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## **POVEZANOST RS143383 MUTACIJE GDF5 GENA S RAZVOJEM OSTEOPOROZE**

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Osteoporozna je progresivna, sistemska metabolička bolest koju karakterizira smanjena masa koštanog tkiva, poremećena mikroarhitektura koštane strukture te povećanje lomljivosti kosti, što za posljedicu ima rizik od nastajanja fraktura. Posljedica ove bolesti su česti lomovi pojedinih kostiju, najčešće kompresivni prijelomi kralježnice, prijelom vrata bedrene kosti i distalne podlaktice. Dosadašnja istraživanja pokazala su da je osteoporozna kompleksna bolest koja je uz određene etiološke čimbenike posljedica i mutacije pojedinih gena. GDF5 gen kodira za protein istog imena. Dosada su mutacije GDF5 gena povezane sa slijedećim bolestima: akromezomelična displazija (Hunter-Thompson tip), bahidaktilija tipa C i hondrodisplazija (Grebe tip). Povezanost s ovim bolestima upućuje da je genski produkt GDF5 gena uključen u razvoj skeleta. GDF5 spada u porodicu koštanih morfogenetskih proteina koji su uključeni u formaciju hrskavice i kosti, a u njegovom nedostatku moguć je razvoj skraćenih kosti nogu te tvorba kosti slabije konzistencije i pojačane lomljivosti. Mutacija rs143383 je polimorfizam jedne baze (SNP) u kojem je zamijenjen citozin (C) s timinom (T) unutar 5'-UTR GDF5 gena. Ovaj polimorfizam se prvi put spominje u vezi s osteoartritisom kuka u japanskoj populaciji 2007. godine. Kasnija istraživanja pokazala istinitost povezanosti rs143383 mutacije s razvojem osteoartritisa kuka, koljena i kralježaka u populacijama bijelaca i azijata. Dosadašnja istraživanja pokazala su usku povezanost osteoartritisa s pojedinim genetskim mutacijama. Jedna od najpoznatijih je SNP rs143383 mutacija GDF5 gena. S obzirom da su osteoartritis i osteoporozna koštane bolesti sličnih kliničkih slika, molekularnih i patofizioloških osnova, logično je pretpostaviti da su određene genetske mutacije jednake u pozadini obiju bolesti. Stoga su postavljena slijedeće hipoteze i ciljevi ovoga rada: dokazati povezanost SNP rs143383 mutacije GDF5 gena s razvojem osteoporoze u slovenskoj populaciji, pronaći povezanost s promjenama BMD-a i

## **ASSOCIATION BETWEEN THE RS143383 MUTATION IN THE GENE GDF5 AND THE DEVELOPMENT OF OSTEOPOROSIS**

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Osteoporosis is a progressive, systemic metabolic disorder characterized by decreased bone mass, disturbed microarchitecture of the bones and increased bone brittleness, the consequence of which is a risk of fractures. The disease results in frequent fractures of certain bones, most often compressive spinal fractures, femoral neck and distal radius fractures. Research has shown that osteoporosis is a complex disease that is, along with specific etiologic factors, also due to mutations in certain genes. The GDF5 gene codes for a protein of the same name. So far, GDF5 mutations have been linked to the following diseases: acromesomelic dysplasia (Hunter-Thompson type), brachydactyly type C and chondrodysplasia (Grebe type). The association with these diseases suggests that the gene product of GDF5 is involved in skeletal development. GDF5 belongs to the family of bone morphogenetic proteins involved in the formation of cartilage and bone, and its deficiency may lead to the shortening of leg bones as well as formation of bones with reduced density and increased brittleness. The mutation rs143383 is a single nucleotide polymorphism (SNP) in which cytosine (C) is replaced by thymine (T) within the 5'-UTR of GDF5. This polymorphism was first mentioned in 2007 in relation to hip osteoarthritis in a Japanese population. Later investigations showed a true association between rs143383 and the development of hip, knee and spinal osteoarthritis in Caucasian and Asian populations. Studies completed thus far have revealed a close association between osteoarthritis and certain genetic mutations. One of the best known is the SNP rs143383 mutation in the GDF5 gene. Since osteoarthritis and osteoporosis are bone diseases with similar clinical pictures, molecular and pathophysiological bases, it seems logical to assume that the same specific genetic mutations are behind both diseases. Therefore, the following hypotheses have been proposed also as the aims of this paper: to prove the association of SNP rs143383 mutation in

biokemijskih biljega koštane pregradnje te potvrditi dosadašnje hipoteze o povezanosti SNP rs143383 mutacije s razvojem osteoartritisa. U tu svrhu je molekularnim tehnikama genotipizirano ukupno 900 uzoraka od kojih je za 750 unaprijed postavljena dijagnoza osteoporoze, a za 150 osteoartritisa. Potencijalnim dokazivanjem povezanosti SNP rs143383 mutacije GDF5 gena s razvojem osteoporoze, postavili bi se dokazi o novim patofiziološkim mehanizmima te bi se olakšalo razumijevanje molekularnih osnova same bolesti. Doprinijelo bi se novim spoznajama u vidu prevencije i liječenja osteoporoze te otvoreni novi aspekti u postavljanju hipoteza i daljnjem istraživanju osteoporoze.

*Ključne reči:* osteoporoza, mutacije GDF5, polimorfizam

GDF58 with osteoporosis development in a Slavic population, to establish a correlation with the changes in BMD and the biochemical markers of bone turnover, and to confirm the current hypothesis of an association between the SNP rs143383 mutation and development of osteoarthritis. To this end, using molecular techniques, we genotyped a total of 900 samples, of which in 750 a diagnosis of osteoporosis and in 150 of osteoarthritis had been previously established. Potential evidence of an association between the SNP rs143383 mutation in the GDF5 gene and the development of osteoporosis would provide data about new pathophysiological mechanisms and contribute to the understanding of the molecular basis of this disorder. Also, it would enable new insights valuable for the prevention and treatment of osteoporosis and open new aspects in proposing hypotheses and in further investigations of osteoporosis.

*Keywords:* osteoporosis, GDF5 mutation, polymorphism

## **ODREĐIVANJE ADIPONEKTINA, REZISTINA I IL-6 KOD PACIJENATA SA KORONARNOM ARTERIJSKOM BOLEŠĆU**

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Ateroskleroza je hronična inflamatorna bolest koja se karakteriše akumulacijom lipida i inflamatornih ćelija duž zidova arterija, i smatra se glavnim uzrokom koronarne arterijske bolesti (KAB). Rezistin je adipokin koji se primarno sekretuje iz monocita i makrofaga i poseduje značajna proinflamatorna svojstva. Proinflamatorni medijatori, kao što je IL-6 u in vitro uslovima dovode do povećanog lučenja rezistina i vice versa. Adiponektin je adipokin koji se primarno luči iz adipocita, poboljšava osetljivost na insulin, poseduje značajna antiaterogena i antiinflamatorna svojstva. Određivanje nivoa rezistina, adiponektina i IL-6 kod pacijenata sa KAB-om u cilju rasvetljavanja njihove uloge u razvoju bolesti. Adiponektin, rezistin i IL-6 su određivani u plazmi 78 pacijenata sa KAB (41 žena i 37 muškaraca), starosti od 32 do 82 godine, ELISA metodom. Pacijenti su na osnovu nalaza koronarografije razvrstani prema prisustvu (jednosudovna, dvosudovna, trosudovna KAB) i odustru stenozne. Dobijene vrednosti analizirane su neparametarskim Mann-Whitney testom i prikazane kao

## **ASSESSMENT OF ADIPONECTIN, RESISTIN AND IL-6 IN PATIENTS WITH CORONARY ARTERY DISEASE**

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Atherosclerosis is chronic inflammatory disease characterized by the accumulation of lipids and inflammatory cells along the inner walls of arteries. It is considered to be one of major causes of coronary artery disease (CAD). Resistin, adipokine primarily secreted by monocytes and macrophages, shows significant proinflammatory properties. Proinflammatory mediators, such as IL-6 lead to increased resistin secretion in vitro and vice versa. Adiponectin, adipocyte-derived protein, improves insulin sensitivity and shows important antiatherogenic and antiinflammatory activity. Measurement of resistin, adiponectin and IL-6 levels in patients with CAD in order to assess their involvement in disease development. Resistin, adiponectin and IL-6 concentrations were measured in plasma of 78 patients with CAD (41 female and 37 male), aged from 32 to 82 year, using ELISA method. Patients have been classified by presence (single, double or triple-vessel stenosis) or absence of coronary stenosis, based on coronaryography results. Results were analyzed using nonparametric Mann-

medijane sa interkvartilnim rasponima. Nivoi rezistina kod pacijenata bez stenozne [11,8(9,4–17,9) ng/mL] su bili značajno niži u odnosu na sve pacijente sa stenozom [15,3(12,3–21,1) ng/mL],  $p=0,018$  i u odnosu na pacijente sa trosudovnom KAB [16,4(15,7–23,6) ng/mL],  $p=0,011$ . Koncentracija adiponektina je bila značajno niža kod pacijenta sa trosudovnom stenozom [11,2(8,9–13,6) ng/mL] u odnosu na pacijente bez stenozne [15,9(10,9–23,3) ng/mL],  $p=0,042$ . Nivoi adiponektina su bili značajno niži kod pacijenata na terapiji statinima [12,2(8,8–20,4) ng/mL],  $p=0,025$  u odnosu na pacijente koji nisu uzimali terapiju [16,1(11,1–24,9) ng/mL]. Nivoi IL-6 se nisu značajno razlikovali između pacijenata sa [1,21(0,79–7,25) pg/mL] i bez stenozne [1,94(0,95–5,31) pg/mL]. Povećane koncentracije proinflatornog rezistina i snižene koncentracije vaskuloprotektivnog adiponektina kod pacijenta sa prisutnom stenozom ukazuju na njihovu moguću ulogu u razvoju ateroskleroze. Snižene vrednosti adiponektina kod pacijenata na terapiji statinima ukazuju na negativni plejotropni efekat ovih lekova. U ovom istraživanju nije dokazana direktna povezanost IL-6 i stepena razvoja bolesti.

*Ključne reči:* rezistin, adiponektin, IL-6, KAB

Whitney test and shown as median values with interquartile range. Resistin levels in patients without stenosis [11.8(9.4–17.9) ng/mL] were significantly lower compared with patients with stenosis [15.3(12.3–21.1) ng/mL],  $p=0,018$ , and compared with those with triple-vessel stenosis [16.4(15.7–23.6) ng/mL],  $p=0,011$ . Adiponectin levels were significantly lower in patients with triple-vessel stenosis [11.2(8.9–13.6) ng/mL] compared with patients without stenosis [15.9(10.9–23.3) ng/mL],  $p=0.042$ . Patients on statin therapy also had significantly lower adiponectin levels [12.2(8.8–20.4) ng/mL], compared with those without therapy [16.1(11.1–24.9) ng/mL]  $p=0,025$ . Difference in IL-6 levels was not significant between patients with [1,21(0.79–7.25) pg/mL] and without stenosis [1.94(0.95–5.31) pg/mL]. Increased concentrations of proinflammatory resistin and decreased concentrations of vasculo-protective adiponectin indicate their potential role in atherosclerosis development. Decreased adiponectin levels in patients on statin therapy suggest negative pleiotropic effect of these drugs. In this study direct correlation between IL-6 and degree of the disease has not been proven.

*Key words:* resistin, adiponectin, IL-6

## **POZIVANJE MEMORIJE U MORISOVOM VODENOM LAVIRINTU U MODELU LIPOPOLISAHARIDOM-IZAZVANOG NEURORAZVOJNOG OŠTEĆENJA KOD PACOVA**

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Prenatalno izlaganje mladunaca pacova lipopolisaharidu (LPS) i posledična inflamacija dovode do neurorazvojnih oštećenja koja su prepoznata kao mogući animalni model šizofrenije. U ovom radu sproveden je segment validacije LPS modela kroz test pozivanja memorije u Morisovom vodenom lavirintu (MVL). Mladunci pacova soja Wistar bili su prenatalno izloženi LPS-u ili fiziološkom rastvoru (FIZ) u 15. i 16. danu embriogeneze. Nakon rođenja mužjaci (M) su odvojeni od ženki (F) i formirano je osam grupa ( $n=11-12$ ) u zavisnosti od pola, intrauterinog tretmana i uzrasta u kome je bilo predviđeno da, u okviru MVL eksperimenta, uđu u test pozivanja memorije i

## **MEMORY RETRIEVAL IN MORRIS WATER MAZE IN THE RAT MODEL OF LIPOPOLYSACCHARIDE-INDUCED NEURODEVELOPMENTAL IMPAIRMENT**

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Prenatal exposure to lipopolysaccharide (LPS) and consequential inflammation lead to neurodevelopmental impairment in offspring rats that has been recognized as a putative animal schizophrenia model. This study was conducted as a segment of LPS model validation using memory retrieval test (probe trial) in Morris water maze (MWM). Offspring Wistar rats were exposed to LPS or saline (SAL) at prenatal days 15 and 16. After birth, males (M) and females (F) were separated, and eight groups ( $n=11-12$ ) were formed with respect to the sex, intrauterine treatment and the planned age for memory retrieval testing, at postnatal days 40 and 60,

to 40. odnosno 60. dana postnatalno (P40, P60). Tokom pet dana eksperimenta životinje su učile da u MVL-u pronađu platformu ispod površine vode. Test pozivanja memorije je izvođen šestog dana, bez platforme i analizirani su parametri deklarativne memorije: efikasnost putanje i latencija do ulaska u ciljnu zonu, kao i procenat tigmotaksičnog kretanja kao parametar proceduralne memorije. Kod P40 životinja nije bilo značajnih razlika u praćenim parametrima. Studentov T- test je pokazao da su pacovi u grupi M/LPS/P60 imali statistički značajno manju efikasnost putanje do ulaska u ciljnu zonu ( $r=0,015$ ) kao i veću latenciju do ulaska u ciljnu zonu ( $r=0,002$ ) u odnosu na grupu M/FIZ/P60, dok u datim parametrima među grupama F/LPS/P60 i F/FIZ/P60 nije bilo statističke razlike. Trend povećanja procenta vremena provedenog u tigmotaksičnoj zoni pokazan je pri poređenju F/LPS/P60 sa F/FIZ/P60 ( $p=0,068$ ), ali ne i pri poređenju M/LPS/P60 sa M/FIZ/P60. Dobijeni rezultati ukazuju na to da se neurorazvojna oštećenja u LPS modelu mogu manifestovati kognitivnim deficitima u periodu kasne adolescencije (P60). Može se postaviti hipoteza da su mladunci pacova muškog roda podložniji LPS-indukovanim inflamatornim procesima u centralnom nervnom sistemu, što je moglo da rezultuje zapaženim deficitom memorije.

*Gljučne reči:* lipopolisaharid, šizofrenija, neurorazvojni model, MVL, pacov

respectively (P40, P60). During five days of Morris water maze acquisition, animals have been learning to find a platform, hidden beneath water surface. The probe trial without platform was performed on the sixth day. The analysis used included the declarative memory parameters: path efficiency to the target zone and latency to the target zone, as well as percentage of thigmotaxis locomotion, as a procedural memory parameter. P40 animals did not differ in any of analyzed parameters. The Student's t test showed that M/LPS/P60 animals had significantly decreased path efficiency to the target zone ( $p=0.015$ ), and prolonged latency to the target zone ( $p=0.002$ ), compared to M/SAL/P60 group, while for these parameters F/LPS/P60 and F/SAL/P60 groups showed no statistical difference. A trend of increase in the percentage of time spent in thigmotaxis area was shown for F/LPS/P60 in comparison to F/SAL/P60 group ( $p=0.068$ ), but not when comparing male groups. The results demonstrate that neurodevelopmental impairment in the LPS model can be manifested through cognitive deficits during the late adolescence (P60). We hypothesize that rat male pups are more susceptible to the LPS-induced inflammatory processes in central nervous system, which may have resulted in the observed memory deficit.

*Keywords:* lipopolysaccharide, schizophrenia, neurodevelopmental model, MWM, rat

## **GENOTIPIZACIJA POLIMORFIZAMA U GENU OCT1 I NJIHOV ODNOS SA TRETMANOM IMATINIBOM KOD OBOLELIH OD HRONIČNE MIJELOIDNE LEUKEMIJE**

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Imatinib je inhibitor tirozinske kinaze koji se koristi za lečenje hronične mijeloidne leukemije (HML) (1). Nedavno objavljeni podaci u literaturi pokazuju da efikasnost lečenja imatinibom možda zavisi od aktivnosti prenosioca leka, transportera organskih katjona 1 (OCT1) (2). Cilj naše studije bio je da se istraži povezanost efikasnosti lečenja imatinibom sa pet odabranih genskih polimorfizama i nivoom genske ekspresije OCT1 gena SLC22A1 kod obolelih od HML. Naša teza bila je da odabrani polimorfizmi u genu za OCT1 utiču na aktivnost prenosioca imatiniba što ima za posledicu slabiji odgovor na lečenje kod bolesnika sa varijantnim alelima u poređenju sa subjektima bez mutacija. Takođe smo želeli da utvrdimo da li su za postizanje zna-

## **GENOTYPING POLYMORPHISMS IN OCT1 GENE AND THEIR RELATION TO TREATMENT WITH IMATINIB IN CML PATIENTS**

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Imatinib is a tyrosine kinase inhibitor used for treatment of chronic myeloid leukemia (CML) (1). The recently published literature data shows that treatment efficacy of imatinib could depend on activity of drug transporter, organic cation transporter 1 (OCT1) (2). The aim of our study was to research the association of imatinib treatment efficacy with five selected gene polymorphisms and the level of gene expression of OCT1 gene SLC22A1 in CML patients. We postulated that the selected polymorphisms in the gene for OCT1 affect the imatinib transporter activity and result in poorer response to treatment in patients with variant alleles compared to wild type individuals. We also wanted to determine, if higher doses of imatinib are needed to achieve the

čajnog molekularnog odgovora kod pacijenata sa mutacijama u genu OCT1 potrebne veće doze imatiniba. Genotipizacija je izvršena za 5 polimorfizama u genu OCT1: rs 12208357, rs72552763, rs34130495, rs628031 i rs683369 korišćenjem tehnologije TaqMan. Aktivnosti prenosioca imatiniba u granulocitima i mononuklearnim ćelijama periferne krvi nisu se razlikovale između genotipova ni u jednom slučaju testiranih polimorfizama. Pored toga, na osnovu genotipova OCT1 za testirane polimorfizme, kod bolesnika nisu otkrivene značajne razlike u postizanju odnosno nedovoljnom postizanju molekularnog odgovora tokom 18 meseci. Međutim, bolesnicima sa dva varijanta alela za polimorfizam rs683369 bile su potrebne značajno veće doze imatiniba da bi se postigao značajan molekularni odgovor u poređenju s heterozigotima i subjektima bez mutacija. Prema našim rezultatima, razlog za to nije uticaj na aktivnost prenosioca imatiniba. Možemo zaključiti da nismo pronašli nikakvu vezu između polimorfizma u genu OCT1 i aktivnosti prenosioca imatiniba ili postizanja značajnog molekularnog odgovora. U budućim studijama trebalo bi istražiti moguću povezanost između rs683369 i doze imatiniba.

*Ključne reči:* genotipizacija polimorfizma, imatinib, hronična mijeloidna leukemija

major molecular response in patients with mutations in the OCT1 gene. Genotyping was performed for 5 polymorphisms in the OCT1 gene rs12208357, rs72552763, rs34130495, rs628031 and rs683369 using TaqMan genotyping technology. Activity of imatinib transporter in granulocytes and peripheral blood mononuclear cells did not differ between genotypes for any of the tested polymorphisms. In addition, no significant differences in achievement / underachievement of molecular response within 18 months were discovered between patients according to OCT1 genotypes for the tested polymorphisms. However, patients with two variant alleles for rs683369 polymorphism required significantly higher doses of imatinib for reaching major molecular response compared to heterozygous and wild-type individuals. According to our results, this is not due to influence on imatinib transporter activity. In conclusion we did not find any association of polymorphism in the OCT1 gene with imatinib transporter activity or achievement of major molecular response. The possible association of rs683369 with imatinib dose should be investigated in future studies.

*Keywords:* genotyping, polymorphisms, Imatinib, chronic myeloid leukemia

## **UČESTALOST TRADICIONALNIH I NOVIH KARDIOVASKULARNIH FAKTORA RIZIKA U RAZLIČITIM PATOFIZIOLOŠKIM STANJIMA POVEZANIM SA ATEROSKLEROZOM**

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Ishemijske bolesti mozga i srca predstavljaju vodeći uzrok smrtnosti u svetu. U njihovoj osnovi leži razvoj ateroskleroze, pa je primarna prevencija orijentisana na identifikaciju faktora značajnih za rano prepoznavanje i procenu rizika za razvoj bolesti. Pored tradicionalnih faktora rizika (starost, pol, gojaznost, pušenje, hipertenzija, hiperlipidemija), uvedeni su i novi parametri kao što su veličina i raspodela subfrakcije lipoproteina niske gustine (engl. Low density lipoprotein, LDL) i lipoproteina visoke gustine (engl. High density lipoprotein, HDL). Ispitati učestalost tradicionalnih i novih lipidnih i nelipidnih parametara za razvoj ateroskleroze kod pacijenata sa ishemijskim bolestima srca i mozga i proceniti njihovu dijagnostičku tačnost. U istraživanju je učestvovalo 168 paci-

## **PREVALENCE OF TRADITIONAL AND NOVEL CARDIOVASCULAR RISK FACTORS IN ATHEROSCLEROSIS – RELATED DISEASES**

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Ischemic heart and brain diseases are the main causes of mortality worldwide. Since development of atherosclerosis is a common basis for these conditions, primary prevention is oriented towards identification of risk factors which are useful in disease prediction and early recognition. Beside traditional risk factors (age, gender, obesity, smoking, hypertension, hyperlipidemia), new parameters, such as size and distribution of low-density lipoprotein (LDL) and high-density lipoproteins (HDL) subfractions, are investigated. To determine the prevalence of traditional and new lipid and non-lipid cardiovascular risk factors in patients with ischemic heart and brain diseases and to estimate their clinical accuracy. The study involved 168 patients with coronary artery dis-



jenata sa angiografski dokazanom koronarnom bolešću, 167 pacijenata sa akutnim ishemijskim moždanim udarom i 174 zdrava ispitanika. Svim ispitanicima određene su antropometrijske karakteristike, lipidni profil i raspodela lipoproteinskih subfrakcija metodom elektroforeze na gradijentu poliakrilamida. Dijagnostička tačnost je ispitivana metodom »Receiver Operating Characteristic (ROC)« krive. Pacijenti sa kardiovaskularnim bolestima (KVB) imaju značajno više vrednosti indeksa telesne mase i koncentracije triglicerida (engl. TG), a statistički značajno manje koncentracije HDL-holesterola, dijametar LDL i dijametar HDL čestica u odnosu na kontrolnu grupu. Pacijenti sa cerebrovaskularnim bolestima (CVB) imaju značajno više koncentracije TG i koncentracije LDL-holesterola, a manje koncentracije HDL-holesterola, dijametar LDL čestica, te veći udeo malih HDL i LDL subfrakcija od zdravih ispitanika. Učestalost tradicionalnih faktora rizika je i kod KVB i kod CVB pacijenata veća u odnosu na zdrave ispitanike. ROC analizom smo utvrdili da se dodatkom dijametra LDL i HDL čestica dijagnostička tačnost tradicionalnih faktora rizika značajno poboljšala (površina ispod ROC krive=0,900,  $p < 0,05$ ). Učestalost tradicionalnih lipidnih i nelipidnih faktora rizika kod KVB i kod CVB pacijenata je veća u odnosu na zdrave ispitanike. Određivanje LDL i HDL subfrakcija doprinosi dijagnostičkoj tačnosti.

*Cljučne reči:* LDL čestice, HDL čestice, kardiovaskularne bolesti, cerebrovaskularne bolesti

ease (CAD), 167 patients with acute ischemic stroke (AIS) and 174 healthy participants. Lipoprotein subfractions were assessed by polyacrylamide gradient gel electrophoresis. Clinical accuracy was tested with Receiver Operating Characteristic (ROC) curve. CAD patients have significantly higher values of body mass index and concentrations of triglyceride (TG), but significantly lower concentrations of HDL-cholesterol, and diameters of LDL particle and HDL particles compared to the controls. AIS patients have significantly higher concentrations of TG and LDL-cholesterol, but lower HDL-cholesterol and LDL particle size and a larger proportion of small HDL and LDL subfractions than healthy subjects. Prevalence of traditional risk factors is higher in CAD and AIS patients than in healthy subjects. ROC analysis showed that by adding the diameter of LDL and HDL particles clinical accuracy of traditional risk factors is significantly improved (area under the ROC curve = 0.900,  $p < 0.05$ ). Prevalence of traditional lipid and non-lipid risk factors is higher in patients with ischemic heart and brain diseases than in healthy subjects. The determination of LDL and HDL subfractions improves clinical accuracy.

*Keywords:* LDL particles, HDL particles, cardiovascular disease, cerebrovascular disease.

## **POVEZANOST SALIVARNIH BIOKEMIJSKIH BILJEGA STRESA S PSIHOLOŠKIM POKAZATELJIMA I STUPNJEM TJELESNE AKTIVNOSTI U STUDENSKOJ POPULACIJI**

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Slina kao uzorak je pogodna zbog jednostavnosti uzorkovanja, a u njoj se analiti nalaze u slobodnom tj. aktivnom obliku. Stres je stanje u kojem je poremećena psihofiziološka ravnoteža organizma. Aktivira osovinu hipotalamus–hipofiza-kora nadbubrežne žlijezde pa je salivarni kortizol (sCORT) indikator slobodnog kortizola, dobro korelira sa serumskom vrijednošću i odražava slobodnu frakciju koja je biološki aktivna. Istovremeno, stresna stanja stimuliraju simpatički neuronski sustav što uzrokuje promjenu sekrecije salivarne  $\alpha$ -amilaze (sAA) iz sali-

## **CORRELATION BETWEEN SALIVARY BIOCHEMICAL MARKERS OF STRESS AND PSYCHOLOGICAL INDICATORS OF PHYSICAL ACTIVITY LEVELS IN STUDENT POPULATION**

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Saliva as a sample is suitable for easy sampling and analytes are found in free, active form. Stress is a condition in which is disturbed inner (psychophysiological) balance of the organism. Activates the hypothalamic – pituitary – adrenal cortex and salivary cortisol (sCORT) is an indicator of free cortisol, correlates well with serum free value and the reflected fraction is biologically active. At the same time, stressful situation stimulate the sympathetic neural system which causes a change in the secretion of salivary alpha amylase from salivary gland (sAA). The hypothesis of

varnih žlijezda. Hipoteza ovog istraživanja bila je da su studenti sa svakodnevnom intenzivnom fizičkom aktivnošću izloženi kroničnom stresu u odnosu na druge studente koji imaju minimalne fizičke aktivnosti. Svi ispitanici najprije su ispunili psihološki test, a potom su pristupiti davanju uzorka silne za određivanje koncentracija sCORT i sAA. Utvrđena je umjerena negativna povezanost između koncentracija sCORT i sAA kod svih studenata neovisno o fizičkoj aktivnosti, ali nije dokazana statistička značajnost. Najbolja povezanost koncentracije sCORT i sAA i najčvršća statistička značajnost pokazala se u skupini studenata s umjerenom fizičkom aktivnosti. Aktivnije i pozitivnije suočavanje sa stresnim stanjima zapaženo je kod studenata kineziologije na temelju psihološkog testiranja. Usporedbom biokemijskih i psiholoških pokazatelja u odnosu na stres zaključujemo da su dvije skupine studenata izjednačene unatoč bitno različitim fizičkim aktivnostima.

*Ključne riječi:* slina, stres, kortizol, amilaza, psihološko testiranje

this study was that students with intensive daily physical activity are exposed to chronic stress compared to other students who have a minimum of physical activity. All participants first filled out a psychological test, and then gave saliva samples to determine the concentration of sCORT and sAA. There was a weak to moderate negative correlation between the concentration of salivary cortisol and sAA in all students, but not statistically significant. The best correlation between cortisol and sAA and the strongest statistical significance showed in a group of students who have moderate physical activity. There was no statistically significant correlation between biochemical indicators of stress and results obtained using psychological testing. Actively and positively coping with stressful situations is observed in physical education students on the basis of psychological testing, coping with stressful situations. On a comparison of biochemical and physiological parameters in relation to stress, we conclude that the two groups of students are equal despite the very different physical activities.

*Keywords:* saliva, stress, amilase, cortisol, psychological testing

## **BIOLOŠKI UČINCI METIL-TRANSFERAZE RMTc U BAKTERIJSKIM STANICAMA ESCHERICHIA COLI DH5 $\alpha$**

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Pojava otpornosti na antibiotike jedan je od najvećih problema u liječenju bolesti uzrokovanih bakterijama zbog smanjenja broja djelotvornih lijekova. Jedan od mehanizama otpornosti je modifikacija ciljnog mjesta djelovanja lijeka. Enzimi metil-transferaze metiliraju 16S rRNA ribosoma te onemogućuju vezanje i djelovanje aminoglikozidnih antibiotika. Taj mehanizam uobičajen je za sojeve bakterija koji su prirodni proizvođači antibiotika, ali se ubrzano širi i kod patogenih bakterijskih sojeva otpornih na antibiotike. Cilj ovog rada bio je ispitati biološki učinak metil-transferaze RmtC iz porodice Arm u stanicama *E. coli* DH5 $\alpha$ . U tu svrhu provedeni su testovi kojima su ispitane karakteristike rasta stanica s enzimom u odnosu na stanice bez enzima usporedbom generacijskih vremena i provedbom testa konkurencije rasta stanica.  $\beta$ -galaktozidaznim testom ispitan je utjecaj

## **BIOLOGICAL EFFECTS OF METHYLTRANSFERASE RMTc IN ESCHERICHIA COLI DH5 $\alpha$**

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Appearance of antimicrobial resistance is one of the greatest issues in treatment of diseases caused by bacteria due to reduction in number of effective antibiotics. One mechanism of resistance is modification of target sites of action of the drug. Enzymes methyltransferases perform methylation of 16S rRNA in ribosomes and thereby inhibit binding and action of aminoglycoside antibiotics. This mechanism is common for bacterial strains that are natural producers of antibiotics, but it is spreading rapidly in pathogenic strains resistant to antibiotics. The aim of this work was to examine biological effects of methyltransferase RmtC from Arm family in *E. coli* DH5 $\alpha$ . To examine characteristic of growth of *E. coli* cells expressing RmtC in relation to cells without enzyme, determination and comparison of generation time and growth competition assay were done. The effect



enzima na točnost dekodiranja mRNA tijekom sinteze proteina. Rezultati su pokazali da stanice *E. coli* DH5 $\alpha$  koje eksprimiraju metil-transferazu RmtC rastu sporije od stanica bez enzima. U uvjetima u kojima su se morale natjecati za hranjive tvari i životni prostor, stanice s enzimom su pokazale da mogu konkurirati stanicama bez enzima nakon početnog perioda prilagodbe. Prisutnost metil-transferaze RmtC uzrokuje učestalije pogreške prilikom dekodiranja na A- i P-mjestu ribosoma tijekom translacije. Proučavanje mehanizama otpornosti od velike je važnosti u borbi protiv širenja otpornosti na aminoglikozidne antibiotike i pronalasku novih načina liječenja bolesti uzrokovanim otpornim sojevima bakterija. Dobiveni rezultati mogu poslužiti kao podloga za daljnja istraživanja, iako predstavljaju tek malen korak na putu za ostvarenje ovoga cilja.

*Cljučne riječi:* aminoglikozidni antibiotici, otpornost, metil transferaza RmtC

of enzyme to accuracy of decoding mRNA during proces of protein translation was examined using  $\beta$ -galactosidase assay. Results showed that bacteria *E. coli* DH5 $\alpha$  expressing methyltransferase RmtC grow slower than cells without the enzyme. In conditions when they had to compete for nutrients and living space, cells with enzyme showed that they can compete with cells without enzyme but after initial adjustment period. The presence of methyltransferase RmtC causes more frequent errors in decoding on P- and A-site during translation. The results of this work can serve as a basis for further research, which could help to find new opportunities in combating antibiotic resistance based on ribosomal RNA methyltransferases.

*Keywords:* aminoglycoside antibiotics, resistance, methyltransferase RmtC

## BIOHEMIJSKI MARKERI POREMEĆAJA OKSIDATIVNO- STRESNOG STATUSA KOD DECE KOJA ŽIVE U PETROHEMIJSKOJ INDUSTRIJSKOJ SREDINI

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Negativan efekat zagađenog vazduha životne sredine na krvne sudove smatra se važnim uzročnikom nastanka kardiovaskularnih bolesti. U posebno osetljive grupe spadaju gerijatrijska i pedijatrijska populacija. Studija je sprovedena sa ciljem da bi se ispitalo da li postoji uticaj zagađenog vazduha životne sredine na markere oksidativnog stresa (OS) i antioksidativne zaštite (AOZ) kao faktore rizika za razvoj kardiovaskularnih oboljenja. Markeri OS statusa, lipidnog statusa, koncentracija glukoze i broj leukocita su određivani kod 40 zdrave, školske dece. Jednu grupu je činilo 20 dece (10 devojčica i 10 dečaka) uzrasta od 12 do 13 godina iz Pančeva (najveće industrijske zone u Srbiji). Drugu grupu je činilo preostalih 20 dece oba pola podjednako zastupljenih, uzrasta od 13 do 15 godina, iz Kovačice (varošice

## BIOCHEMICAL MARKERS OF OXIDATIVE STRESS STATUS IN CHILDREN LIVING IN PETROCHEMICAL INDUSTRIAL AREA

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Negative effect of environmental air pollution on blood vessels is considered to be an important cause of cardiovascular diseases. Geriatric and pediatric populations are particularly vulnerable groups. This study was conducted in order to determine possible influence of environmental air pollution on oxidative stress (OS) and antioxidative defense (AOD) status markers as risk factors for cardiovascular diseases development. OS and lipid status markers, glucose concentration and leukocytes count were measured in the group of 40 healthy schoolchildren. One group of 20 children (10 girls and 10 boys), who were 12 to 13 years old, were from Pančevo (the biggest industrial area in Serbia). The second group consisted of the rest of 20 children both gender equally presented who were 13 to 15 years old from

udaljenoj 30 kilometara severno od Pančeva). Odabrane grupe dece su bile homogene, tj. nisu postojale statistički značajne razlike u ispitivanim markerima između dečaka i devojčica u okviru svake grupe. Deca iz Kovačice su imala značajno veći indeks telesne mase (ITM) i bila su starija od dece iz Pančeva. Statistički značajna razlika nije postojala u vrednostima biohemijskih i lipidnih markera između grupa. Povećan nivo OS prikazan kroz povećane koncentracije tiobarbituratna kiselina-reagujućih supstanci (TBKRS) ( $p < 0,001$ ) utvrđen je kod dece koja su živela u industrijskoj u odnosu na decu koja su živela u ruralnoj sredini. Takođe, smanjen nivo AOZ kao posledica smanjene aktivnosti superoksid-dismutaze (SOD) ( $p < 0,01$ ) je pokazan kod dece koja su živela u industrijskoj sredini. Razlike u koncentraciji TBKRS i aktivnostima SOD su zadržale statističku značajnost i nakon analize kovarijanse za ITM i uzrast. Povećan nivo OS pokazan je kod dece koja su odrastala u industrijskoj sredini. Dugotrajno izlaganje povećanim koncentracijama zagađivača u vazduhu predstavlja potencijalan faktor rizika po zdravlje dece.

*Cljučne reči:* oksidativni stres, antioksidativna zaštita, SOD, TBKRS, zagađenje vazduha

Kovačica (village located 30 km north of Pančevo). Groups of children were homogeneous; no significant differences were determined in tested markers between boys and girl within each group. Children from Kovačica had statistically significant higher body mass index (BMI) and they were older than children from Pančevo. No statistically significant differences were found in biochemical and lipid status markers between groups. High level of OS demonstrated as significantly higher concentration of tiobarbituric acid-reacting substances (TBARS) ( $p < 0.001$ ) was found in children from industrial environment comparing to children from rural environment. Low level of AOD demonstrated by lower superoxide dismutase (SOD) activity ( $p < 0.01$ ) was observed in group of children from industrial area. Differences in TBARS level and SOD activity remained statistically significant after analysis of covariance for BMI and age. Increased level of OS was found in children who lived in an industrial area. Long-term exposition to increased air pollutants concentrations presents a potential risk factor for children's health.

*Keywords:* oxidative stress, antioxidative defense, TBARS, SOD, air pollution

## ZNAČAJ ODREĐIVANJA ADIPONEKTINA I REZISTINA KOD PACIJENATA NA HEMODIJALIZI

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Hronična bubrežna insuficijencija (HBI) je sindrom koji nastaje kao posledica progresivnog, ireverzibilnog smanjenja glomerularne filtracije do konačnog stadijuma uremije. Bolesnici sa HBI imaju znatno veći rizik od kardiovaskularne smrti nego što je to slučaj sa opštom populacijom. Adiponektin je multifunkcionalni protein adipocita sa antiaterogenom i antiinflamatornom aktivnošću. Rezistin, kod ljudi pronađen u ćelijama bele loze, uključen je u patološke procese poput inflamacije, endotelne disfunkcije i angiogeneze. Određivanje adiponektina i rezistina u plazmi kod pacijenata na hemodijalizi (DP) i zdravih ispitanika u cilju razmatranja povezanosti ovih parametara sa HBI. Adiponektin i rezistin su određivani u uzorcima plazme kod ukupno 67 ispitanika od kojih su 33 DP (14 žena, 19 muškaraca) i 34 zdrava ispitanika (18 žena, 16 muškaraca), starosti 27–76 godina, ELISA metodom. Dobijene vred-

## THE SIGNIFICANCE OF ADIPONECTIN AND RESISTIN IN PATIENTS ON HEMODIALYSIS

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Chronic kidney disease (CKD) is a syndrome that occurs as a result of progressive, irreversible decrease in the glomerular filtration rate until the final stage, uremia. The patients with CKD have a significantly higher risk of cardiovascular death than general population. Adiponectin is a multifunctional adipocyte protein with antiatherogenic and anti-inflammatory properties. Resistin, in humans found in white blood cells, is involved in pathological processes such as inflammation, endothelial dysfunction and angiogenesis. Determination of adiponectin and resistin concentrations in patients on dialysis (PD) and healthy subjects in order to access the relationship of these parameters with CKD. Adiponectin and resistin were measured in plasma of 67 patients, 33 PD (14 women, 19 men) and 34 healthy subjects (18 women, 16 men), aged 27–76 years, using ELISA method. The obtained data were analyzed by

nosti su analizirane studentovim t-testom i prikazane kao srednje vrednosti sa standardnim devijacijama. Nivoi rezistina i adiponektina su bili značajno viši kod DP ( $60,1 \pm 12,6$  ng/mL,  $16,0 \pm 8,4$  ng/mL, respektivno) u odnosu na zdrave ispitanike ( $17,4 \pm 5,8$  ng/mL,  $p < 0,001$ ;  $12,0 \pm 7,4$  ng/mL,  $p = 0,041$ ). DP su podeljeni u dve grupe prema vrednostima HDL holesterola (HDL-h) (granična vrednost: CHDL-h =  $0,91$  mmol/L). Pacijenti sa većim vrednostima HDL-h su imali značajno viši nivo adiponektina u odnosu na DP sa nižim vrednostima HDL-h ( $p = 0,028$ ). Kod DP pokazana je korelacija adiponektina sa HDL-h ( $r = 0,618$ ,  $p < 0,001$ ), trigliceridima ( $r = -0,457$ ,  $p = 0,007$ ) i serumskim kreatininom ( $r = -0,368$ ,  $p = 0,035$ ), a višestrukom regresionom analizom pokazano je da je HDL-h jedini nezavisni prediktor adiponektina ( $\beta = 0,618$ ,  $\text{adj}R^2 = 0,361$ ,  $p < 0,001$ ). Razlike u nivoima rezistina i adiponektina u odnosu na zdrave ispitanike ukazuju na značaj koji ovi adipokini mogu imati u razvoju HBI. Pozitivna korelacija adiponektina sa HDL-h i veće vrednosti adiponektina kod pacijenata sa većim HDL-h ukazuju na njegov potencijalno zaštitni efekat kod pacijenata na hemodijalizi.

*Ključne reči:* adiponektin, rezistin, hemodijaliza

Student's t-test and presented as means with standard deviations. Adiponectin and resistin levels were significantly higher in PD ( $60.1 \pm 12.6$  ng/mL,  $16.0 \pm 8.4$  ng/mL, respectively) compared with healthy subjects ( $17.4 \pm 5.8$  ng/mL,  $p < 0.001$ ;  $12.0 \pm 7.4$  ng/mL,  $p = 0.041$ ). PD were divided into two groups according to the values of HDL-cholesterol (HDL-c) (cut-off value: CHDL-C =  $0.91$  mmol/L). Patients with higher HDL-c had a significantly higher level of adiponectin compared with DP with lower HDL-c ( $p = 0.028$ ). In PD group adiponectin correlated with HDL-c ( $r = 0.618$ ,  $p < 0.001$ ), triglycerides ( $r = -0.457$ ,  $p = 0.007$ ) and serum creatinine ( $r = -0.368$ ,  $p = 0.035$ ), and multiple regression analysis showed that HDL-c is the only independent predictor of adiponectin ( $\beta = 0.618$ ,  $\text{adj}R^2 = 0.361$ ,  $p < 0.001$ ). Different levels of resistin and adiponectin found in PD compared to healthy subjects suggest the importance of these adipokines in development of CKD. The positive correlation of adiponectin with HDL-c, and higher values of adiponectin in patients with higher HDL-c indicates potential protective effect of adiponectin in patients on hemodialysis.

*Keywords:* adiponectin, resistin, hemodialysis

# Poster Sessions

**SP001**  
**UPOTREBA QSAR STUDIJA U**  
**DIZAJNIRANJU NOVIH DVOSTRUKIH**  
**INHIBITORA PI3K/MTOR KINAZE**

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PI3K/mTOR kinaze spadaju u porodicu PIKK kinaza (kinaze povezane sa fosfatidilinositol-3-kinazom). Signalni put PI3K/mTOR kinaze igra veoma važnu ulogu u ćelijskom rastu, preživljavanju i regulaciji proliferacije. Uočeno je da pokazuju znatnu homologiju u strukturi svojih aktivnih mesta i izvesno bi mogle postati potencijalne mete za nove antineoplastike. Kako bi se popravila in vitro biološka aktivnost zajedno sa fizičko-hemijskim karakteristikama i odredile farmakokinetičke karakteristike struktura izabranih iz uzorka od 120 molekula, na osnovu pozitivnih interakcija na enzimima PI3K/mTOR. Cilj je razviti moćnije 3D-strukture farmakofora za dvostruke inhibitore PI3K/mTOR kinaze pomoću kompjuterski pomognutog dizajna lekova. Prethodno formirani 3D-QSAR modeli koriste se za predviđanje aktivnosti novih, optimizovanih struktura, MarvinSketch, ChemOffice paket i Gaussian 98W bili su programi za crtanje i optimizovanje molekula a za evaluaciju farmakokinetičkih parametara izabranog jedinjenja postojao je ADME/tox. Birana su i modifikovana jedinjenja sa odgovarajućim pIC50 vrednostima za PI3K i mTOR kako bi se intenzivirale pozitivne interakcije. Pravilnim menjanjem hemijskih struktura u okviru supstituenata, uz poštovanje strategija u dizajnu lekova, formiran je skup molekula sa sličnim ili čak povišenim aktivnostima. Pojačavanje aktivnosti bilo je prioritet za mTOR kinaze; dok su aktivnosti za PI3K kinaze podešavane da budu stalne ili povišene. Upotreba QSAR studija u dizajniranju novih dvostrukih inhibitora PI3K/mTOR kinaze pokazale se korisnom za analizu karakteristika i popravljavanje bioloških aktivnosti jedinjenja. Ovo se može činiti kao dobar osnov za odabir potencijalnih kandidata za sintezu i njihovu analizu u budućim istraživanjima.

*Ključne reči:* 3D-QSAR, PI3K/mTOR kinaze, dvostruki inhibitori, dizajn lekova

**SP001**  
**THE USAGE OF QSAR STUDIES IN**  
**THE DESIGN OF NEW DUAL**  
**PI3K/MTOR KINASE INHIBITORS**

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PI3K/mTOR kinases belong to the PIKK (phosphatidylinositol-3-kinase-related kinase) family of kinases. PI3K/mTOR kinase signaling pathway plays a very important role in cell growth, survival and proliferation regulation. It has been noticed that they contain a considerable homology in the structure of their active sites and there is a certain possibility for them to become potential target places for new anti-neoplastics. To enhance in vitro biological activity altogether with physicochemical characteristics and to determine the pharmacokinetic characteristics of structures chosen from a sample of 120 molecules, based on positive interactions on PI3K/mTOR enzymes. The goal is to develop more potent 3D-structures of pharm cophores for dual PI3K/mTOR kinase inhibitors with the help of computer-aided drug design. Previously formed 3D-QSAR models have been used to predict the activities of new, optimized structures (Pentacle), MarvinSketch, ChemOffice package, and Gaussian 98W were tools for drawing and optimizing the molecules, and for the evaluation of the chosen compound's pharmacokinetic parameters there was ADME/tox. Compounds with adequate pIC50 values for PI3K and mTOR were chosen and modified in order to intensify the positive interactions. With the correct alteration of chemical structures within substituents, while respecting the strategies of drug design, a set of molecules with corresponding or even higher activities has been formed. Enhancing the activities for mTOR kinases was a priority; while activities for PI3K kinases were opt to be constant or higher. The usage of QSAR studies in the design of new, dual PI3K/mTOR kinase inhibitors was proven to be useful for the analysis of characteristics and the improvement of biological activities of compounds. This may seem to be a good basis for the choice of possible candidates for synthesis and their analysis in further research.

*Keywords:* 3D-QSAR, PI3K/mTOR kinases, dual inhibitors, drug design



## SP002 TROVANJE ALKOHOLNIM PIĆIMA SA POVEĆANIM SADRŽAJEM METANOLA

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Povećane količine metanola u alkoholnim pićima mogu biti rezultat neadekvatne proizvodnje prirodnih alkoholnih pića ili falsifikovanja, i pri tome mogu izazvati najčešće masovna trovanja sa mogućim teškim efektima kao što su oštećenje vida i acidoza. Prema podacima SZO od 1998. do 2012. godine je zabeleženo ukupno 59 slučajeva masovnih trovanja metanolom, a u Srbiji je 1998. godine zabeleženo čak 43 smrtna ishoda. Ispitivanje sadržaja metanola i etanola u krvi pacijenata koji su konzumirali alkoholna pića. Uzorci krvi 14 pacijenata koji su primljeni na VMA tokom 2013. godine u alkoholisanim stanju su analizirani headspace gasnom hromatografijom sa plameno-jonizujućim detektorom pod sledećim uslovima: kolona Rtx-BAC1, 30 m × 0,32 mm, debljina filma 1,8 μm, temperatura 40 °C, uz protok gasa 2 mL/min, a kao interni standard korišćen je 1‰ rastvor 2-propanol. Dužina trajanja analize iznosila je 5 minuta. Analizom uzoraka krvi izmerene su koncentracije metanola u rasponu od 0,1–0,26‰, dok su se koncentracije etanola kretale od 0,80–5,18‰ što ukazuje na alkoholisanost ali i povećan unos metanola. Prema literaturnim podacima, već niske koncentracije metanola u krvi (bez prisustva etanola) mogu rezultirati značajnim toksičnim efektima, a koncentracije veće od 0,10‰ mogu izazvati i letalni ishod. Kod dva pacijenta su zabeleženi nivoi metanola u krvi veći i od 0,20‰ (0,22 i 0,26‰). U istim uzorcima krvi je i sadržaj etanola bio najviši i iznosio je 4,52, odnosno 5,18‰. Rezultati Nacionalnog Centra za kontrolu trovanja VMA, prikazani u ovom radu, ukazuju da je tokom 2013. godine registrovano više slučajeva trovanja alkoholnim pićima sa povećanim sadržajem metanola, iako nije bilo smrtnih ishoda. Navedeni rezultati pokazali su da alkoholna pića mogu sadržati povećane koncentracije metanola što potvrđuje značaj određivanja metanola u krvi osoba u alkoholisanim stanju.

*Ključne reči:* alkoholna pića, metanol, trovanje, headspace gasna hromatografija

## SP002 POISONING WITH ALCOHOLIC BEVERAGES CONTAINING INCREASED LEVELS OF METHANOL

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The increased amount of methanol in alcohol beverages can be the result of inadequate production of natural alcoholic drinks or its falsification and can cause massive poisonings with possible severe toxic effects such as visual impairments and acidosis. According to WHO 59 cases of massive methanol poisonings were reported from 1998 to 2012, and in Serbia methanol caused death of 43 persons in 1998. Analysis of methanol and ethanol contents in blood of patients who consumed alcoholic beverages. Blood samples of 14 alcohol-intoxicated patients who were admitted to the MMA during 2013 were analyzed with headspace gas chromatography with flame ionization detector under the following conditions: Rtx-BAC1 column, 30 m × 0.32 mm, film thickness 1.8 μm, temperature 40 °C, gas flow 2 mL/min, internal standard 1‰ 2-propanol. Run time was 5 minutes. The concentrations of methanol in analyzed blood samples were in the range between 0.10 and 0.26‰ while ethanol concentrations were 0.80–5.18‰ indicating increased intake of alcoholic beverages which contained increased levels of methanol. According to literature data, rather low methanol levels (in absence of ethanol) can cause development of significant toxic effects, and even lethality with concentrations higher than 0.10‰. Two patients had methanol concentrations even higher than 0.20‰ (0.22 and 0.26‰). Furthermore, in these samples ethanol levels were the highest as well, 4.52 and 5.18‰ respectively. The results of the National Poison Control Centre of MMA presented in this study indicate that several cases of poisoning with alcoholic drinks containing increased amount of methanol occurred during 2013, although with no death outcomes. Obtained results have shown that alcoholic beverages can contain increased levels of methanol justifying the importance of methanol analysis in the blood of alcohol-intoxicated patients.

*Keywords:* alcoholic beverages, poisoning, methanol, headspace gas chromatography

**SP003**  
**FORMULACIJA I PROCENA**  
**VIŠESTRUKIH V/U/V**  
**EMULZIJA PRIMENOM**  
**EKSPERIMENTALNOG DIZAJNA**

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Multiple emulzije (v/u/v ili u/v/u) su kompleksni disperzni sistemi, poznati i kao »emulzije emulzija«. Neke od prednosti višestrukih emulzija v/u/v tipa, koje ih čine pogodnim za primenu u farmaciji i kozmetologiji, su: relativno velika mogućnost inkorporiranja hidrofilnih supstanci, zaštita inkapsuliranih supstanci od degradacije, mogućnost inkorporiranja inkompatibilnih supstanci u isti sistem, kao i produženo oslobađanje aktivnih sastojaka. Međutim, višestruke emulzije su termodinamički izuzetno nestabilni sistemi. Cilj ovog rada bila je procena uticaja različitih formulacijskih faktora na reološke karakteristike i fizičku stabilnost v/u/v višestrukih emulzija, primenom metode 24-1 frakcionog faktorijskog dizajna, kao i njihova optimizacija. Primenom dvostepenog postupka izrađeno je osam formulacija višestrukih emulzija (F1 – F8), koje su se razlikovale u koncentracijama: primarnog emulgatora PEG-30 dipolihidroksisteearata (1% i 3%), sekundarnog emulgatora poloksamera 407 (0,8% i 1,2%), magnezijum-sulfata, heptahidrata (0,1% i 0,5%) i natrijum-hlorida (0,1% i 0,5%). Prisustvo složenih kapi je potvrđeno posmatranjem uzoraka optičkim mikroskopom. Rezultati, dobijeni 72h posle izrade (vrednosti električne provodljivosti, volumeni odvojenog sloja nakon centrifugiranja, prividni viskoziteti i histerezne površine) su analizirani primenom softvera Design-Expert 7.0. Druga faza eksperimentalnog rada bila je posvećena optimizaciji formulacija višestrukih v/u/v emulzija. Sve izrađene višestruke emulzije bile su homogene, bele boje, konzistencije kremova ili losiona. Vrednosti električne provodljivosti su bile u rasponu od 113  $\mu\text{S}/\text{cm}$  do 1563  $\mu\text{S}/\text{cm}$ , a zapremina odvojenog sloja nakon centrifugiranja bila je 0 do 0,5 ml. Rezultati reoloških merenja su pokazali da sve ispitivane emulzije ispoljavaju nenjutnovsko tiksotropno proticanje. Vrednosti maksimalnog prividnog viskoziteta su bile u rasponu od 421 mPas do 45433 mPas. Karakterizacija i ispitivanje fizičke stabilnosti optimizovanih formulacija (izrađenih u skladu sa predlogom softvera), dali su sledeće rezultate: nakon testa centrifugiranja nije uočena separacija faza, vrednosti električne provodljivosti bile su 158  $\mu\text{S}/\text{cm}$  i 192  $\mu\text{S}/\text{cm}$ , a maksimalnog prividnog viskoziteta 19800 mPas odnosno 22300 mPas (za formulacije

**SP003**  
**FORMULATION AND**  
**EVALUATION OF W/O/W**  
**MULTIPLE EMULSIONS USING**  
**EXPERIMENTAL DESIGN**

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Multiple emulsions (w/o/w or o/w/o) are complex dispersion systems, known also as »emulsions of emulsions«. Relatively high entrapment capacity for hydrophilic compounds, protection of the encapsulated substances towards degradation, the ability to introduce incompatible substances into the same system and sustained active substance release are some of the advantages of w/o/w type of these emulsion systems that make them potentially interesting for application in pharmaceuticals and cosmetics. The aim of this study was to evaluate significance of the influence of different formulation parameters on the rheological properties and physical stability of the w/o/w multiple emulsions (using 24-1 fractional factorial design) as well as their optimization. Eight samples (F1 – F8) were prepared using two-step procedure varying the following factors: concentrations of primary emulsifier PEG 30-dipolyhydroxystearate (1% and 3%), secondary emulsifier Poloxamer 407 (0.8% and 1.2%), magnesium sulfate heptahydrate (0.1% and 0.5%) and sodium chloride (0.1% or 0.5%). Multiple emulsions were analyzed under microscope to confirm the multiple characters. Results obtained 72h after the preparation (values of electrical conductivity, the volumes of separate layers after centrifugation test, apparent viscosities and hysteresis area) were analyzed using software Design-Expert 7.0. In the second phase of experiments, optimization of the w/o/w multiple emulsions was performed. All prepared multiple emulsions appeared as white and homogenous creams or lotions. The conductivity values were in range from 113–1563  $\mu\text{S}/\text{cm}$ , and the volumes of after centrifugation test were 0 to 0.5 ml. Results of the rheological measurements have shown that the investigated emulsions exhibited non-Newtonian thixotropic behavior. The maximal apparent viscosities were from 421–45433 mPas. When it comes to optimized formulations (which were prepared based on software recommendation), there was no phase separation after centrifugation. The conductivity values were 158  $\mu\text{S}/\text{cm}$  and 192  $\mu\text{S}/\text{cm}$ ; the maximal apparent viscosities were 19800 mPas and 22300 mPas (for formulations F9 and F10, respectively). Based on the results obtained using the fractional factorial design it was concluded

F9 i F10, respektivno). Na osnovu rezultata, dobijenih primenom metode frakcionog faktorijalnog dizajna, može se zaključiti da najveći uticaj na reološke karakteristike i fizičku stabilnost ispitivanih v/u/v višestrukih emulzija imaju koncentracije primarnog emulgatora PEG-30 dipolihidroksistearata i magnezijum-sulfata, heptahidrata. Eksperimentalno dobijeni rezultati optimizovanih formulacija V/U/V emulzija bili su veoma bliski predviđenim, te primena metoda eksperimentalnog dizajna može biti od velikog praktičnog značaja u razvoju novih formulacija farmaceutskih i kozmetičkih emulzija.

*Ključne reči:* v/u/v emulzije, polimerni emulgatori, eksperimentalni dizajn

#### **SP004 DEFINISANJE KINETIKE REAKCIJE DEGRADACIJE MIKOFENOLAT MOFETILA**

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Mikofenolat mofetil je imunosupresiv koji se najčešće koristi nakon transplantacije organa. Studije forsirane degradacije izvode se prema smernicama ICH regulative Q1A. Procena uspešnosti transfera HPLC na U-HPLC metodu mora biti u skladu sa Ph. Eur. 7 i USP 30. Skratiti vreme analize transferom HPLC na U-HPLC metodu, a zatim primeniti U-HPLC metodu za praćenje kinetike degradacije mikofenolat mofetila pri kontrolisanim stres uslovima. Kinetika reakcija degradacije mikofenolat mofetila ispitivana je u baznoj sredini (0,01 mol/L NaOH), kiseloj sredini (1 mol/L HCl), pod uticajem oksidativnog agensa (3 % H<sub>2</sub>O<sub>2</sub>) i na povišenoj temperaturi (70 °C). Hromatografski uslovi obuhvatali su: Atlantis C18 (150 × 4,6 mm, 3 μm) kolonu, talasnu dužinu detekcije od 215 nm, mobilnu fazu (10 mmol/L amonijum-acetat pH 6 : acetonitril (60 :40, v/v)) sa protokom od 0,7 mL min<sup>-1</sup>; Prilikom transfera na U-HPLC metodu korišćena je Hypersil Gold aq kolona (100 × 2,1 mm, 1,9 μm), protok je smanjen na 0,23 mL min<sup>-1</sup> i injekciona zapremina je smanjena sa 10 na 1,39 μL. Izlaganje mikofenolat mofetila oksidativnom stresu dovodi do nastanka četiri moguća degradaciona proizvoda, zbog čega je transfer metode rađen sa ovim uzorkom. Prilikom transfera bilo je važno da stacionarna faza ostane hemijski ista. Na osnovu svojih karakteristika, predložena U-HPLC metoda se može smatrati novom metodom i zato

that the concentrations of primary emulsifier PEG 30-dipolyhydroxystearate and magnesium sulfate heptahydrate had the greatest influence on the rheological properties and physical stability of the investigated w/o/w multiple emulsions. Experimentally obtained results of the optimized formulations were very close to predicted values. Therefore, application of an experimental design can significantly improve the development of pharmaceutical and cosmetic emulsions.

*Keywords:* w/o/w emulsions, polymeric emulsifiers, experimental design

#### **SP004 DEFINITION OF KINETIC DEGRADATION OF MYCOPHENOLATE MOFETIL**

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Mycophenolate mofetil is an immunosuppressant most used after organ transplantation. Forced degradation studies are conducted by guidelines of ICH Q1A. Evaluation of the success of transfer from HPLC to U-HPLC must be in accordance with the Ph. Eur. 7 and USP 30. Shorten the analysis time using the transfer from HPLC to U-HPLC, and then use U-HPLC method to follow the kinetics of degradation of mycophenolate mofetil in controlled stress conditions. Kinetic of reaction of degradation of mycophenolate mofetil was tested in base environment (0.01 mol/L NaOH), in acid environment (1 mol/L HCl), in the conditions of oxidative stress (3% H<sub>2</sub>O<sub>2</sub>) and at elevated temperature (70 °C). Chromatographic conditions included: Atlantis C18 (150 × 4,6 mm, 3 μm) column, wavelength of detection in 215 nm, mobile phase (10 mmol/L ammonium-acetate pH 6 : acetonitrile (60 :40, v/v)) with flow of 0.7 mL min<sup>-1</sup>; During the transfer to U-HPLC method was used Hypersil Gold aq column (100 × 2.1 mm, 1.9 μm), flow was reduced to 0.23 mL min<sup>-1</sup> and the injection volume was reduced from 10 to 1.39 μL. Exposure of mycophenolate mofetil to oxidative stress leads to the creation of 4 degradation products which is why the transfer of method was made with this sample. During the transfer, it was important that the stationary phase remains chemically the same. Based on its characteristics, proposed U-HPLC method can

zahteva kompletnu validaciju. U-HPLC metoda uspešno je primenjena za praćenje kinetike degradacije mikofenolat mofetila. Transferom HPLC metode skraćeno je vreme hromatografske analize sa 22 na 6 minuta. U-HPLC metoda korišćena je za praćenje kinetike degradacije mikofenolat mofetila. Na osnovu dobijenih rezultata može se zaključiti da mikofenolat mofetil prati kinetiku nultog reda pri oksidativnoj degradaciji, prvog reda pri baznoj degradaciji i reakciju drugog reda pri kiseljoj i termičkoj degradaciji.

*Cljučne reči:* mikofenolat mofetil, kinetika reakcija degradacije, transfer HPLC u U-HPLC.

be regarded as a new method and therefore requires the complete validation. U-HPLC method has been successfully applied to monitor the kinetics of degradation of mycophenolate mofetil. With the transfer of HPLC method, was shortened the time of chromatographic analysis from 22 to 6 minutes. Based on the obtained results it can be concluded that the mycophenolate mofetil follows zero-order reaction in oxidative stress degradation, first-order reaction in base degradation, and second-order reaction in acid and thermal degradation.

*Keywords:* mycophenolate mofetil, kinetics of degradation reactions, HPLC to U-HPLC method transfer

**SP005**  
**OKSIDATIVNO-STRESNI STATUS**  
**LDL LIPOPROTEINSKE FRAKCIJE**  
**KOD PACIJENATA SA AKUTNIM**  
**INFARKTOM MIOKARDA**

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Oksidativna modifikacija lipoproteina male gustine (LDL) ima značajnu ulogu u patogenezi ateroskleroze koja predstavlja komplikovan vaskularni poremećaj i vodeći uzrok nastanka infarkta miokarda. Praćenje efekata oksidativnog stresa na modifikaciju LDL čestica kod pacijenata sa akutnim infarktom miokarda. U izolovanoj LDL frakciji seruma 16 pacijenata sa akutnim infarktom miokarda i 10 zdravih osoba određivani su parametri lipidnog statusa, oksidativnog stresa-totalni oksidativni status (TOS) i totalni antioksidativni status (TAS), kao i koncentracije malondialdehida (MDA), pre i posle oksidacije vodonik-peroksidom u prisustvu soli bakra. Pokretljivost LDL frakcija pre i nakon egzogene oksidacije detektovana je metodom elektroforeze. Koncentracija malondialdehida pre oksidacije LDL frakcije nije bila značajno različita kod pacijenata u odnosu na kontrolnu grupu, dok je koncentracija TAS niža. Nakon in vitro oksidacije LDL frakcije u obe ispitivane grupe dolazi do značajnog povećanja koncentracije malondialdehida, kao i do pada totalnog antioksidantnog statusa ( $p < 0,001$ ), pri čemu je smanjenje koncentracije TAS-a izraženije kod zdravih ispitanika. Elektroforetskim merenjem pokretljivosti izolovanih LDL frakcija kod pacijenata sa akutnim infarktom miokarda i zdravih osoba utvrđena je inicijalna zastupljenost oksidovanog LDL-a, kao i povećanje njego-

**SP005**  
**OXIDATIVE STRESS STATUS**  
**OF LDL LIPOPROTEIN FRACTION**  
**IN PATIENTS WITH ACUTE**  
**MYOCARDIAL INFARCTION**

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Oxidative modification of low density lipoprotein (LDL) plays an important role in the pathogenesis of atherosclerosis, which is a complex vascular disorder and a leading cause of myocardial infarction. Monitoring the effects of oxidative stress on the modification of LDL particles in patients with acute myocardial infarction. The LDL fraction was isolated from serum of 16 patients with acute myocardial infarction, and 10 healthy subjects. Lipid status parameters, oxidative stress-total oxidative status (TOS) and total antioxidative status (TAS), also malondialdehyde concentration (MDA) were determined before and after hydrogen peroxide oxidation in the presence of a copper salt. Mobility of LDL fractions before and after oxidation was detected using electrophoresis. Malondialdehyde concentration before LDL fraction oxidation was not different comparing to control group, while TAS concentration was significantly lower. After in vitro oxidation of LDL fraction in both groups there was a significant increase in MDA, and a decline in the total antioxidant status ( $p < 0.001$ ), whereby the decrease in TAS is more pronounced in healthy subjects. The initial presence of oxidized LDL, and its increase after the in vitro oxidation was determined measuring the electrophoretic mobility of isolated LDL fraction in patients with acute myocardial infarction and in healthy persons.

vog udela nakon in vitro oksidacije. Oksidovane LDL čestice pokazale su veću elektroforetsku pokretljivost u odnosu na neoksidovane. Migraciona daljina povećala se za 19% kod obolelih, a za približno 40% kod kontrolne grupe. Na osnovu rezultata ovog ispitivanja može se zaključiti da je kontrolna grupa sadržala veći udeo neizmenjenog LDL-a pa je naknadna, egzogena oksidacija u većem procentu uticala na modifikaciju ovih čestica. Manji procenat dodatne modifikacije ovih čestica kod pacijenata nakon oksidacije, ukazuje da su usled bolesti LDL čestice već bile značajno modifikovane endogenom oksidacijom.

*Ključne reči:* infarkt miokarda, oksidovani LDL

Oxidized LDL particles showed a higher electrophoretic mobility compared to unoxidized. Migration distance was increased by 19 % in the patients, and by about 40 % in the control group. Based on the results of this study conclusion is that the control group contains a higher amount of unmodified LDL and the subsequent, exogenous oxidation resulted in a higher percentage of particles' modification. Lower percent of additional LDL particles modification in patients' group showed that these particles were already endogenously modified because of the disease process.

*Keywords:* myocardial infarction, oxidized LDL

**SP006  
ZNAJJE STUDENATA  
U VEZI SA REGISTROM  
DAVALACA KOSTNE SRŽI  
SRBIJE**

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Registar davalaca kostne srži (RDKS) sadrži podatke o osobama koje žele da se prijave za potencijalne donore kostne srži. Trenutno je u RDKS prijavljeno preko 4000 potencijalnih davalaca. Cilj je ispitati znanje, stavove i ponašanje studentske populacije Farmaceutskog, Stomatološkog i Medicinskog fakulteta Univerziteta u Beogradu, Srbija, u vezi sa dobrovoljnim davanjem kostne srži i postojanjem RDKS, uporediti dobijene rezultate među fakultetima zdravstvene struke i istražiti moguće uzroke nedovoljnog broja potencijalnih davalaca u RDKS. Urađena je prospektivna studija preseka na uzorku studenata farmacije, medicine i stomatologije, korišćenjem namenski kreiranog upitnika. Anketiranje studenata je urađeno na Univerzitetu u Beogradu. Rezultati su analizirani deskriptivnom statistikom koristeći MS Excel 2013. Anketirano je ukupno 574 studenata: 195 (34%) studenata farmacije, 190 (33,1%) studenata medicine i 189 (32,9%) studenata stomatologije. Od anketiranih studenata, njih 320 (55,7%) zna da postoji Svetski registar davalaca kostne srži (BMDW), dok za postojanje RDKS zna 141 (24,6%) student. Među anketiranim studentima bilo je i 199 (34,7%) dobrovoljnih davaoca krvi, od kojih je četvoro (0,7%) prijavljeno u RDKS, 32 (5,6%) studenata poznaje osobu iz okruženja, upisanu u RDKS. Rezultati ukazuju da je samo 20,0% ispitanika razmišljalo da se prijavi u RDKS, a 64,7% nije uopšte razmišljalo ili nije sigurno. Skoro četvrtina ispitanika

**SP006  
BIOMEDICINE STUDENTS'  
KNOWLEDGE RELATED  
TO BONE MARROW DONATION  
REGISTRY IN SERBIA**

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Serbian Bone Marrow Donor Registry (SBMDR) contains information concerning potential bone marrow donors. Currently there are over 4000 potential donors listed in the SBMDR. The aim is to examine the knowledge, attitudes and behavior of students in the health professions at the University of Belgrade, Serbia, concerning bone marrow donation and the existence of SBMDR. Also the aim is to compare the results between different faculties and to discover possible causes of insufficient bone marrow donors in the SBMDR. A prospective cross sectional study was conducted among students of pharmacy, medicine and dentistry, using a specifically designed questionnaire. Data collection was conducted at the University of Belgrade. Results were analyzed by descriptive statistics using MS Excel 2013. A total of 574 students were asked to fill in the questionnaire: 195 (34%) from the Faculty of Pharmacy, 190 (33.1%) from the Faculty of Medicine and 189 (32.9%) from the Faculty of Dentistry. Among surveyed students, 320 (55.7%) of them know that there is the Bone Marrow Donors Worldwide (BMDW), while 141 (24.6%) know of the existence of SBMDR. Amid 34.7% (199 students) of study participants who are blood donors, only four (0.7%) are also registered with SBMDR. Only 32 students (5.6%) know someone who is registered with SBMDR. Results point to the fact that only 20% of interviewees have considered registering with



je izjavilo da bi voleli da budu kontaktirani od strane zaposlenih u Registru kako bi se prijavili. Najčešći razlog za neprijavljivanje u RDKS je strah od bola, kod 30,1% studenata. Veliki broj studenata je upoznat sa postojanjem Svetskog registra, a dosta manje sa postojanjem nacionalnog RDKS. Relativno mali broj studenata je razmišljao o prijavljivanju u isti. Poželjno bi bilo da se rade promocije i edukacije u cilju informisanja i proširenja svesti o postojanju i važnosti upisivanja u RDKS.

*Ključne reči:* registar davalaca kostne srži, donori, studenti

### **SP007 ISHEMIJOM MODIFIKOVAN ALBUMIN KOD PACIJENATA SA AKUTNIM INFARKTOM MIOKARDA**

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Dijagnoza akutnog infarkta miokarda u hitnim slučajevima je često teška zbog nejasne kliničke slike i nedostatka brzog i pouzdanog dijagnostičkog testa. Otud potiče i potencijalni značaj ishemijom modifikovanog albumina (IMA) kao osetljivog biomarkera ishemije miokarda koji se pojavljuje pre razvoja nekroze ćelija srčanog mišića. Utvrditi značaj IMA kao potencijalnog biomarkera u ranom otkrivanju infarkta miokarda, kao i njegovu korelaciju sa parametrima oksidativnog stresa. Kod 109 pacijenata sa akutnim bolom u grudima i 60 zdravih ispitanika određivana je koncentracija IMA, metodom vezivanja kobalta za albumin. Ispitanicima je određena i koncentracija troponina I (TnI), kao i parametri oksidativno – stresnog statusa: totalni antioksidantni status (TAS), totalni oksidantni status (TOS), paraoksonazna aktivnost (PON1), prooksidativno – antioksidativni balans (PAB). Rezultati ovog ispitivanja pokazali su značajno više vrednosti koncentracije IMA ( $p < 0,01$ ) kod pacijenata u odnosu na zdrave osobe. Podelom pacijenata prema koncentracijama troponina I, specifičnog markera akutnog infarkta miokarda prema graničnim koncentracijama za kliničku odluku o postojanju infarkta miokarda utvrđene su više koncentracije IMA kod pacijenata koji su imali klinički značajno više koncentracije TnI, i ta razlika je bila statistički značajna ( $p < 0,05$ ). Vrednosti PAB su bile značajno više kod obolelih pacijenata u poređenju sa

SBMDR, while 64.7% have not at all considered or are uncertain about it. Almost a quarter of the sample report they would like to be contacted in order to apply for registration. The most common reason for not applying for registration with SBMDR is fear of pain (30.1% students). A large number of students are acquainted with the existence of the BMDW, although far fewer know of the existence of the SBMDR. Relatively few students have considered joining the national registry. Promotion and education with aim to inform and increase awareness about the existence and importance of joining the SBMDR are highly desirable.

*Keywords:* bone marrow donor registry, donors, students

### **SP007 ISCHEMIA – MODIFIED ALBUMIN IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION**

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The diagnosis of acute myocardial infarction in emergency patients with acute coronary symptoms is often difficult due to an unclear clinical presentation and lack of a rapid, reliable diagnostic test. Therefore, ischemia – modified albumin (IMA) might have potential value as a sensitive biomarker of ischemia, possibly detected before myocardial cell necrosis. To determine the significance of IMA as a potential biomarker in early myocardial infarction diagnosis and its correlation with oxidative stress parameters. IMA concentration has been determined in 109 patients with acute chest pain and 60 controls, by cobalt binding method. Also, troponin I (TnI) concentration has been measured and oxidative stress parameters: total antioxidant status (TAS), total oxidant status (TOS), paraoxonase (PON1), pro oxidant/antioxidant balance (PAB). Results have shown significantly higher IMA concentrations in patients compared to control group ( $p < 0.01$ ). Dividing patients according to troponin I concentration limits for establishing myocardial infarction diagnosis, higher IMA concentrations have been established in patients with significantly higher TnI concentrations. Furthermore, that difference has been significant ( $p < 0.05$ ). PAB values have been significantly higher in patients compared to control group ( $p < 0.01$ ), also TOS values ( $p < 0.05$ ). PON1 activity has been significantly lower in patients ( $p < 0.01$ ), while TAS values

zdravim sredovečnim osobama koje su činile kontrolnu grupu ( $p < 0,01$ ), kao i vrednosti TOS ( $p < 0,05$ ). Aktivnost PON1 je bila značajno niža kod pacijenata ( $p < 0,01$ ), dok se koncentracija TAS nije značajno razlikovala. Primenom Spearman-ove neparametarske korelacije utvrđena je značajna korelacija između koncentracija IMA kao markera ishemije i koncentracije TOS kao markera prooksidativnih procesa ( $r = +0,368$ ,  $p < 0,001$ ). Na osnovu rezultata ove studije može se zaključiti da IMA nije marker specifičniji od klasičnih biomarkera za akutni infarkt miokarda. Korelacija ovog parametra sa prooksidansima u krvi ukazuje na ulogu slobodnih radikala u njegovom nastanku.

*Cljučne reči:* ishemijom modifikovan albumin, infarkt miokarda

have not been significantly different. Spearman's non-parametric correlation has shown significant correlation between IMA, marker of ischemia, and TOS, marker of pro oxidant processes, has been determined ( $r = +0.368$ ,  $p < 0.001$ ). Based on results of the current study it could be concluded that IMA is not more specific marker than other classic myocardial infarction markers. The correlation of this marker with blood pro oxidants implies the role of free radicals in his producement in blood.

*Keywords:* ischemia – modified albumin, myocardial infarction

## SP008 ZNAJANJE I STAVOVI STUDENATA U VEZI SA FARMACEUTSKIM OTPADOM

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Farmaceutski otpad (FO) čine svi lekovi i medicinska sredstva zajedno sa primarnom ambalažom, koji su neupotrebljivi zbog isteka roka upotrebe, neispravnosti u pogledu njihovog kvaliteta, kontaminacije ili rasipanja. Ispitati znanja i stavove studenata Beogradskog univerziteta o pojmu FO, njegovim upravljanjem i ulozi studenata Farmaceutskog fakulteta (FF) u ovoj oblasti. Deskriptivno istraživanje sa namenski kreiranim upitnikom za ovu studiju koji su dobrovoljno popunjavali studenti različitih godina Univerziteta u Beogradu tokom meseca februara 2014. godine. Od ukupnog broja ispitanih studenata njih 198 je popunilo upitnik što daje stepen odgovora od 99%. Veoma mali procenat studenata, svega 2%, zna šta se podrazumeva pod pojmom FO, dok daleko veći zna koje su boje kontejneri za odlaganje FO (30,8%), koji je krajnji korak upravljanja kontejnerima (25,3%). Svi studenti su jedinstvenog stava da je svest građana u vezi sa FO nedovoljno razvijena a velika većina ispitanika (72,1 %) smatra da bi studenti FF trebalo da podstiču aktivnosti u oblasti FO: edukacijom građana (39,4%) i organizovnim prikupljanjem FO (4,5%). Saznanja studenata u vezi sa upravljanjem FO nisu dovoljno razvijena, te je potrebno preduzimati edukacije studenata Beogradskog Univerziteta, a studenti FF bi mogli uzeti učešće u

## SP008 STUDENTS' KNOWLEDGE AND ATTITUDES ABOUT PHARMACEUTICAL WASTE

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Pharmaceutical waste includes all drugs and medical devices, including primary packaging, unusable due to their expiration date, failure in the terms of their quality, contamination or dissipation. Enquire into the knowledge and attitudes of Belgrade University students about the term of pharmaceutical waste, managing of it, and Faculty of Pharmacy students' participation in this area. Materials and methods: Descriptive research with purposefully made questionnaire for this study which has been filled out voluntarily by University of Belgrade students from different study levels, in February 2014. Out of the total number of the examinees 198 have filled out the questionnaire which provides us with the response level of 99%. Very low percent of students, only 2%, know what the term of pharmaceutical waste implies, whereas a larger number know what the colour of containers for pharmaceutical waste disposal is (30.8%), which is the last step of containers managing (25.3%). All of them are unanimous in their attitude that citizens' awareness about pharmaceutical isn't developed well enough. Most of the examinees (72.1 %) think that students of Faculty of Pharmacy should stimulate the activities in this area: with education of citizens (39.4%) and organized pharmaceutical waste collection (4.5%). Students'

programu edukacije opšte javnosti, koja prema mišljenju studenata nema dovoljno razvijenu svest o ovom pitanju.

*Cljučne reči:* farmaceutski otpad, znanja i stavovi, studenti farmacije

### **SP009 VALIDACIJA POPULACIONOG FARMAKOKINETIČKOG MODELA TOPIRAMATA**

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Populaciono modelovanje omogućava ispitivanje uticaja faktora varijabilnosti na farmakokinetiku leka i posledično njegovu koncentraciju. Da bi mogao da se koristi, populacioni farmakokinetički model je potrebno validirati nekim od tehnika interne ili eksterne validacije. Validacija populacionog farmakokinetičkog modela topiramata radi njegove primene u individualizaciji režima doziranja. Model je izgrađen na podacima dobijenim od 49 odraslih pacijenata sa epilepsijom koji su na monoterapiji topiramatom ili kombinovanoj terapiji nekim drugim antiepileptikom. Validacija finalnog modela je vršena tehnikama interne validacije, koje su obuhvatile: grafičku procenu (goodness of fit), numeričku (numerical predictive check, NPC) i vizuelnu procenu predikcije (visual predictive check, VPC), kao i bootstrap analizu. Eksterna validacija je rađena na setu podataka dobijenom od 29 pacijenata. Odnos populacionih/individualnih predviđenih koncentracija i merenih koncentracija pokazuje visok stepen korelacije. Takođe, i grafik zavisnosti kondicionalnih ponderisanih reziduala (conditional weighted residuals, CWRES) u zavisnosti od vremena nakon primene leka je pokazao da su vrednosti većine reziduala u rasponu od  $-2$  do  $+2$ . Numeričkom i vizuelnom procenom predikcije je pokazano da se merene vrednosti nalaze uglavnom u okviru intervala pouzdanosti simuliranih podataka. Bootstrap analizom nije pokazana značajna razlika u srednjim vrednostima, standardnim greškama i 95% intervalu pouzdanosti između bootstrap replikata i originalnih podataka. Eksternom validacijom je pokazana dobra korelacija između predviđenih i merenih vrednosti. Rezultati potvrđuju da je model stabilan i da se može koristiti u identifikaciji i kvantifikaciji faktora farmakokinetičke varijabilnosti sa ciljem njegove primene u individualizaciji režima doziranja topiramata.

*Cljučne reči:* validacija, populaciono modelovanje, topiramata, NONMEM®

knowledge about pharmaceutical waste managing isn't developed well enough, thus it's necessary to educate students of Belgrade University, and students of Faculty of Pharmacy should be involved in education programs of general public.

*Keywords:* pharmaceutical waste, knowledge and attitudes, pharmacy students

### **SP009 VALIDATION OF A POPULATION PHARMACOKINETIC MODEL OF TOPIRAMATE**

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Population modeling allows investigation of influence of variability factors on drug pharmacokinetics and consequently on drug level. For the model to be used, it is needed to perform its validation, using techniques of internal or external validation. Validation of the population pharmacokinetic model of topiramate for its use in the individualization of the dosage regimen. Data were collected from 49 adult epileptic patients on mono- or co-therapy of TPM and other antiepileptic drug(s). The validation of the final model was performed by the techniques of internal validation, which included: goodness of fit, numerical predictive check, visual predictive check, and bootstrap method. For external validation was used dataset from 29 patients. Population/individual predicted concentrations correlated well with the observed values. Also, conditional weighted residuals vs. time after dose plot showed that the most of the residuals were within  $-2$  to  $+2$  range. Numerical and visual predictive check showed that observed concentrations were mostly within confidence intervals (CIs) for the simulated data. Bootstrap analysis didn't show any significant difference in mean values, standard errors and 95% CIs. External validation showed good correlation between predicted and observed values. The results confirmed that the model is stable and that it can be used in identification and quantification of pharmacokinetic variability factors, with a goal of individualisation of topiramate dosage regimen.

*Keywords:* validation, population modeling, topiramate, NONMEM®

**SP010**  
**ODREĐIVANJE AKTIVNOSTI**  
**ENZIMA LECITIN-HOLESTEROL**  
**ACILTRANSFERAZE (ENGL. LCAT)**  
**I HOLESTEROL-ESTAR TRANS-**  
**FERNOG PROTEINA (ENGL. CETP)**  
**KOD GOJAZNE DECE**

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Gojaznost predstavlja jedan od osnovnih zdravstvenih problema u savremenom svetu, a sve češće se javlja i u pedijatrijskoj populaciji. Poremećaji koncentracije serumskih lipida su u velikoj meri uslovljeni promenjenim aktivnostima enzima lecitin-holesterol aciltransferaze (engl. LCAT) i holesterol-estar transfernog proteina (engl. CETP). Ispitati lipidni profil, aktivnost enzima LCAT i CETP kod gojazne dece i adolescenata i uporediti sa kontrolnom grupom normalno uhranjene dece. U ispitivanju je učestvovalo 90 gojazne dece i 27 normalno uhranjene dece, koji su ambulantno lečeni na Univerzitetnoj dečjoj klinici Tiršova. Lipidni profil je određen rutinskim metodama. Aktivnosti LCAT i CETP su određene metodom po Asztalos-u i saradnicima. Gojazna deca imala su značajno viši indeks telesne mase i koncentraciju triglicerida ( $P < 0,001$ ), a nižu koncentraciju holesterola sadržanog u lipoproteinima niske gustine (engl. High-density lipoprotein, HDL) ( $R < 0,001$ ) u odnosu na negojaznu decu. Nije nađena statistički značajna razlika u aktivnostima enzima LCAT i CETP kod gojazne i normalno uhranjene dece. Kod gojazne dece aktivnost LCAT je bila u statistički značajnoj korelaciji sa koncentracijom ukupnog holesterola ( $P < 0,05$ ) i aktivnošću CETP ( $R < 0,01$ ), dok je aktivnost CETP bila u značajnoj korelaciji sa glikoziliranim hemoglobinom (engl. HbA1C) ( $P < 0,05$ ). Aktivnost enzima CETP bila je značajno povišena kod gojazne dece sa hipertenzijom u odnosu na normotenzivne ispitanike ( $P < 0,05$ ). Aktivnosti LCAT i CETP nisu se značajno razlikovale između gojazne i normalno uhranjene dece, ali su gojazna deca sa hipertenzijom imala značajno više aktivnosti CETP.

*Ključne reči:* gojazna deca, lipidni profil, LCAT, CETP.

**SP010**  
**ASSESSMENT OF THE ACTIVITY**  
**OF LECITHIN-CHOLESTEROL**  
**ACYLTRANSFERASE AND**  
**CHOLESTEROL ESTER**  
**TRANSFER PROTEIN**  
**IN OBESE CHILDREN**

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Obesity is one of the major health problems in the modern world and it has been more and more frequent in pediatric population. Disturbances of serum lipid concentrations in obese individuals are highly related to changes of activities of lecithin:cholesterol acyltransferase (LCAT) and cholesterol ester transfer protein (CETP). To examine lipid profile, activities of LCAT and CETP in obese children and adolescents and compare the results with a control group of normal weight children. The study included 90 obese children and 27 normal weight children who are outpatients treated at the University Children's Hospital Tiršova. Lipid profile is determined by routine methods. LCAT and CETP activities were determined by the method introduced by Asztalos and associates. Compared to normal weight children, obese children had significantly higher body-mass index and concentrations of triglycerides ( $P < 0.001$ ), while lower concentration of high-density lipoprotein (HDL) cholesterol ( $P < 0.001$ ). We found no differences in activities of LCAT and CETP between obese and normal-weight children. In obese children, LCAT activity significantly correlated with concentrations of total cholesterol ( $P < 0.05$ ) and the activity of CETP ( $P < 0.01$ ). The activity of CETP and glycosylated hemoglobin (HbA1c) were in statistically significant correlation ( $P < 0.05$ ). Activity of CETP was significantly elevated in obese children with hypertension compared to normotensive subjects ( $P < 0.05$ ). LCAT and CETP activities were not significantly different between obese and normal weight children, but obese children with hypertension had significantly higher CETP activity.

*Keywords:* obese children, lipid profile, LCAT, CETP.

**SP011**  
**ATEROPROTEKTIVNA ULOGA**  
**ENZIMA PARAOKSONAZE 1**  
**U HRONIČNOJ BUBREŽNOJ**  
**INSUFICIJENCIJI**

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Hronična bubrežna insuficijencija (HBI) predstavlja hronično, ireverzibilno gubljenje bubrežne funkcije koje je blisko povezano sa ubrzanim razvojem ateroskleroze. Rezultati prethodnih istraživanja nedvosmisleno ukazuju na postojanje veze između oksidativnog stresa i HBI. Parametri oksidativnog stresa i antioksidativne zaštite se sve više proučavaju kao potencijalni novi markeri za procenu toka i ishoda ove bolesti. Cilj ovog istraživanja bio je da se ispita ateroprotektivna uloga antioksidativnog enzima paraoksonaze 1 (PON 1) kod pacijenata sa HBI. U ovoj studiji je učestvovalo 20 predijaliznih, 20 dijaliznih pacijenata i 20 zdravih ispitanika. Kod svih ispitanika u serumu su određeni parametri lipidnog profila, totalni oksidativni status (TOS) i status enzima PON 1. Iz seruma ispitanika izdvojena je subfrakcija seruma sa holesterolom sa lipoproteinskih čestica visoke gustine (HDL-C) u kojima je kvantifikovana količina paraoksonaze-1 i paraoksonaze-3 imunohemijskom metodom (ELISA test). Koncentracije triglicerida bile su statistički značajno veće kod pacijenata u odnosu na kontrolnu grupu ( $p < 0,05$ ), dok je koncentracija holesterola u česticama visoke gustine (HDL-C) bila značajno niža ( $p < 0,05$ ). TOS je značajno povišen ( $p < 0,05$ ), dok je aktivnost enzima PON 1 bila značajno niža kod pacijenata. Ispitivanjem koncentracije PON 1 u HDL subfrakcijama uočeno je da je koncentracija značajno manja kod pacijenata u odnosu na kontrolnu grupu, dok je koncentracija PON 3 bila približno ista u obe ispitivane grupe. Na osnovu rezultata ove studije može se zaključiti da se pacijenti sa HBI nalaze u stanju uznapredovalog oksidativnog stresa, snižene koncentracije PON 1 i PON 3, kao i aktivnosti enzima paraoksonaze 1, ukazuju na promene u strukturi HDL-a i rezultujuću sniženu antioksidativnu zaštitu.

*Ključne reči:* PON-1, PON-3, HBI, Oksidativni stres

**SP011**  
**THE ATHEROPROTECTIVE ROLE**  
**OF PARAOXONASE 1 ENZYME**  
**IN CHRONIC RENAL FAILURE**

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The chronic renal failure (CRF) is chronic, irreversible lost of kidney function associated with progressive development of atherosclerosis. Results of previous studies have unambiguously showed the relation between oxidative stress and CRF. The oxidative stress and antioxidant parameters are increasingly being investigated as potential new markers for the assessment of progress and outcome of this disease. The aim of this study was to investigate the atheroprotective role of paraoxonase-1 (PON-1), an antioxidant enzyme in patients with CRF. This study included 20 predialysis patients, 20 patients on dialysis and 20 healthy participants. In the serum of all participants the lipid profile parameters were assessed, total oxidative status (TOS) and status of the PON-1 enzyme. The concentrations of paraoxonase-1 and paraoxonase-3 enzymes in the isolated subfractions of high density lipoprotein cholesterol (HDL) were quantified by immunoassay method (ELISA). The concentrations triglycerides were statistically significantly higher in patients compared with control group ( $p < 0.05$ ), while the concentrations of high density lipoprotein cholesterol (HDL-C) were significantly lower ( $p < 0.05$ ). TOS was significantly higher ( $p < 0.05$ ), while the enzyme activity of PON-1 was significantly lower in patients. Results of assessed PON-1 concentration on HDL subfractions showed that the concentrations were significantly lower in patients compared with control group, while the PON-3 concentrations were approximately the same in the both groups. Based on results of this study we can conclude that patients with CRF are in state of advanced oxidative stress, with decreased concentration of PON-1 and PON-3, decreased paraoxonase 1 activity, which indicates the change in HDL structure and consequent compromised antioxidative protection.

*Keywords:* PON-1, PON-3, CRF, Oxidative stress