P6	**0.756**	0.329
P7	**0.550**	0.523
P8	0.299	**0.718**
P9	0.545	**0.653**
P10	**0.712**	0.115
P11	**0.645**	0.416
P12	**0.587**	0.488
P13	0.470	**0.673**
P14	**0.713**	0.222
P15	**0.672**	0.353

Table 3. Factor and meaning, questions pertaining to factor, Cronbach α and the mean value of the score

Factor	Question	Cronbach α	Median value score
Factor 1 Fear of patients related to the relationship of family and friends and the continuation of life after radiation	1, 3, 5, 6, 7, 10, 11, 12, 14, 15	0.926	12.935
Factor 2 Fear linked to disease prognosis and adverse effects of radiation	2, 4, 8, 9, 13	0.881	7.628

Table 2 shows the questions based on the weight load belonging to a particular factor after rotation. Name factors with issues, Cronbach coefficient α and a median score are shown in Table 3. List of the questions in final version of the questionnaire for assessing fear of radiotherapy in oncology patients is shown in Table 4.

Convergent validity was investigated in the context of criterion validity. A high degree correlation was found between the VAS scale and recent responses to questions from the QAFRT (r=0.807, p=0.000), confirming convergent validity.

Table 4. List of questions of final version of The questionnaire for assessment fear of radiotherapy in oncology patients

1.	Do you have a fear that radiation can affect the appearance of a new tumor?
2.	Are you afraid that radiation therapy can damage other organs, which are not subjected to radiotherapy?
3.	Do you have a fear that you will endanger your family because you are in radiation therapy?
4.	Are you afraid that radiotherapy will cause burns at the site of application of radiation?
5.	Are you afraid that radiation therapy will be hinder your everyday activities?
6.	Do you have a fear that friends will change their relationship toward you because you are being treated with radiotherapy?
7.	Were you afraid when you were told that you would continue the treatment of radiation therapy?
8.	Do you feel disturbed while expecting the application of radiotherapy?
9.	Are you afraid that radiation therapy can cause permanent damage to the region of irradiation?
10.	Do you have a fear that you partner will change their relationship toward you because you are being treated with radiotherapy?
11.	Do you think more often than usual about your illness while on radiation therapy?
12.	Do you have a fear that radiation therapy will not be effective against your illness?
13.	Do you have a fear that you have not received all the necessary information about the potential adverse effects of radiotherapy?
14.	Are you afraid to handle electrical appliances, when you are in radiation therapy?
15.	Are you preoccupied by thinking about radiotherapy during the whole day?

Temporal stability was tested by correlating the total score responses from QAFRT after the first and fifth session of radiotherapy. Retest after the fifth session of radio-therapy was undertaken by 86 patients. A high correlation between QAFRT scores was determined (r=0.925, p=0.000), which indicates good temporal stability.

DISCUSSION

The results of the study confirm that the QAFRT in OPs is a reliable and valid instrument for measuring the level of fear of RT. The questionnaire consists of 15 questions and has a good coexistence of internal structure which is summarized in two factors. The identified factors were: "fear related to the attitude of family and friends and the continuation of life after irradiation", and "fear factor related to the prognosis of the disease and the side effects of RT". The Cronbach coefficient α (0.946) confirmed the reliability of the questionnaire. Since the Cronbach coefficient before correction issues had a high value (0.952), 16 questions with similar content and with insufficient criteria for questionnaire inclusion were removed. A shorter version of the questionnaire was then drafted with a somewhat lower Cronbach coefficient (0.946). The criterion validity of the questionnaire was confirmed by examination of convergent validity with the VAS scale. The new questionnaire has also shown exceptional temporal stability and test-retest reliability. In the methodological development of the questionnaire, participants were interviewed in two ways, because this was part of the validation procedure. The good correlation which was achieved between the two tests proved that the questionnaire was valid for use.

The first factor called "fear of patients related to the attitude of family and friends and the continuation of life after irradiation" and composed of 10 questions, confirmed high internal coexistence (Cronbach α 0.926). This factor accounted for 35.931% of the variability of the questionnaire. Questions related to the patients fear during and/or after RT such as changing family relationships, partners and friends, fear connected to daily activities and life after completing treatment.

In the study conducted by Sundaresan and associates, it was observed that more than half of the patients were concerned about the possible impact of RT on family life, living and working obligations and the later effects of radiation (10). Turner and contributors also confirmed that patients in the course of implementation of RT were concerned for their family and the future (12).

The second factor "fear linked to disease prognosis and the adverse effects of radiation" was formed in five questions. The value of the Cronbach coefficient α for this factor was 0.881 and explained 64.349% of the variance. The questions included in this factor related to the fear of patients' lack of necessary information about the potential adverse effects of RT, fear of disease progression and damage to other organs, as well as the fear of the radiation procedure itself.

Fear in patients can be a major obstacle to the implementation of the prescribed treatment (17). Earlier studies showed that 10 to 20% of the patients feel anxiety before the start of the RT, and that 20-50% of patients are anxious during the first day of RT. Fear and anxiety are commonly associated with a lack of information, side effects of treatment and the radiation procedure (2, 5, 10, 17). Sehlen and contributors found that most patients are afraid of the possible side effects of radiation (18). In order to avoid additional psychological and physical stress, in these vulnerable categories of patients, it is necessary to organize screening at the beginning of RT treatment that would allow identification of patients with increased risk of mental instability during treatment (19). The ideal would be to separate risk patients for radiotherapy simulation, or before starting radiation (17).

Our study had several limitations: it was conducted in a single centre, and the test-retest reliability assessment was carried out after seven days. In addition divergent stability was not examined. The majority of respondents were older and less educated, so the reliability of the questionnaire should also be examined in the other categories of patients. Also, it was not possible to ensure the presence of a psychiatrist and an overview of each participant after examination or to compare his findings with the results of responses to the questionnaire. The reliability of the questionnaire should be examined after an extended time period after completion of RT treatment, and the results compared with the results of our study. It is possible that the level of fear in the same patient changes over time and decreases as the therapy nears completion. There is no relevant questionnaire that measures the level of fear of radiotherapy with which we can compare our results.

The results of our study show that the used questionnaire was a unique, reliable and valid instrument for assessing the level of fear of RT in OPs, the application of which makes it possible to identify patients with elevated levels of fear of RT. Patients with a high total score would require special attention and adequate psychological or psychiatric support, with a maximum reduction of adverse events, allowing the treatment to be implemented fully and adequately.

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THE ANALYSIS OF NUTRITIONAL PREDICTORS OF ANEMIA COMBINED WITH OBESITY IN PRIMARY SCHOOL-AGE CHILDREN

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ANALIZA NUTRITIVNIH PREDIKTORA ANEMIJE I GOJAZNOSTI KOD DECE MLAĐEG ŠKOLSKOG UZRASTA

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ABSTRACT

The consumption and amounts of a variety of food products used in a diet affect the incidence of anemia and different levels of nutritional status among school-age children. The prevalence of food intake comprised of fats, carbohydrates and sodium (salt) is a significant contributing factor to the incidence of excessive weight. Apart from nutrition, a leisure-time physical activity and the time spent in front of the TV or computer may contribute to an increase in anemia and obesity rates. The objective of this paper was to examine nutritional status, dietary habits and anemia among school-age children in the central Serbia region (the city of Kragujevac). It was established that 47.3% of the surveyed children fell into the normal weight group, 24.5% of the children are considered to be at risk of being overweight, 21.4 % of the children are considered as obese, whereas 6.8% of the children fell into the under-weight group. The incidence of anemia was noted in 10.8% of the cases, whereas anemia in obese children was observed in 21.6% of the cases (n=114; during the school year of 2014-2015). The obtained results show a statistically significant correlation between an increase in the consumption of fast food and anemia in children, whereas the amount of time children spend in front of the TV is also associated with the higher percentage of anemia and obesity.

Keywords: *anemia, obesity, school children*

SAŽETAK

Upotreba i količina različitih vrsta namirnica u ishrani utiče na pojavu anemije i različitih stanja uhranjenosti kod osnovnoškolske dece. Učestalost uzimanja hrane koja je puna masti, ugljenih hidrata i soli značajno utiče na pojavu prekomerne težine. Osim navika u ishrani na pojavu gojaznosti utiče i stepen fizičke aktivnosti, vreme koje deca provedu ispred ekrana, kao i navike koje propagiraju u ishrani. Cilj ovog rada je bio da se proceni učestalost anemije, nutritivni status i navike u ishrani dece iz osnovnih školi u Kragujevcu, centralnoj Srbiji. Ovim istraživanjem utvrđeno je da 47,3% anketirane dece spada u grupu normalno uhranjene, 24,5% je predgojazno, 21,4% ih je gojazno, a 6,8% pripada grupi pothranjene dece. Podaci pokazuju da se učestalost anemije među decom školskog uzrasta javlja u 10,8% slučajeva, dok se anemija kod gojazne dece pojavljuje u 21,6% slučajeva (n=114; period školske 2014/2015. godine). Studijom je utvrđena statistički značajna povezanost između povećane upotrebe brze hrane i anemije kod dece, dok je vreme provedeno kraj televizije povezano i sa većim procentom anemije i gojaznosti.

Ključne reči: *anemija, gojaznost, deca školskog uzrasta*

INTRODUCTION

Bad eating habits, inadequate amounts of physical activity and excessive media use (such as: watching television, and viewing computer screens) may have a significant effect on the incidence of anemia and obesity. The most common cause of nutritional anemia is iron deficiency. Therefore, the recommended daily iron intake from foods is 6 mg/day in children aged 7–10 years (1). As regards the relationship between micronutrient deficiencies and children's cognitive functioning, iron deficiency have been associated not only to cognitive deficits but potential long-term behavioral changes as well (2). Examples of bad eating habits are: the consumption of higher calorie-density of foods, eating meals in front of the TV screen, breakfast skipping, lack of physical activity (3, 4). Over the course of the past few years there has been a trend toward decreased physical activity that plays a significant role in the

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regulation of body mass, compared with excessive energy intake which is a contributing factor to childhood obesity (5). Worldwide, there has been a startling increase in rates of obesity. It has an epidemic-like nature (6). Currently 10% of school-age children worldwide are overweight, one in four children are obese, whereas approximately two thirds of the USA developing population are either overweight or obese (3). In Europe, one in five school-age children are overweight (6). Nutritional deficiency disorders are associated with a variety of health problems of children, and they can also lead to the onset of many age-related diseases (7). Obesity can thereby increase the risk of the development of the major noncommunicable diseases such as hypertension, type 2 diabetes, coronary artery diseases and certain types of cancers (8).

Numerous studies have shown that obesity might also increase the risk of iron deficiency (9). Despite their excessive dietary and caloric intake, obese children and adolescents can be at risk of iron deficiency (ID), because they tend to consume unbalanced meals, particularly rich in carbohydrates and fat (6). Iron deficiency and anemia can lead to fatigue and additionally decreased exercise capacity that may create favourable environmental conditions predisposing people to weight gain (10). The widest range of nutritional deficits includes insufficient dietary intake and absorption of iron. Iron deficiency (ID) is estimated to affect 1.5 billion people worldwide. 10% of children and adolescents in the USA suffer from iron deficiency (11). 25.4% of school-age children worldwide is estimated to suffer from anemia (12). Considering the fact that children's eating patterns and food preferences are established in their early childhood, parents have a significant role in their development (3). Therefore, children's behaviour patterns directly affect not only their current but overall health state as well. In May 2004, the 57th World Health Assembly (WHA) endorsed the World Health Organization (WHO) Global Strategy on Diet, Physical Activity and Health in order to enhance and sustain health and encourage the implementation of action plans to improve diets and increase physical activity (13). The objectives of this paper were established in order to examine nutritional status, dietary habits and anemia prevalence among school-age children in the central Serbia region (the city of Kragujevac).

MATERIALS AND METHODS

The research was carried out in the 4 elementary schools in the territory of the city of Kragujevac. The study sample consisted of 114 children (56 boys and 58 girls) attending the first (55 children) and the fourth (59 children) grade. The participation of at least one parent/guardian of each student was mandatory. The research was conducted during the 2014-2015 school year, in regular classes, whereas the doctors who conducted the research were present there as well. Respondents voluntarily participated in the survey. Survey respondents were assured that their contributions would remain anonymous. The opportunity to participate in the study was denied in the cases of children with incomplete medical records, which entailed not providing sufficient information on blood hemoglobin concentration. Children diagnosed with the anemia that was not mainly nutritional in origin were also excluded from the current analysis.

The collected data were processed using G*Power software, version 3.0.10., a statistical power analysis program. In order to ensure more precise results, the study sample was examined at various levels by analysing numerous parameters such as the following: the probability of Type I error ($p=0.05$), the power of the test ($1-\beta$) of 95% and the correlation coefficient of $C=0.6$.

The data collection was done by combining surveys, anthropometric measurements and biochemical parameters. The survey (a questionnaire used in the survey) was designed in a heterogenous manner and based on open-ended and closed-ended questions, which were arranged in such a manner so that the topic should be reached gradually and selected according to their difficulty levels not only for parents but for their children as well. The parents had multiple choice questions, questions with a set of alternatives or possible answers and fill-in-the-blank questions (consisting of a numeric response box adjacent to the test item indicating where a respondent should provide his/her numeric response or of a blank line where a written response should be provided). The children involved in the survey participated by circling a letter adjacent to the answer that fitted best after reading instructions.

Research materials included questionnaires designed for parents – for family nutrition assessment, and those designed for early elementary school students (that is, first graders) – for eating habits assessment. The utilized questionnaires were formal standardised questionnaires designed according to the principle of the food-frequency approach, that is, food frequency questionnaires, ratified by the World Health Organization (WHO). A parent-based questionnaire consisted of 20 questions, whereas questionnaires for children included 10 simple questions. The forms of anthropometric measurement included children being properly measured for height-for-age and weight-for-age under adequate conditions. The obtained results were converted to body mass index (BMI) and closely aligned to the corerresponding reference values given in the charts of the WHO Child Growth Standards. The children with a BMI-for-age over the 95th percentile (age specific and gender specific) were considered obese; the children with a BMI-for-age between the 85th and 95th percentiles were classified as healthy weight or at risk of overweight; the children with a BMI-for-age between the 10th and 25th percentiles were considered to have normal healthy weight; whereas those with a BMI-for-age less than the 5 percentile were considered underweight (14). Biochemical measurements included determination of hemoglobin concentration in blood as an indicator for anemia. Anemia was considered

to be present in school-age children in the cases when hemoglobin concentration was less than 115 g/l (15). Hemoglobin values were extracted from the children's medical records kept at the Children's Department of the Health Care Center Kragujevac. The abovementioned values were obtained at the regular examinations.

After data collection, the data were entered into SPSS software version 20.0 and were analyzed using descriptive and comparative analytical/statistical methods. Descriptive statistics included measures of central tendency and variability. The results were classified according to the expected research variables, shown in this paper through the following types of visuals: charts and graphs. The analyses on categorical variables were carried out by using χ^2 - test and the findings were considered statistically significant at $p<0.05$.

RESULTS

The mean age of the respondents was 8.75 ± 1.58 years. 56.9% of the children had normal weight, 20.8% of the children were at the risk of being overweight, 16.7% were classified as obese, whereas 5.6% were classified as underweight (Figure 1). The prevalence of anemia among school-age children in the total sample size was 10.8%, whereas 21.6% of the obese children had anemia (Figure 2).

The study showed the cause-effect relationship between the number of daily meals and the occurrence of anemia. Consequently, the occurrence of anemia was reported in 100% of the noted cases of children having more than 5 meals per day, whereas not a single case of anemia was reported in the cases of children who had only 3 meals per day (Figure 3).

The highest level of obesity was found in the children who had 4 or 5 meals during one day. The children who had 3 daily meals (11.6% of the sampled children) were most likely to be found underweight. On the other hand, the children having more than 5 meals during one day were not found to be underweight. However, the prevalence of nutritional deficiency disorders was not statistically significant compared with the number of meals the children had on a daily basis. $\chi^2= 2.503$, DF=2, p >0.05.

The occurrence of anemia and nutritional status were studied concerning the variety of food included in a family diet, methods of food preparation and eating habits (Table 1).

The obtained results showed that anemia was most frequently present in children who had fish once a week (14%). The percentage of normal-weight group of children was observed in the case of those eating fish once a week (55.2%), whereas the greatest percentage of those at the risk of being overweight (66.7%) and obese children (33.3%) were observed in the case of not having any fish in children's diet.

The highest percentage of the occurrence of anemia was observed in children not having nuts in their diet, whereas 50% of the sampled children who occasionally ate nuts were found to be obese.

The study found the correlation between the anemia prevalence rates and a higher intake of sweets and snacks. The highest percentage of children diagnosed with anemia (55.2%) occasionally ate sweets and snacks, whereas 45.8% of the children included in the study consumed that kind of food every day. In the under-weight group, the highest percentage of the sampled children consumed sweets and snacks every day.

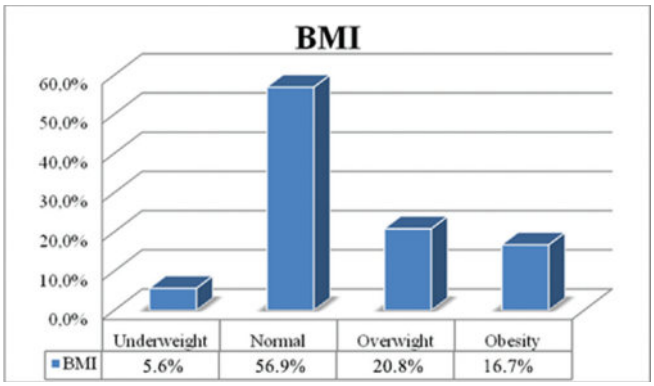


Figure 1. Distribution of the nutritional status of the sampled children

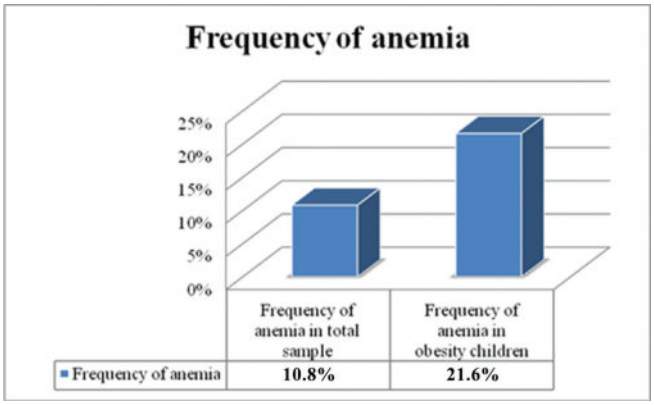


Figure 2. The prevalence of anemia in children

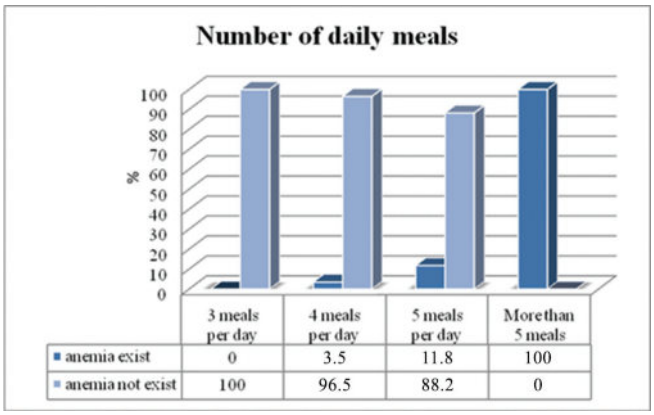


Figure 3. The relationship between the anemia prevalence rates and the number of family meals on a daily basis

Table 1. The relationship between anemia prevalence rates and nutritional status according to the variety of food included in a diet, methods of food preparation and children's eating habits.

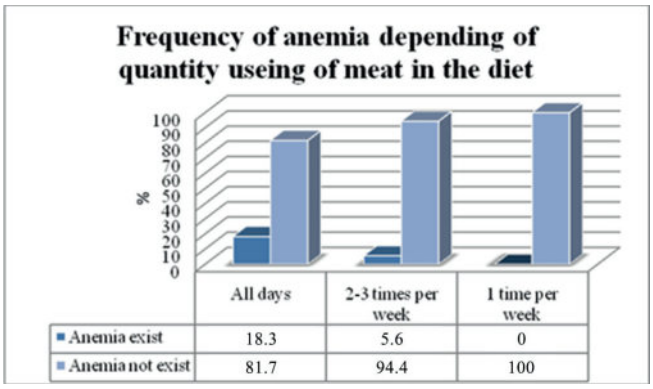
Variables		The prevalence of anemia		χ^2 тест	Nutritional status				χ^2 тест
	Number of children	Anemia is present %	Anemia is not present %	Anemia	Under-weight %	Normal-weight %	Over-weight %	Obesity	Nutritional status
Fish intake consumption in a diet									
2-3 times a week	13	0	100		19.4	36.4	27.3	18.2	
once a week	22	9	91	χ^2=1.94	0	55.2	22	21.6	χ^2= 13.45
2-3 times a month	75	14	86	DF=3	5.7	52.9	21.1	19.3	DF=9
Not eating fish	4	0	0	p>0.05	0	0	66.7	33.3	p >0.05
The consumption of nuts in a diet									
Yes	100	10	90	χ^2=16.79	6	52	24	18	χ^2=2.57
No	3	35	65	DF=2	0	75	25	0	DF=6
Sometimes	5	20	80	p<0.05	0	50	0	50	p >0.05
Sweets and snacks consumption									
On a daily basis	52	16	84	χ^2=1.08	10	45	21	20	χ^2=6.74
Sometimes	62	10	90	DF=1	3	57	23	17	DF=3
No	0	0	0	p>0.05	0	0	0	0	p <0.05
Food preparation methods									
Frying	24	15	85		0	53.8	25	21.2	
Roasting	8	30	70	χ^2=16.87	0	80	0	20	χ^2= 3.82
Boiling	81	4	96	DF=3	7.4	53.7	22.2	16.7	DF=9
Purchasing ready-cooked meals and fast food	1	100	0	p<0.05	0	100	0	0	p >0.05
Skipping a meal									
Breakfast only	30	20	80		0	50	20	30	
Lunch only	4	0	100	χ^2=2.88	0	66.7	33.3	0	χ^2=5.26
Dinner only	3	0	100	DF=3	0	44.4	33.4	22.2	DF=9
No meals skipping	77	8	92	p>0.05	8	60	18	14	p >0.05

The highest percentage of anemia was observed in children having ready-cooked meals and fast food (100%), and the least percentage of anemia was noted in children who ate home-made food (4%). The children who ate ready-made products and fast food were of healthy weight in 100% of the cases. The children who ate fried food (25%) were at risk of being overweight, and as for those who ate roasted food only 20% of them were considered as obese.

The study found that the highest percentage of anemia was observed in children who did not have breakfast in their family diet (20%), on the other hand, even 30% of the children with the same eating habits were obese. The results indicated that obesity was not found in children whose family members did not have lunch (Table 1).

The children eating meat every day were found to be underweight (10.3% of the sampled children). Obesity was most frequently found in children who had meat once a week (28.3%), consequently it was found in the children who had a daily intake of meat in their diet (21.4%). There was no statistically signficant correlation between nutritional status disorders prevalence rates and meat consumption frequency. We have established that anemia was mostly present in children who ate meat on a daily basis (18.3%). However, there was no statistical significance (Figure 4).

Apart from nutrition it is leisure-time physical activity that has its effect on anemia and obesity rates. The study demonstrated that 45% of the sampled children were physically active almost every day, although it was not part of their training, just a form of their recreational activities, whereas only 15% of the children showed their physical activity participation (2 – 3 times in a week, as part of their extracurricular activities). The amount of time children spent watching television screens was also analysed. The obtained results demonstrated that the greatest percentage of the sampled children (48%) spent more than 3 hours in front of the TV screens, whereas only 15% of the sampled children spent less than one hour in front of the TV screens (Table 2).



χ^2= 2.728, DF=2, p >0.05

Figure 4. The prevalence of anemia according to red meat consumption frequency

Table 2. Outdoor physical activities for children complete with the amount of time they spend watching television or using a computer during one day.

Variables	The prevalence of anemia		χ^2 тест	Nutritional status		χ^2 тест
	Anemia is present %	Anemia is not present %	Anemia %	Normal weight and under-weight children's group %	At the risk of being overweight and obese %	Nutritional status
Physical activity						
No physical activities	0	61.8	χ^2=14.16 DF=1	32.4	0	χ^2= 15.49 DF=1
Occasionally there are some physical activities or training	100	38.2	p<0.01	67.6	100	p<0.01
Time spent in front of the TV or computer screens						
< 3hours	0	57.8	χ^2=14.16 DF=1	83.1	0	χ^2= 12.2 DF=1
> 3hours	100	42.2	p<0.01	16.9	100	p<0.01
Number of children	12	102		71	43	

DISCUSSION

Anemia and obesity can result from unhealthy eating habits, family habits effects and environmenal factors (16). Our study results indicate that 16.7% of children is considered as obese which is approximate to the prevalence of obesity in France (14%) (17), then it is higher when compared with the study coming from Sweden (3%) (18), and less then the prevalence of obesity as depicted in the study of the USA (25%) (19). Our examination results show 10.8% of the children with anemia, which is considerably less when compared with the Ethiopian study results (39.1%) (20), and more when compared with the Brazilian study (6.3%) (21). A potential cause of the occurrence of anemia in the higher percentage of children in Ethiopia, based on the results of our particular study, is mainly related to less availability and consumption of dietary iron-rich foods, products of animal origin in particular as well as low income affecting a variety of food choices. The less prevalence of anemia observed among children in Brazil as opposed to our respondents probably resulted from the lack of a variety of food choices offered to our respondents. The number of daily meals is one of the factors relevant for maintaining energy bilance. The WHO recommended five daily meals, meaning: three main courses and two snacks. We have established that an increase in the number of daily meals is followed by an increase in anemia level. The reason for such a claim may be related to the low nutritional value of foods utilized and the consumption of inadequately prepared foods (fast food, sweets, snacks). Breakfast is considered to be the most significant meal that should provide adequate intake of carbohydrates, vitamins and minerals. Breakfast skipping increases risk of anemia and obesity development rates. Our reasearch findings indicate that anemia is mostly present in children whose family eating habits include skipping breakfast (20% of the respondents). The research of Bahaa Abalkhail and Sherine Shawky shows the presence of anemia in 27.7% of the children skipping breakfast (22). We have noted that 50% of the children classified into the excess weight gain group and obese children group would skip breakfast, unlike the results of the study Monika Arora et al. which noted 38.1% of children skipping breakfast (23).

There are numerous food preparation methods, classified into healthy and unhealthy preparation forms. Food prepared by roasting, frying or fast food has the high level of energy density, but it is not considered as the adequate source of micronutritients necessary for normal body functioning. In our study, the least percentage of anemia was present in children whose parents chose boiling as the most present food preparation form. A greater percentage of anemia was recorded among children who consumed fried and roasted food (30%). Anemia was present in all the sampled children who consumed ready-cooked meals and fast food. A frequent consumption of fast food and a frying method utilized for food preparation can be a contributing factor to obesity development. Unlike some of the previous studies Ayranci U et al. (24) and Pereira et al. (25), our study did not confirm the correlation between these bad dietary habits and the respondents' nutritional status. Only the children who had normal, healthy weight consumed fast food. The study showed that children who consumed fast food were at normal weight, probably because of the less frequent consumption of fast food or the fact that they did not consume large amounts of such food. There is also a possibility that there was sufficient physical activity that led to the expenditure of excess calories. Of the sampled children, 42% was considered to be at risk of excess weight gain or considered as obese, whereas the highest percentage of children eating fried food (58%) had a normal BMI. There were no statistically significant differences between the prevalence of anemia and nutritional status disorders compared with the food preparation method.

The consumption of certain foods can play a role in the prevention or predisposition of anemia and obesity development rates. Red meat falls into the category of more important nutrient dense foods. Due to the chemical form of iron contained in meat, which is more easily susceptible to absorption and use, the consumption of this type of food is important for the prevention of anemia development. In our research the obtained results show that the greatest percentage of children consuming meat 2-3 times a week (5.6%), which is evidently less when compared with the data of 24.8% of the children involved (19). Obesity is mostly present in children eating meat once a week (28.3%), and the reason for stating this is probably related to the fact that types of higher nutritient-dense foods such as carbohydrates and fats are prevailing in their diet. Our research showed that anemia was not present in children who had intake of fish 3 times a week. In favour of the previous argument is the fact that anemia is less present in children who consumed fish more frequently in their diet, which is substantiated by the study results (26). Our study demonstrated that the greatest percentage of obese children did not consume fish in their diet. 19% of the obese children consumed fish in their diet at least 2 or 3 times a week, which is in correlation with the study results (27).

Nuts present excellent sources of energy, plant-based proteins, monounsaturated and polyunsaturated fatty acids, dietary fibres, magnesium, potassium, folate and vitamin E. Certain types of the nuts contain greater amounts of iron which is a necessary component in the reduction and treatment of anemia. Nuts contain a bit of natrium and they do not contain any cholesterol which can reduce the risk of cardiovascular diseases and obesity in the combination with antioxidants contained in the abovementioned nuts (28). We have detected a statistical significance between the prevalence of anemia and nuts consumption. Anemia was present in 35% of the sampled children who did not consume any nuts. Among the children who consumed nuts in their daily diets, the majority of children fell into the normal, healthy weight group

(51%), whereas the minority fell into the underweight group (7%). The study results conducted by O'Neil CE et al. were in accordance with these results. Their study indicated that those who consumed nuts were associated with a lower risk of obesity later in life (28). This particular study revealed no statistically significant difference in the prevalence of nutritional status disorders compared with the consumption of nuts. According to studies, sweets consumed by children every day are reported to have a negative impact on their health state and well-being (29). Anemia was less present (45.8%) among respondents who ate sweets on a daily basis, but there were no statistical differences. On the other hand, the results obtained in the study conducted by Crnevic and Radovic Lj. indicated less percentage of anemia present in children (12.7%) (30). One of the possible explanations may lead to sugars generally increasing the absorption of Fe. However, the consumption of sweets containing a lot of 'empty calories' distracts the intake of nutrient-dense foods with a high nutritional value, increasing the risk of obesity development (31). Our research proved that there was 19% of the obese children who consumed sweets and snacks on a daily basis, which was similar to the study data given (31).

Various types of lifestyles also have their own influence on children's development, health and well-being. The amount of time children spend in front of the TV screen is the period of their physical inactivity which is a contributing factor to childhood obesity. Anemia and obesity were present in all the children who spent more than three hours a day in front of the TV, which is contrary to the study results obtained in Cuprija, where only 0.58% of the obese children spent three hours a day in front of the TV (32).

Frequent physical activity increases total energy demands, causing fatigue and due to the inappropriate macro and micronutritients intake it creates a basis for obesity development. All children diagnosed with anemia were physically active. The reason lies in the fact that physical activity of such children along with training and insufficient intake of iron-rich foods lead to the additional expenditure of nutrients. Unlike our results, the study administered by Crncevic and Radovic Lj indicates a lower level of anemia present in this particular children group (30).

Regular physical activity shows a possitive impact on the reduction of the incidence of obesity (33). We have come to the conclusion that 67.5% of the children who were of normal were physically active every day, healthy weight or fell into the underweight group, which was twice as much comparing with the study that revealed only 28.8% of such children. The consumption of fast and unhealthy food could be one of the possible reasons.

CONCLUSION

The obtained results show a statistically significant correlation between an increase in the consumption of fast food and anemia in children, whereas the amount of time children spend in front of the TV is also associated with the higher percentage of anemia and obesity. 21.6% of obese school-age children presented anemia. Additionally, it has been established that inadequate eating behaviour patterns, including not only the continual consumption of fast food, sweets and snacks but irregular intake of fish as well, complete with excessive consumption of meat and nuts – present young children's acquired habits and food preferences mostly influenced by their parents and caregivers. Therefore, it is very important to encourage children and their parents towards a healthier lifestyle by eating well and exercising regularly, as key measures directed at the prevention and control of obesity and and anemia.

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CARDIORENAL SYNDROME TYPE 1: DEFINITION, ETIOPATHOGENESIS, DIAGNOSTICS AND TREATMENT

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KARDIO RENALNI SINDROM TIP 1: DEFINICIJA, ETIOPATOGENEZA, DIJAGNOSTIKA I LEČENJE

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ABSTRACT

Cardiorenal Syndrome Type 1 (CRS-1) is defined as an acute worsening of heart function leading to acute kidney injury and/or dysfunction. It is an important cause of hospitalization which affects the diagnosis as well as the prognosis and treatment of patients. The purpose of this paper is to analyze causes that lead to the development of cardiorenal syndrome type 1 and its clinical consequences, as well as to emphasize the clinical importance of its early detection. The clinical studies and professional papers dealing with ***etiopathogenesis****, diagnosis and treatment of cardiorenal syndrome type 1, have been analyzed. The most important role in the occurrence of cardio renal syndrome type 1 is played by hemodynamic mechanisms, activation of neurohumoral systems, inflammation and imbalance between the production of reactive oxygen species (ROS) and nitric oxide (NO). Diagnosis of cardiorenal syndrome type 1 involves biomarkers of acute renal injury among which the most important are:* ***neutrophil gelatinase-associated lipocalin*** *(NGAL), cystatin C, kidney injury molecule 1 (KIM-1), liver-type fatty acid binding protein (L-FABP), IL-18 and the values of nitrogen compounds in serum. In addition to a pharmacological therapy, various modalities of extracorporeal ultrafiltration are applied in treatment of CRS-1, particularly if there is resistance to the use of diuretic therapy. As opposed to the experimental models, in clinical practice acute renal injury is often diagnosed late so that the measures taken do not give the expected results and the protective role shown in experimental conditions do not give the same results. For all these reasons, it is necessary to analyze the pathophysiology of renal impairment in cardiorenal syndrome as well as detect early indicators of kidney injury that could have clinical benefit and positive impact on reducing the cost of treatment.*

Keywords: *cardiorenal syndrome, diagnosis, treatment, therapy*

SAŽETAK

Kardiorenalni sindrom tip 1 (KRS - 1) se definiše kao akutno pogoršanje funkcije srca koje dovodi do akutnog oštećenja i/ili poremećaja funkcije bubrega. Predstavlja važan uzrok hospitalizacije koji utiče kako na dijagnozu, tako i na prognozu i lečenje pacijenata. Rad je imao za cilj da analizira faktore koji dovode do nastanka kardiorenalnog sindroma tip 1, kliničke posledice i da ukaže na klinički značaj njegovog ranog otkrivanja. Analizirane su kliničke studije i stručni radovi koji se bave etiopatogenezom, dijagnostikom i lečenjem kardiorenalnog sindroma tip 1. U nastanku kardiorenalnog sindroma tip 1 najznačajniju ulogu imaju hemodinamski mehanizmi, aktivacija neurohumoralnih sistema, inflamacija i poremećaj ravnoteže između stvaranja slobodnih radikala kiseonika (ROS) i azot monoksida (NO). Dijagnostika kardiorenalnog sindroma tip 1 uključuje određivanje biomarkera oštećenja bubrega u serumu među kojima su najznačajniji: NGAL (serumski lipokalin povezan sa želatinozom neutrofila), cistatin C, molekul oštećenja bubrega 1 (KIM-1), jetreni transportni protein masnih kiselina (L-FABP), IL -18 kao i vrednosti azotnih materija u serumu. Pored primene farmakološke terapije u lečenju se primenjuju i različiti modaliteti vantelesne ultrafiltracije, naročito ukoliko postoji rezistencija na primenu diuretske terapije. Nasuprot eksperimentalnim modelima u kliničkoj praksi se akutno oštećenje bubrega kasno dijagnostikuje tako da mere koje imaju benefita i protektivnu ulogu u njegovom nastanku u eksperimentalnim uslovima ne daju iste rezultate u kliničkoj praksi. Zbog svega pomenutog potrebno je analizirati patofiziologiju renalnog oštećenja u KRS-u kao i otkriti raniji indikator oštećenja bubrega što bi moglo imati ranu kliničku korist i uticalo na smanjenje troškova lečenja.

Ključne reči: *kardiorenalni sindrom, dijagnostika, lečenje, terapija*

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ABBREVIATIONS

ADHF - acute decompensated heart failure
CRRT – continuous renal replacement therapy
SCUF - slow continuous ultrafiltration
CVVHF - continued veno-venous hemofiltration
CRS-1 – cardiorenal syndroma type 1
GFR - glomerular filtration rate
KIM-1 - kidney injury molecule 1
L-FABP - liver-type fatty acid binding protein
IL-1, IL-6, IL-18 – interleukin 1, 6, 18
NGAL - neutrophil gelatinase-associated lipocalin
NO - nitric oxide
RAAS - renin angiotensin aldosterone system
ROS - reactive oxygen species
SCr – serum creatinine
TGF - tubuloglomerular feedback mechanism

INTRODUCTION

Cardiorenal syndrome type 1 (CRS-1) is defined as an acute worsening of the heart function leading to an acute kidney injury and/or renal dysfunction. It is an important cause of hospitalization which affects the diagnosis as well as the prognosis and treatment of patients, increasing the risk of cardiovascular mortality and morbidity, cerebrovascular insult, duration of hospitalization and the frequent need of rehospitalization. It occurs in 25-33% of patients hospitalized for acute heart failure (1, 2). Conditions that lead to rapid deterioration of cardiac function are acute hypertensive pulmonary edema with preserved systolic function, acute decompensation of chronic heart failure, cardiogenic shock and acute predominant right ventricular failure (3-10) (Table 1).

Etiopathogenesis

There are direct and indirect effects of heart failure that are designated as primary for the occurance and incidents of acute renal injury as well as renal dysfunction. The factors hidden behind the classic hemodynamic mechanisms play the main role in the pathogenesis of renal impairment. The factors involved in the development of cardiorenal syndrome type 1 are: venous congestion, dysfunction of the sympathetic nervous system, anemia, activation of the renin angiotensin aldosterone system (RAAS), disruption of the hypothalamic-pituitary axis and significant changes in immune and somatic cell signaling (Table 1) (11). In addition to the aforementioned mechanisms, renal impairment occurs as a result of renal hypoperfusion, which occurs as a consequence of reduced cardiac output and systemic arterial pressure. A sudden decrease of intravascular volume activates the renin angiotensin aldosterone system (RAAS), which leads to an increase in angiotensin 2, which stimulates creation and release of endothelin 1 in kidneys, a strong profibrotic inflammatory and vasoactive peptide which plays an important role in most pathogenetic mechanisms of acute kidney injury (3-9). All this leads to a decrease in glomerular filtration rate and development of acute kidney injury.

Venous congestion

Venous congestion is one of the most important hemodynamic determinants of cardiorenal syndrome which is

Table 1. Ethipathogenesis and biomarkers in Cardiorenal syndroma type 1

Cardio renal sindroma type 1		
The main couses	**Pathogenesis**	**Biomarkers**
1. Acute hypertensive pulmonary edema with preserved systolic function 2. Acute decompensation of chronic heart failure 3. Cardiogenic shock 4. Acute predominant right ventricular failure	*Factors that lead to renal hypoperfusion*: 1. Dysfunction of the sympathetic nervous system 2. Venous congestion 3. Anemia 4. Activation of the RAAS 5. Disruption of the hypothalamic-pituitary axis 6. Significant changes in immune and somatic cell signaling 7. Imbalance between the production of reactive oxygen species (ROS) and nitric oxide (NO) 8. Inflamation (c-reactive protein, pentraxin 3, tumor necrosis factor α, IL-1, and IL-6 9. Apoptotic factors in plasma (caspase-3 and caspase 8)	1. NGAL 2. Cystatin C 3. KIM-1 4. L-FABP 5. IL -18

RAAS - renin angiotensin aldosterone system
NGAL- Neutrophil gelatinase-associated lipocalin
KIM-1 Kidney injury molecule
L-FABP- Liver fatty acid binding protein
IL-18- interleukin 18
IL-1 interleukin 1
IL-6 interleukin 6

associated with a renal dysfunction within the acute decompensated heart failure (12). Neurohormonal activation flooding atrio-renal reflexes in patients with heart failure is manifested by persistent renal retention of sodium and water in spite of atrial pressure. Transmission of venous congestion to renal veins continues to threaten glomerular filtration rate (GFR) (13). One of the possible mechanisms that connects venous congestion and GFR is an increase in renal interstitial pressure that leads to an increased concentrations of angiotensin II which results in a reduction in the glomerular filtration rate, either directly or via modulation of the sympathetic nervous system. The stimulation of the adrenergic receptors on the proximal tubule renal cells increases the reabsorption of sodium, while the stimulation of the adrenergic receptors in the juxtaglomerular apparatus stimulates RAAS (14).

The most important non-hemodynamic mechanisms involved in the development of CRS-1 are: renin angiotensin aldosterone system (RAAS), the sympathetic nervous system (SNS), inflammation and an imbalance between the production of *reactive oxygen species* (ROS) and nitric oxide (NO) (15).

Inflamation and immune cell signaling

Inflammation plays an important role in the development of all types of cardiorenal syndrome, including type one. Numerous studies have demonstrated the activation of different levels of inflammation in patients with heart failure. Acute heart failure leads to an immune activation and production of cytokines, complement system, TNF-alpha, adhesion molecules and immune cells, which cause damage to distant organs such as kidneys, leading to acute renal injury, exacerbating the heart failure. The inflammatory cytokines which can be created in the myocytes as a result of an ischemic or mechanical stimuli or innate immune response that is represented as a "toll-like" receptor, "pentraxin-like" C-reactive protein and pentraxin 3 (16-18) provide an inflammatory etiology of heart failure. The increase of cytokines, as well as inflammatory markers, in the blood have been proven in patients with acute decompensated heart failure (19). There is evidence which supports the prognostic value of various circulating markers of inflammation, particularly C-reactive protein, pentraxin 3, tumor necrosis factor α, IL-1, and IL-6 (20). For example, it has been shown that high levels of C-reactive protein, an acute phase reactant, are registered in the last stages of renal failure (21). A study conducted by Pastori and his colleagues showed that the development of CRS-1 is immune-mediated and that loss of normal regulation of the immune system may play a role in the pathogenesis of CRS-1. The study pointed to a high activity of apoptotic factors in plasma of patients with CRS-1 (caspase-3 and caspase 8 – inducing apoptosis of monocytic cells) as well as high levels of IL-6 and IL-18 (22). These findings suggest an immunological basis for CRS-1 because various cardiorenal mediators can induce early pathological apoptosis of monocytes.

The predisposing factors for type 1 CRS

There are variety of contributing factors that pose a risk for the development of cardiorenal syndrome type 1. Obesity and cardiometabolic changes in the cardiovascular system, including diabetes, hypertension, and subsequently, during the illness cachexia, biochemical and hormonal changes caused by disorders of bone and mineral metabolism, retention uremic molecules, proteinuria and anemia – these all are involved in the development of CRS-1. The development of this syndrome can cause permanent renal impairment and the need for dialysis or a partial renal recovery. Coronary revascularization also leads to a development of cardiorenal syndrome type 1. Before surgical vascularization each patient undergoes coronary angiography. Iodinated contrast agents cause renal vasoconstriction and direct toxicity of the renal tubules. As a result, contrast-induced nephropathy occurs in 15% of patients (23). During surgical revascularisation of the heart, kidneys are exposed to hypothermia, absence of pulse, perfusion reduction of 30-90 minutes, which can lead to progression of ischemic injury in the ambient of proinflammatory condition. It is possible that extracorporeal circulation used in the cardiovascular surgery triggers systemic factors that further lead to the acute renal injury. Attempts to reduce exposure to this risk did not lead to a reduction in incidence of acute kidney injury (24). Cardiac bypass surgery with acute renal impairment occurs in 30% of patients. The cardiorenal syndrome type 1 causes an increase in mortality rate three to four times despite the possibility of dialysis (25).

Diagnostics of cardiorenal syndrome type 1

The latest biochemical biomarkers, whose values are increased even at a slight reduction in kidney function, are used in the early diagnosis of type 1 CRS. What should not be ignored is the increase in serum creatinine in the diagnosis of acute kidney injury. Thus cardiorenal syndrome type 1 is defined as an increase in serum creatinine compared to the value at the time of admission to 26.5 μmol/L and more or 44.2 μmol/L and more or 25% and more, as well as an increase in the value of the SCr by 50% SCr values at the time of admission or as a combined increase in SCr of 26.5 μmol/L and more or 25% and more (26). In the last decade, several risk scores for acute kidney injury in patients with acute heart failure, acute myocardial infarction and in patients undergoing cardiac surgery or coronary angiography have been published. The application of these scores in clinical work has shown great benefit in the prevention of CRS-1 (27). Forman (28) and Mehran (29) risk scores are widely used in the world. They are very valuable for predicting the emerging acute renal injury in patients with acute decompensated heart failure and those who underwent coronary angiography. Risk factors included in risk scores were: age, serum creatinine levels, systolic arterial pressure, glomerular filtration rate, diabetes mellitus, use of furosemide and many others.

Biochemical markers of acute renal injury

Serum creatinine, glomerular filtration rate determined by creatinine clearance, the concentration of sodium in the urine, fractional excretion of sodium, fractional excretion of urea, and urine osmolality are used as standard laboratory parameters in clinical practice. In addition to the parameters listed, for early detection of acute kidney injury in recent years some new biomarkers have been used: KIM-1 - kidney injury molecule 1, L-FABP - the liver fatty acid binding protein, IL -18, NGAL and cystatin C (30). A larger number of studies have examined the role of biomarkers in predicting the occurrence of cardiorenal syndrome type 1 in patients with acute decompensated cardiac insufficiency (Table 1) (31). In their study Pfister and colleagues found elevated basal NT-proBNP as a predictor for developing acute renal impairment (32).

NGAL

In one of the numerous studies, on admission, among patients who had developed acute renal failure, the increased serum neutrophil gelatinase-associated lipocalin (NGAL) was shown. NGAL is a protein mass of 25 kDa, and is one of the important indicators of acute renal injury and is very often used in clinical practice (33). If the values of NGAL in the urine are greater than 100 ng/ml two hours after the initial event, it indicates the development of acute renal impairment (34). NGAL serum values are determined by the impairment of renal function rather than myocardial function (35). *In p*atients with ADHF (acute decompensated heart failure) serum NGAL largely correlates with renal functional markers. High values of NGAL on hospital admission are associated with adverse cardiovascular outcomes or death.

Cystatin C

Serum cystatin C measured on admission of patients with acute decompensated heart failure is a better long-term predictor of mortality and rehospitalization than serum creatinine or BNP (36). Cystatin C is a cysteine protease inhibitor which is completely reabsorbed in the proximal tubule cells. It proved to be a very sensitive indicator of small changes in glomerular filtration rate. This biomarker is an independent predictor of adverse cardiac events in patients with acute coronary syndrome (37). Its concentration is increased up to two days before the increase of serum creatinine concentration. Due to its predictive values and biochemical characteristics serum cystatin C may also be of great relevance for the diagnosis of early loss of kidney excretory function in CRS-1.

Treatment of CRS-1

The treatment of CRS type 1 is rather complex and the consensus on the choice of an appropriate therapeutic approach has not been reached yet. It was agreed that the preservation of renal function should have the same priority as the preservation of heart function (38). Standard therapeutic strategies in clinical practice imply medical therapy and open ultrafiltration dialysis supportive therapy.

Medical therapy

The usage of medications for the treatment of heart failure, such as diuretics, ACE inhibitors, and angiotensin receptor blockers have a deleterious effect on renal function. Diuretics improve symptoms in acute decompensated heart failure and in the long run they do not affect morbidity and mortality, but they may lead to a deterioration of renal function, due to neurohumoral activation. These patients often experience resistance to these drugs as well as the absence of desired effect. Resistance occurs due to the reduction of glomerular filtration, activation of the sympathetic nervous system and RAAS system, hypertrophy of epithelial cells of the distal tubules and increased concentration of adenosine. Loop diuretics are the first-line drugs for patients with acute decompensated heart failure, where volume overload is present, with the goal of acheaving a gradual diuresis (39). Dosing of diuretics depends on renal function, systolic blood pressure and previous treatment with diuretics. Diuresis can be enhanced by the addition of thiazide diuretic, acetazolamide, spironolactone or by using continuous infusion of loop diuretics. Continuous infusion of diuretics can be monitored by using the techniques to evaluate the quantity of liquids such as bioelectrical impedance vector analysis (BIVA), measuring proBNP, cardiac output and central venous pressure. According to a study conducted by Felker et al. it has been proven that in patients with acute decompensated heart failure (ADHF) there is no significant difference in improvement of symptoms, the number of rehospitalizations and deaths between those in which diuretics are administered by continuous IV infusion and those who received the bolus, nor were there diffeences in the application of high versus low doses of diuretics (40). Beta blockers are administered with caution, especially in patients with cardiogenic shock. Their application should be started cautiously, when hemodynamic stability of patient is established, particularly in patients with CRS-1 after myocardial infarction (41). ACE inhibitors and angiotensin receptor blockers are underused in the treatment of cardiorenal syndrome due to fears of worsening renal function. Blockade of RAAS alone reduces morbidity and mortality in patients with chronic heart failure and slowing the progression of chronic renal failure, particularly in patients with diabetic nephropathy (42). The use of ACE inhibitors in patients with moderate renal failure has a positve effect on their survival rate despite a transient worsening of renal function that occurs in 30% of cases. The presence of acute renal failure with or without hypokalemia can also reduce the use of the ACE inhibitors and the aldosterone receptor blockers. In

patients with severe renal insufficiency a constant monitoring of renal function is required because of unknown relations between their safety and efficacy. Apart from the drugs mentioned, in the treatment of CRS-type1 the inotropic drugs are used, among including dopamine, dobutamine, norepinephrine, fenodolpan. Dopamine is a remedy which, depending on the dose, may have vasodilator, inotropic and systemic vasoconstrictor effects. It is used in patients with systemic hypotension and in patients with reduced cardiac index (43).

Newer drugs for the treatment of type 1 CRS

New strategies in the treatment of CRS 1 involving use of drugs that improve kidney function in patients with heart failure and those are adenosine receptor antagonists and vasopressin antagonists. The blockade of subtype 1 adenosine receptors can enhance diuresis and natriuresis with maintaining or increasing GFR and reduce the need for loop diuretics (44). The blockade of adenosine receptors leads to a blockade of tubuloglomerular feedback mechanism (TGF) which is responsible for normal homeostasis of electrolytes and fluids that can be changed in heart failure and leads to a diuretic resistance and reduction of glomerular filtration rate. A placebo-controlled, randomized study showed that selective adenosine A1 receptor blockers such as rolofylline in patients hospitalized with ADHF and volume overload in order to assess the effect on congestion and renal function was not better than placebo (45). Although it showed an improvement of dyspnea, rolofilline did not prevent the emergence of kidney damage nor had a significant effect on the overall success of treatment. The blockade of vasopressin V2 receptors in collecting ducts of kidneys leads to an increase in the excretion of free fluid which can theoretically correct fluid retention and hyponatremia in patients with congestive heart failure. Tolvaptan, an oral selective vasopressin V2 receptor antagonist, showed results in improving symptoms, but not in reducing the number of rehospitalizations and mortality in patients with ADHF (46). Conivaptan is antagonist of both vasopressin V1 and V2 receptors which not only reduces the excretion of fluids but it can also reduce systemic vascular resistance and improve systolic function (47). Natriuretic peptides have systemic and renal vasodilator effect, natriuretic, diuretic and myorelaxant effect on smooth muscles and they reduce the need for oxygen in certain segments of the nephron (48). The synthetic natriuretic peptides lead to a dilatation of the arterial and venous systems. The study ASCEND - HF (Acute Study of Clinical Effectiveness of Nesiritide and decompensated Heart Failure) which studied the effect of Nesiritide B, a synthetic natriuretic peptide, in patients with decompensated heart failure showed that Neseritide B has no effect on survival rate, as well as on the number of rehospitalizations (49). Urodilantin A is a natriuretic peptide which can improve symptoms but does not affect the mortality and renal function in patients with ADHF (acute decompensated heart failure) (50).

Ultrafiltration in treatment of type 1 CRS

Ultrafiltration is indicated if the volume overload persists despite an optimal output and the application of diuretic therapy and when there is resistance to its use. The process of ultrafiltration removes the isotonic fluid from the blood of patients, wherein there is no activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system (51, 52). There are several modalities of ultrafiltration used in the treatment of patients with an acute worsening of chronic heart failure and these are Aquadex system, Intermittent Ultrafiltration - IUF and Slow Continuous Ultrafiltration - SCUF. According to the study RAPID-CHF ("Relief for Acutely Fluidity Overload Patients with Decompensated CHF") the application of SCUF showed good results in patients with a sudden worsening of chronic heart failure (53). The Continued Veno - Venous Hemofiltration (CVVHF) has found its application in patients with a sudden worsening of chronic congestive heart failure, metabolic disorders of electrolyte and acid-base balance (hyperkalemia, metabolic acidosis), as well as in the removal of excess fluids.

Comparison of efficacy of diuretic therapy and ultrafiltration by open dialysis supportive therapy in the treatment of type 1 CRS

A study called UNLOAD (The Ultrafiltration versus IV Diuretics for patients Hospitalized for Acute decompensated Congestive Heart Failure) compared the application of ultrafiltration with the application of diuretics in patients with acute decompensated cardiac failure. Ultrafiltration has reduced the number of rehospitalizations and increased weight loss in patients with ADHF (54). Another smaller study, conducted by Rogers and associates showed no difference in the balance of fluid, glomerular filtration rate and renal plasma flow between the application of ultrafiltration and loop diuretics (55). In a four-year period the large study CARRES-HF (2008-2012) showed that the application of the step-approach pharmacological therapy is superior to ultrafiltration regarding preserving renal function during 96 h in patients with acute decompensated heart failure, worsening of renal function and persistent congestion (56). The use of ultrafiltration has been associated with a number of adverse events (bleeding, worsening of renal function, complications associated with IV catheter). Weight loss was similar in both groups. In addition to the aforementioned, two smaller studies have also shown that the use of methods of ultrafiltration or so-called (CRRT) – Continuous Renal Replacement Therapy – is associated with a high mortality rate, especially in the simultaneous administration of vasopressors in patients over 70 years of age (57). One of these studies, conducted in Cleveland, has shown that the application of slow continuing ultrafiltration (SCUF) in patients with acute decompensated heart failure, despite the fact that it has demonstrated significant weight loss and improvement of central and venous hemodynamic parameters, did

not show significant improvement of renal function in reducing serum creatinine and urea (58). According to these studies it can be concluded that the development of cardiorenal syndrome type 1 does not only involve hemodynamic mechanisms, but also some other, still unknown variables, suggesting that the improvement in haemodynamic parameters is not closely related to the amelioration of renal function (59).

Peritoneal dialysis in cardiorenal syndroma type 1

Peritoneal dialysis provides better hemodynamic stability in patients with acute exacerbation of chronic congestive heart failure and acute kidney injury (60). The main advantages of peritoneal dialysis are the preservation of renal function, less variable ultrafiltration, hemodynamic stability, better clearance of large molecules, smaller loss of sodium and preservation of its normal concentration in the serum (61). The disadvantages of peritoneal dialysis are an increased risk for the development of peritonitis, metabolic disorders (hyperlipidemia, increased atherogenic potential). The results of new well-controlled, randomized clinical trials should more accurately determine the significance, place and role of peritoneal dialysis in the treatment of patients with cardio-renal syndrome type 1 (62).

CONCLUSION

The clinical significance of acute kidney injury, when the outcome of treatment of patients is in question, has increased in recent years. Deterioration of renal function in patients with acute decompensated heart failure is an important risk factor and very often results in a rapid deterioration of the clinical picture. In contrast to experimental models, in clinical practice, acute renal failure is often diagnosed too late so that the measures that normally have beneficial effects and protective role under the experimental conditions do not give the same results in clinical practice. For all these reasons it is necessary to analyze the pathophysiology of renal impairment in the CRS as well as detect early indicators of kidney damage. Then the application of various protective molecules could have early clinical benefits.

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COMPREHENSION OF SPATIAL METAPHORS AFTER RIGHT HEMISPHERE STROKE: A CASE REPORT

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RAZUMEVANJE PROSTORNIH METAFORA NAKON MOŽDANOG UDARA U DESNOJ HEMISFERI: PRIKAZ SLUČAJA

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ABSTRACT

Studying how spatial information interacts with figurative language processing in right-hemisphere (RH) stroke patients is a relatively neglected area of research. The goal of the present case study was to establish whether an ischemic lesion in the right temporo-parietal region causing spatial neglect would affect comprehension of sentence-level spatial metaphors, since some evidence indicates the crucial role of the RH in metaphor processing. The patient under study showed some degree of cognitive impairment (e.g., in spatial and verbal working memory, executive control, visuo-spatial matching skills). However, his comprehension of spatial metaphors was preserved. This case illustrates that RH damage does not necessarily affect comprehension of sentence-level spatial metaphors.

Keywords: *spatial metaphors, stroke, right hemisphere, temporo-parietal region, allocentric representations, spatial neglect.*

SAŽETAK

Uticaj spacijalnih informacija na razumevanje figurativnog jezika kod pacijanata sa lezijama u desnoj hemisferi usled moždanog udara je nedovoljno istražena tema. S obzirom na to da dosadašnja istraživanja ukazuju na presudnu ulugu desne hemisfere u razumevanju metafora, cilj naše studije bio je da utvrdi da li ishemijska lezija u desnoj temporalno-parijetalnoj oblasti povezana sa spacijalnim neglektom utiče na razmevanje prostornih metafora u rečenici. Pacijent ZG prikazan u ovoj studiji pokazao je deficit prostorne radne memorije, verbalne radne memorije i vizuelno-prostornih odnosa. Međutim, njegovo razmevanje prostornih metafora je očuvano. Ova studija pokazuje da lezije u desnoj hemisferi ne utiču na razumevanje prostornih metafora.

Ključne reči: *prostorne metafore, moždani udar, desna hemisfera, temporo-parijetalna oblast, alocentrične reprezentacije, spacijalni neglekt.*

INTRODUCTION

Studying how spatial information interacts with figurative language processing in post-stroke patients is a relatively neglected area of research. While often reported deficits in spatial cognition in Alzheimer's disease (AD) patients are associated with early hippocampal deterioration or with basal forebrain atrophy (1), patterns of deficit/sparing of spatial processing in post-stroke patients with right hemisphere damage is less clear. Spatial cognition has been associated with a bilateral fronto-parietal network, in which the right hemisphere (RH) plays the main role in directed attention within extrapersonal space (2, 3). A hierarchical organization in the processing of spatial relations has been proposed, in which egocentric (body-centered) spatial relations require only a subsystem of the resources required by allocentric (body-independent) spatial relations (4). According to this view, the latter require the RH involvement. On the other hand, the neural basis of spatial language, e.g. linguistically coded spatial information, involves the left inferior temporal and parietal areas, occipito-temporal junction, inferior prefrontal region and anterior superior temporal gyrus (5-7). However, some evidence suggests that spatial language processing is supported by the brain regions that support non-linguistic spatial processing (8).

It has been debated whether comprehension of metaphors and other types of figurative language requires RH

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support (9-11). So far, only a small number of studies investigated comprehension of sentence-level metaphors in post-stroke patients. An early study involving patients with RH injury (n=22) reported that these patients had preserved ability to verbally explain metaphoric sentences, but they were correct only half of the times in picture-metaphor matching (12). Another study investigated comprehension of words, familiar phrases (similar to conventional metaphors), and novel sentences in left-hemisphere (LH) aphasic (n=28) and RH-damaged speakers (n=11) and reported that the RH-damaged group had impairment in the comprehension of familiar phrases, but not in the comprehension of words or novel sentences (13). The group with LH damage showed the opposite pattern: good comprehension of familiar phrases, but impaired comprehension of novel sentences.

A series of three case studies that investigated the comprehension of moderately familiar sentential metaphors and closely matched literal sentences in post-stroke aphasic patients showed that all three patients had moderately impaired comprehension of metaphors. Crucially, one of the three patients had a RH lesion, whereas the remaining two were LH-damaged aphasic patients. What sets these cases apart is a differential impairment of metaphoric and literal sentence comprehension. Put differently, these patients exhibited three distinct comprehension patterns: the patient with a RH injury had impaired comprehension of both metaphoric and literal sentences at a comparable level; one of the two patients with LH damage had impaired comprehension of both types of sentences, but with significantly more impaired comprehension of metaphors, while the other had impaired comprehension of metaphoric sentences but spared comprehension of literal sentences (14). Taken together, this evidence indicates the existence of a variety of patterns in metaphor comprehension in post-stroke patients that goes beyond simple dichotomies, such as novelty vs. familiarity, and involves damage to both cerebral hemispheres.

In the present study, we investigated the comprehension of sentence-level spatial metaphors in a RH stroke patient. By "spatial metaphors" we refer to the metaphors containing spatial terms or as previously defined in the literature: "Talking in spatial metaphors means using spatial words to describe nonspatial entities, states, or relationships" (15). Comprehension of spatial metaphors requires information integration based on both linguistically coded spatial information and figurative language, where two previously non-linked concepts are joined in a new meaning. Here we focused on conventional metaphors with spatial terms and wanted to determine whether a RH post-stroke patient with no apparent aphasia had difficulties comprehending metaphoric sentences containing spatial information. We hypothesized that a stroke lesion affecting the right temporo-parietal region and resulting in spatial neglect would be associated with impaired comprehension of spatial relations in the allocentric frame of reference (object-object); however, comprehension of metaphoric sentences with locative predicates and literal sentences with spatial modifiers would be preserved, because the intact left hemisphere would support these tasks. To test these hypotheses, we designed four experiments (section "Evaluative and experimental tests") and tested a right-handed man who had previously suffered a stroke in the right hemisphere (section "Case presentation").

BACKGROUND

Case presentation

A 70-year-old right-handed man, ZG, was admitted to the neurology department due to a sudden weakness of the whole left side of body, confusion, and inability to speak. The patient did not have a history of dementia, other brain diseases, or alcohol/drug abuse. He had been treated for angina pectoris for eight years prior to the incident, but he had not been taking the medication regularly. A CT scan showed an acute right-hemisphere temporo-parietal lesion. Additionally, MRI revealed smaller ischemic lesions in the right basal ganglia and occipital lesions. Nine days later, ZG suffered another stroke, which was caused by occlusion of the right internal carotid artery within the cavernous sinus. There was no evidence of lesions in the left hemisphere. An extracranial Doppler sonogram obtained immediately after the first stroke showed no substantial stenosis in the common carotid artery, external carotid artery and internal carotid artery, although atheromatous changes were present, being in particular prominent at the carotid bifurcation; there were no hemodynamic changes in the vertebral arteries or signs of reverse flow. Furthermore, the Multislice Computed Tomography (MSCT) angiography, which was performed 10 days after the second stroke, showed no pathological changes extracranially, in the common carotid artery, external carotid artery or internal carotid artery. The vertebral arteries showed normal hemodynamics, with sufficient blood velocity. The intracranial segments of the internal carotid artery showed no signs of stenosis. In addition, the MSCT scan showed normal branching of the circle of Willis and no signs of pathology.

The medical records indicate that the two major incidents left the patient with a left-side paresis and spatial neglect, which persisted throughout the chronic stage. In addition, immediately after the incidents, the patient showed language disturbances both in speech production and comprehension. For example, he was not able to talk, except to produce one word, "water", or to respond to the questions asked by the medical staff inquiring about his condition. He was disoriented and could not follow commands. He remained at this verbally nonresponsive, low-level consciousness and confused state during the initial part of his stay at the hospital, but his condition had improved by the time he was discharged.

After leaving the hospital, ZG stayed at a rehabilitation institution for three weeks, where he underwent an intensive program for rehabilitation of motor and cognitive functions. The initial language disturbances were transient and resolved spontaneously, leaving the patient with some reading difficulties (section "Results"). In addition, he exhibited memory problems (forgetfulness, difficulty with retrieval of words and names), but according to his spouse, these were present before the illness. Since leaving the rehabilitation institution, he has continued the intensive physical therapy at home, which he has had four times per week. He has regularly attended neurological follow-up exams as scheduled and visited his general practitioner for a check-up once a month.

At the time of testing for the present study, which was 4 years post-onset, ZG had left-side hemiparesis, no apparent language disturbances, and was using the following prescribed medications: amiodaron to regulate arrhythmia, plavix to prevent platelets from clumping together and forming blood clots, clonazepam to control muscle spasms, and nitroglycerin for the management of angina pectoris. When asked about his condition, ZG complained that he "cannot see things on the left side". He also stated that he needed more time to complete any mentally engaging task than before the illness and that he now preferred a slower rate of speech in conversation, especially with speakers who use longer and more complex sentences.

Before the testing began, the patient signed informed consent. In addition, five neurologically intact subjects participated in the study as a control group. They also signed informed consent. The study was conducted in accordance with the guidelines of the Declaration of Helsinki for studies involving human subjects.

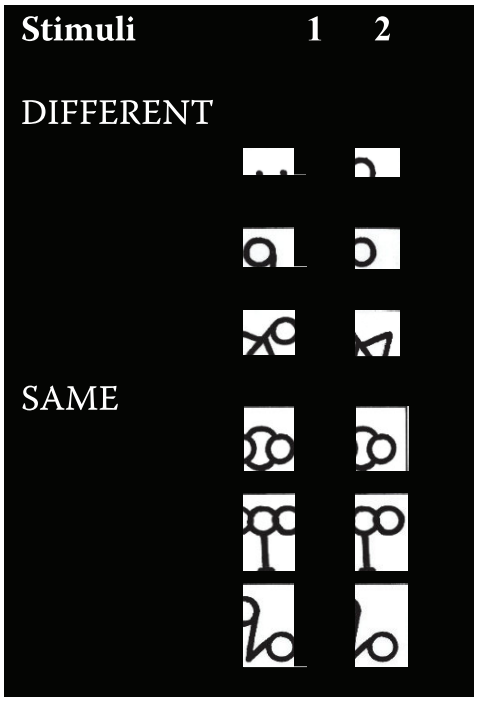
Evaluative and experimental tests

In addition to consulting ZG's medical records, before conducting experiments, we administered a set of evaluative tests: an aphasia screening test validated for Serbian (16) to determine patient's language status, Montreal Cognitive Assessment (MoCA) (17) to evaluate his general cognitive status, the Month ordering test (18) to assess his verbal working memory, and Raven's Coloured Progressive Matrices (19) to assess his visuo-spatial pattern-matching ability and relational reasoning.

Four experiments were designed to test the following abilities of the patient: spatial working memory (Experiment 1), comprehension of basic spatial relations (Experiment 2), use of spatial prepositions in nonfigurative sentences (Experiment 3) and comprehension of sentence-level metaphors with spatial terms (Experiment 4).

Briefly, Experiment 1 consisted of 40 pairs of symbols that were modified from the Glagolitic alphabet, with which the patient was not familiar. One half of the stimuli contained pairs with two identical symbols and the other half contained pairs with two different symbols. The pairs containing different symbols were created by manipulating one symbol along either a vertical reference axis (up, down) or horizontal reference axis (left, right). Crucially, in a pair with two different symbols, the symbols differed in only one spatial feature, which was varied across the upper vs. lower left vs. right quadrants. In this experiment, the symbols in each pair were presented separately for 3 seconds, with the second symbol immediately following the first one (see Table 1 for examples). The task was to decide whether the second symbol was positioned in the same way or differently from the first symbol, i.e. whether the two express the same spatial relation. The order of presentation of the pairs containing same/different symbols was randomized. The stimuli were printed in black color on a white background and positioned in the center of page. The time to respond was not limited.

Table 1. Examples of stimuli for Experiment 1.
The task was to judge whether the second symbol (column 2) of a pair was positioned in the same way or differently from the first symbol (column 1) of that pair. The first three rows in the table illustrate the pairs with differently positioned symbols and the last three rows illustrate the pairs of symbols positioned in the same way.



Experiment 2 tested participants' ability to identify spatial relations among paired objects in drawings (e.g., heart, cross, circle, triangle, etc.). The stimuli consisted of 14 pairs of drawn objects, each testing a different spatial relation in the object-object frame of reference, such as *behind, in front of, on top of, below.* Each pair was presented on a separate sheet of paper, and above each pair a written word indicated the target spatial relation, e.g., "BELOW". The task was to form a sentence expressing the spatial relation between the presented pair of objects based on that

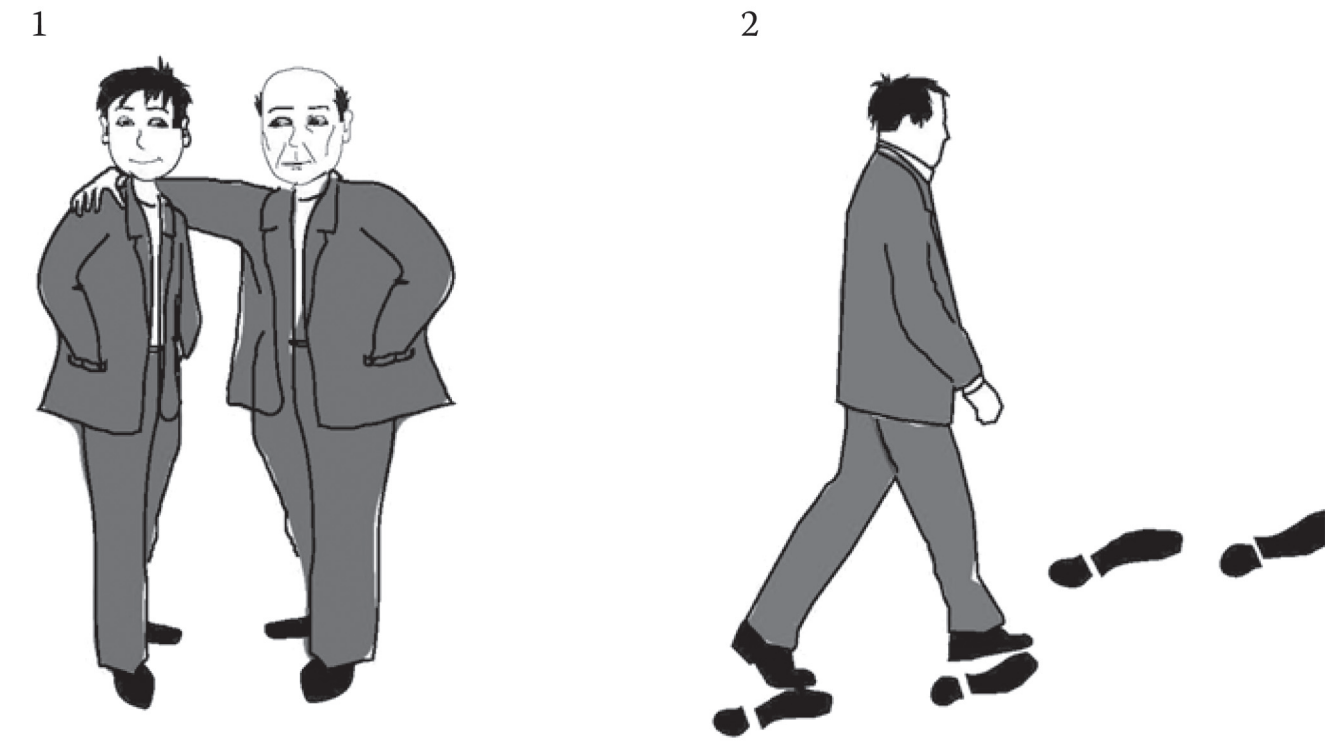


Figure 1. Picture-metaphor matching task: "He is following in his father's footsteps."

word, e.g. "The cross is below the heart". To facilitate the task and avoid interference with possible reading difficulties, the experimenter read the written word.

Experiment 3 tested participants' ability to produce spatial prepositions in simple non-figurative sentences, consisting of a noun expressing the role of subject, verb, and prepositional phrase indicating a spatial relation. In this sentence-completion task (e.g. *He is sitting … the chair.*), which requires comprehension of spatial prepositions for accurate completion, the stimuli consisted of 20 sentences. Examples of spatial prepositions that were required to correctly complete the sentences included: *in, on, below, on top of, in front of,* among others. The experimenter read each sentence making a pause for a missing preposition. The task was to say which preposition was missing, after the sentence was read.

Experiment 4 tested participants' comprehension of sentences with spatial metaphors. In this picture-metaphor matching task, 14 pairs of drawings were created for a selection of 14 sentential spatial metaphors, such as *He is following in his father's footsteps*. Crucially, one drawing depicted the literal meaning and the other drawing depicted the figurative meaning of the sentence (Figure 1). The two drawings of each pair were equal in size and positioned next to each other, with the metaphorical sentence written above them. The experimenter read the sentence. The task was to decide which drawing was a better match for the sentence. The drawings depicting literal and metaphoric meanings were presented on the left vs. right side equal number of times, in a randomized order.

Before each experiment, the patient completed two to four practice trials to demonstrate that he understood the task. The time to respond was not limited in any of the experiments. The patient was tested in a quiet room at his home, in a single session. The study took about 2 hours to complete.

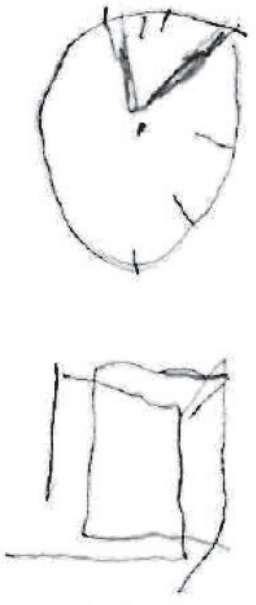


Figure 2. ZG's drawing of a clock face from memory (above) and his copy of a cube (below).

RESULTS

Evaluative measures. ZG achieved maximum scores on all subtests of the aphasia screening test, except for reading, where he failed to read the first two letters in 50% of words and was not able to correctly read sentences. It is likely that the reading difficulty was due to spatial neglect and that correctly reading half of the words stimuli involved a strategy of relying on lexical knowledge, which overruled the effects of neglect on perception of letters (20). This strategy was clearly ineffective in reading sentences.

ZG's score on MoCA was 20/30, revealing difficulties in visuo-spatial, executive, and memory subtests. Figure 2 illustrates lack of detail, consistent with spatial neglect, on the left side of ZG's copy of the cube and especially on the clock drawing, which has correct time setting but contains visuo-spatial errors.

ZG's score on the verbal working memory test was 9/20. The results of this test show that he was able to remember the names of three to four months long enough to correctly report which months appeared in a particular string, but he was not able to reorder them canonically before reporting them back, as required by the task, except in the sequences consisting of two and three months. This indicates a limitation of verbal working memory capacity (18).

Finally, ZG scored 24/36 on Raven's Progressive Color Matrices, achieving 6/12 on component A, 7/12 on component A_B and 11/12 on component B. The tasks in these components differ in the sense that some may be solved by relying purely on visual-pattern matching skills, whereas others require relational reasoning (21), which is more relevant for language processing (7). ZG's scores on this test indicate that his visual-pattern matching abilities are impaired, as also indicated by the subtests of MoCA, whereas his relational reasoning is well-preserved.

Experimental measures. ZG demonstrated good comprehension of spatial metaphors (13/14; 92.8%) and basic spatial relations (14/14; 100%). The modified *t* test, designed for comparing an individual case to a small control sample (22), revealed no statistically significant differences between ZG's performance and the control group's performance on the test of spatial metaphors ($p = 0.08$, one-tailed) and spatial relations ($p = 0.35$, one-tailed). However, ZG's use of spatial prepositions in literal sentences and his score on the spatial working memory test were considerably different from the scores of the control group (spatial prepositions: $p = 0.006$, one-tailed; spatial working memory: $p < 0.005$, one-tailed).

DISCUSSION

The main finding of our study is that the comprehension of familiar sentence-level metaphors with spatial terms is preserved in the presently studied patient, in whom stroke affected the right temporo-parietal region. This finding is consistent with the view that the left hemisphere may support comprehension of familiar sentence-level metaphors either by default or by compensation when the right hemisphere is injured.

ZG's performance pattern does not resemble the pattern of sentence-level metaphor comprehension in RH-damaged patients found by Winner & Gardner (1977), i.e. impairment in picture-metaphor matching. ZG's metaphor comprehension is also unlike the comprehension pattern of Van Lancker and Kempler's (13) RH-damaged patients, who could not comprehend familiar phrases. Finally, ZG's clinical profile is very similar to the profile of patient 444DX from the Ianni et al. (14) study: they both have RH damage, normal language, but compromised memory, executive function and visuo-spatial abilities. Yet, they exhibit different metaphor comprehension patterns: 444DX could not comprehend moderately familiar sentence-level metaphors and literal sentences, which indicates a general sentence level impairment. In contrast, ZG's comprehension of metaphoric sentences was good relative to the control group.

Furthermore, ZG comprehended well object-to-object spatial relations. Since there were only two objects per trial in this task, there was no "clutter" in the scene, a factor that is disruptive to spatial processing in patients with neglect (23). Although both neglect and hemianopia are common following RH damage, there are important differences between the two conditions. Briefly, hemianopia or visual field deficit is a sensory loss caused by damage to the primary visual pathways running between the optic tract and striate cortex, whereas neglect refers to inability to attend to contralesional space, due to cortical lesion (24, 25). Crucially, while the boundary between the intact and blind field in hemianopia is typically perceived as a "cliff", the visual loss in neglect is more gradual and the size of the neglected field appears to depend on the features of the scene (26).

An alternative explanation of ZG's comprehension of object-to-object spatial relations is grounded in recent fMRI findings from healthy adults, which point to a bilateral fronto-parietal network supporting both allocentric and egocentric frames of reference, with the former being more associated with activation in the right parietal lobe, in addition to the bilateral ventrolateral occipito-temporal cortex and the bilateral hippocampal formation (4). If the network of areas that supports allocentric representations is much wider than the right parietal region, functional compensation might have been mediated by these additional areas, resulting in ZG's good performance on this task. Thus, our data is aligned with the network approach to spatial processing, according to which lesions to only posterior parietal region of the right hemisphere cause mild spatial neglect that may not be apparent in spontaneous behavior (2). The network approach to complex functions such as directed attention, memory and

language offers a more plausible explanation of cognitive deficits caused by a stroke than the approach that seeks to determine one-to-one brain-function mapping. One reason is that stroke lesions are typically large, affecting more than one brain area and often spreading subcortically to deep grey matter and white matter.

Our findings are also compatible with a model according to which the right temporal lobe and basal ganglia injury contribute to chronic spatial neglect (27), because both regions were affected in ZG. The model further postulates damage to specific white matter (WM) tracts in relation to spatial neglect. In the absence of data on WM for the present study, we cannot speak of whether WM lesion contributed to ZG's condition, but the possibility remains that in addition to the cortical and subcortical grey matter lesions, white matter was also damaged. The most probable candidate tract would be the superior longitudinal fasciculus (SLF), more specifically (parietal portion of) segment II, which connects inferior parietal and prefrontal regions (28). Damage to this specific tract has been associated with spatial neglect (29). Furthermore, lack of cortical damage to the left prefrontal areas that support working memory cannot explain ZG's poor performance on spatial and verbal working memory tests. However, abnormal functional and/or structural connectivity within the fronto-parietal network is likely to contribute to these deficits, despite the cortical remoteness of intact prefrontal areas from the lesion areas. This explanation gains plausibility in the context of recent fMRI and repetitive transcranial magnetic stimulation findings that implicate Brodmann area 6—which is one of termination loci of SLF segment II—in updating verbal (medial BA 6) and spatial representations (lateral BA 6 in both hemispheres) (30). It appears then that possible damage to this tract could explain ZG's working memory problems, both spatial and semantic, which raises intriguing questions on lateralization of the neural substrates for verbal and spatial working memory.

One limitation of the present study is related to the fact that the patient did not undergo scanning immediately prior to his taking part in the present study and therefore we cannot fully exclude the possibility pointed out by an anonymous reviewer that other ischemic strokes might have taken place during the time preceding the actual study. However, the neurological follow-ups and patient's once-a-month visits to the general practitioner provide no evidence for further deterioration of his cognitive functions.

CONCLUDING REMARKS

In conclusion, we studied comprehension of spatial metaphors in a right-handed patient who had previously suffered a RH stroke in temporo-parietal region. We found that his comprehension of spatial metaphors was intact as well as his comprehension of spatial relations in the allocentric frame of reference. However, his spatial working memory was less preserved, even though it did not affect his performance on the type of spatial tasks used in the present study. This deficit may have a bigger impact on more complex spatial tasks, in which several spatial variables need to be stored and manipulated at the same time, or on visuo-spatial search of "cluttered" scenes that requires the ability to keep track of already searched points, which is difficult for patients with spatial neglect. Regardless, our study provides further evidence for the link between spatial working memory deficit and spatial neglect (23).

Finally, our data indicate that heterogeneity in behavioral output after RH stroke may be explained by the differences in the degree of damage to the network regions and their connections. Future studies will investigate how focal and diffuse lesions in the relevant networks contribute to dysfunction of spatial language, testing hypotheses on the role of spatial working memory in linking the domains of space and language.

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SUCCESSFUL TREATMENT OF CAPD PERITONITIS CAUSED BY MORAXELLA CATARRHALIS

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USPEŠNO LEČENJE MORAXELLA CATARRHALIS-OM PROUZROKOVANOG CAPD PERITONITISA

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ABSTRACT

Peritonitis remains a major complication of peritoneal dialysis which is usually caused by saprophytic gram positive microorganisms originated from skin. Here, I report an unusual case of peritonitis due to Moraxella catarrhalis.

A male, 59 age, on peritoneal dialysis modality because ESRD due to diabetic nephropathy was admitted to our hospital due to CAPD peritonitis. After initial empiric treatment and identification of this infrequent causer, he was submitted to two week antibiotic treatment with complete recovery and good prognosis.

Peritonitis is the major cause of peritoneal dialysis failure which requires prompt recognition of the causative agent for successful treatment.

Keywords: *peritoneal dialysis, peritonitis, diabetic nephropathy, treatment*

SAŽETAK

Peritonitis predstavlja najozbiljniju komplikaciju lečenja peritoneumskom dijalizom i obično je prouzrokovan gram pozitivnim komensalnim mikroorganizmima sa kože. U našem radu prikazujemo slučaj peritonitisa izazvan neuobičajenim uzročnikom Moraxella catarrhalis.

Muškarac star 59 godina, na programu lečenja peritoneumskom dijalizom zbog terminalne bubrežne slabosti izazvane dijabetesnom nefropatijom primljen je u našu bolnicu zbog CAPD peritonitisa. Nakon inicijalno sprovedene empirijske terapije i identifikacije ovog retkog uzročnika, naredne dve sedmice je sprovedeno ciljano lečenje prema antibiogramu sa kompletnim oporavkom pacijenta.

Peritonitis kao najozbiljnija komplikacija peritoneumske dijalize zahteva brzu i optimalnu identifikaciju uzročnika radi uspešnog lečenja.

Ključne reči: *peritoneumska dijaliza, peritonitis, dijabetesna nefropatija, lečenje*

INTRODUCTION

Peritoneal dialysis is ESRD treatment modality complementary with hemodialysis and kidney transplantation, based on simultaneous daily exchanges over peritoneal catheter (1). The main complication of these home dialysis modality is peritonitis. Based on the latest reports of International Society for Peritoneal Dialysis, death outcome is presented in less than 5% of total peritonitis episodes, but indirectly it is contributing factor for death outcome in 16% of peritoneal dialysis patients (2). Also, peritonitis is strongly associated with significant morbidity- transient loss of ultrafiltration, possible permanent membrane damage and transfer to hemodialysis (3-5). The most frequent causers are gram positive organisms originated from the skin (coagulase negative staphylococci) and less often gram negative organisms and fungi (6). Here, I report a case of peritonitis caused by Moraxella species. Until now, on the basis of relevant literature data, there were only 8 published cases of peritonitis due to this pathogen (7-13).

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CASE REPORT

A insulin depended diabetic male, 59 age, on peritoneal dialysis over 12 months due to end-stage renal disease, was admitted to our hospital because of strong abdominal pain followed by cloudy peritoneal fluid. His medical history excluded previous episodes of peritonitis and exit site infections. He conducts four daily exchanges with fill volume of 2000ml (PET test showed d/p creatinine 0.62 -low average transport status) - 3x1.36%, with night exchange with 2.27% of glucose. He uses conventional solutions (Dianeal®; Baxter), and he has satisfied parameters of dialysis adequacy -KT/V was 2.1, weekly creatinine clearance was 72.6 l/7 days; he also has preserved residual diuresis (approximately 1250-1500 ml/24hours).

Actually, initially analysis of blood sample showed C-reactive protein level 129 mg/dL, dialysis fluid showed 266 cell's elements and Gram stain of the fluid showed white blood cells. He was immediately started on empirical therapy -cefazolin and amikacin (our center specific initial treatment protocol for patients without residual renal function). After 48 hours, pains were reduced; effluent became purified with regression of fluid's WBC count (86 elements) when he discontinued aminoglycosides. Initial antibiotic susceptibility testing was unsuccessful due to the slow bacterial growth, but the culture was identified after 5 days as Moraxella catarrhalis *(*BacT/Alert). The organism was sensitive to ampicillin, amoxicillin/clavulanate, cefazolin, ceftazidime, ceftriaxone, erythromicin, trimethoprim/sulfamethoxazole and tetracycline, but it was resistant to vankomicin. Treatment with cefazolin was continued for 14 days which resulted in recovery of the patient's symptoms and complete healing of peritonitis (peritoneal fluid WBC count and culture became negative after 7 days, CRP level completely normalized after 10 days). We did not find the source of infection (nasal, sputum and exit site culture results were negative; US scan of the catheter's tunnel and X ray of lungs were correct). Repeated cultures of peritoneal fluid over the next 3 months have remained negative. Patient was continued on CAPD in good clinical condition.

DISCUSSION

Moraxella species are gram negative, aerobic catalase negative, oxidase-positive diploccocci which were first described in 1896. Human beings are exclusive hosts of this organism which is normally present in the oropharynx, mucous membranes, skin, and genital tract - almost 75% of children and 1-3% of healthy adults are carriers of the bacterium (14). These organisms usually cause respiratory tract infections but can also cause bacteraemia, meningitis, suppurative arthritis, osteomyelitis, endocarditis, keratitis, periorbital cellulitis and urethritis (15-20).

Until now, there were only 8 published cases of peritonitis due to this pathogen (7-13). Identification of these organisms requires culture on blood or chocolate agar plates and usually takes 24 to 48 hrs. Most strains (>90%) are susceptible to penicillin with exception of Moraxella catarrhalis which is susceptible to amoxicillin-clavulanate, expanded-spectrum or broad-spectrum cephalosporins, tetracyclines, rifampin and erythromycin (15-18, 21). Empirical choice of antibiotic therapy with cephalosporins which is recommended by the International Society of Peritoneal Dialysis guidelines/recommendations is sufficient and appropriate for the initial treatment of Moraxella related peritonitis (1). Further treatment should be continued with an appropriate antibiotic for 14 days, based on ISPD recommendations.

CONCLUSIONS

Peritonitis remains a most serious complication of peritoneal dialysis. Accurate and prompt identification of the causative organism, along with previous starting of appropriate empiric treatment, is necessary for positive outcome of this complication of peritoneal dialysis patients.

I have presented a case of infrequent Moraxella catarrhalis peritonitis and commented on the methods of diagnosis and appropriate treatment without catheter removal.

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Roll, W.P. (1976). ESP and memory. In J.M.O. Wheatley & H.L. Edge (Eds.), *Philosophical dimensions of parapsychology* (pp. 154-184). Springfield, IL: American Psychiatric Press.

d. Proceedings from a conference

Format:

Author(s). (Year of publication). Title. In Conference name, Date (Page range). Place of publication: Publisher.

Example:

Field, G. (2001). Rethinking reference rethought. In Revelling in Reference: Reference and Information Services Section Symposium, 12-14 October 2001 (pp. 59-64). Melbourne, Victoria, Australia: Australian Library and Information Association.

e. ebook

Format:

Author(s). (Year of publication). *Title*. Publisher. Retrieving date, http address. DOI.

Example:

Johnson, A. (2000). *Abstract Computing Machines*. Springer Berlin Heidelberg. Retrieved March 30, 2006, from SpringerLink http://springerlink.com/content/w25154. DOI: 10.1007/b138965.

f. Thesis

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Example:

Begg, M. M. (2001). *Dairy farm women in the Waikato 1946-1996: Fifty years of social and structural change*. Unpublished doctoral dissertation, University of Waikato, Hamilton, New Zealand.

g. Report

Format:

Author(s). (Year of publication). *Title*. Place of publication: Publisher. (Report number)

Example:

Osgood, D. W., & Wilson, J. K. (1990). *Covariation of adolescent health problems*. Lincoln: University of Nebraska. (NTIS No. PB 91-154 377/AS)

h. Government publication

Format:

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Example:

Ministerial Council on Drug Strategy. (1997). *The national drug strategy: Mapping the future*. Canberra: Australian Government Publishing Service.

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Both letters concerning and those not concerning the articles that have been published in Serbian Journal of Experimental and Clinical Research will be considered for publication. They may contain one table or figure and up to five references.

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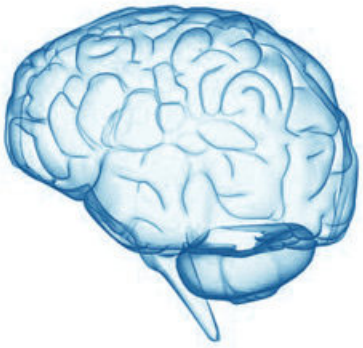
All manuscripts will be carefully revised by the publisher desk editor. Only in case of extensive corrections will the manuscript be returned to the authors for final approval. In order to speed up publication no proof will be sent to the authors, but will be read by the editor and the desk editor.



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