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OSIGURANJE KVALITETA U KLINIČKOJ HEMIJI: NEZNATNO STATISTIKE I DOSTA ZDRAVOG RAZUMA

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Radeći u kliničko-hemijskoj laboratoriji, može nam se učiniti da je naš lični doprinos kvalitetu mali i da glavne uloge pripadaju statističkim modelima i proizvođačima. Retko se u dovoljnoj meri odaje priznanje ličnom znanju, veštinama i zdravoj logici, koji su od presudne važnosti za osiguranje kvaliteta u interesu pacijenata. Zaposleni, okolina i procedure koje se obavljaju u laboratoriji, uključujući njenu interakciju sa klijentima, ključne su za konačni rezultat u ukupnom lancu testiranja. Kako se sistemi merenja, reagensi i procedure postepeno unapređuju, rad na preanalitičkoj, postanalitičkoj i kliničkoj fazi verovatno će se najznačajnije isplatiti u vidu postizanja daljeg napretka u kvalitetu. Ovo znači da treba promeniti stavove i ponašanje, pre svega korisnika laboratorije. Potrebno je razumeti ljude i to na koji način se oni mogu uključiti u zajedničke procese poboljšanja. Moramo upotrebiti svoje znanje i zdravu logiku upotpunjene novim veštinama npr. iz oblasti društvenih nauka, upravljanja, poslovanja i menjati nauke kako bismo ovo postigli zajedno sa korisnicima laboratorije.

QUALITY ASSURANCE IN CLINICAL CHEMISTRY: A TOUCH OF STATISTICS AND A LOT OF COMMON SENSE

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Working in laboratories of clinical chemistry, we risk feeling that our personal contribution to quality is small and that statistical models and manufacturers play the major roles. It is seldom sufficiently acknowledged that personal knowledge, skills and common sense are crucial for quality assurance in the interest of patients. The employees, environment and procedures inherent to the laboratory including its interactions with the clients are crucial for the overall result of the total testing chain. As the measurement systems, reagents and procedures are gradually improved, work on the preanalytical, postanalytical and clinical phases is likely to pay the most substantial dividends in accomplishing further quality improvements. This means changing attitudes and behaviour, especially of the users of the laboratory. It requires understanding people and how to engage them in joint improvement processes. We need to use our knowledge and common sense expanded with new skills e.g. from the humanities, management, business and change sciences in order to bring this about together with the users of the laboratory.

IZUČAVANJE GENOMSKIH ASOCIJACIJA U OTKRIVANJU NOVIH BIOMARKERA: SLUČAJ OSTEOPOROZE

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Osteoporoza je poligenetska i multifaktorijalna bolest kostiju koja pogađa jednu od tri žene posle 50 godina starosti. Kao hronična bolest često se dijagnostikuje u progresivnoj fazi, kada je teško obezbediti efikasno lečenje. Iz tog razloga su za rano dijagnostikovanje, prognozu i efikasnost lečenja veoma važni rani markeri, posebno genetski markeri, koji se mogu detektovati tokom čitavog života i na koje ne utiču drugi faktori poput ishrane ili životnih navika. Najveći konzorcijum koji traga za alelima rizika za osteoporozu, GEFOS FP 7 projekat EU, sproveo je metaanalizu 5 studija koje su izučavale genomske asocijacije za dve osobine, mineralnu gustinu kostiju (BMD) i koštane frakture. Studije genomskih asocijacija za BMD u lumbalnom delu kičme (BMD-ls) i BMD vrata butne kosti (BMD-fn) obuhvatile su sve zajedno 19.195 subjekata. Ukupno 20 lokusa, potencijalnih genetskih markera, dostiglo je potrebnu meru značajnosti na nivou celog genoma ($P<5\times10^{-8}$) za BMD, od čega se 13 mapira na regione koji prethodno nisu bili dovedeni u vezu sa BMD. Ovi lokusi objašnjavaju 2,9% varijacije u BMD-ls i 1,9% u BMD-fn. Međutim, genomske asocijacije za rizik od fraktura nisu mogle da ostvare svoj pun potencijal u ovom okruženju. Zato je konzorcijum GEFOS proširen na konzorcijum GENOMOS u kom je naša skupina SLOPREVAL učestvovala sa preko 800 subjekata. Estrada K. et al. (2012). Nat Genet, 44(1), 491–501. Rezultati su pokazali 96 najbolje rangiranih SNP-a koji su dalje testirani u 31.016 slučajeva fraktura i kod 102.444 kontrolnih subjekata. Najzad smo bili u mogućnosti da identifikujemo 6 SNP-a (potencijalnih genetskih markera) koji su bili povezani sa rizikom od fraktura na nivou značajnosti od $p<5\times10^{-8}$: 18p11.21 (FAM210A), 7q21.3 (SLC25A13), 11q13.2 (LRP5), 4q22.1 (MEPE), 2p16.2 (SPTBN1) i 10q21.1 (DKK1). Analiziranjem 63 SNP-a, objašnjeno je 5,8% ukupnih genetskih varijacija u FN-BMD. Uz ove rezultate, naša istraživačka grupa takođe je izvršila profilisanje genske ekspresije celog genoma analizom primarnih osteoblasta dobijenih iz koštanog tkiva sa i bez osteoporoze. Trošt Z. et al. (2010) Bone, 46(1), 72–80. Diferencijalnu ekspresiju između osteoblasta iz osteoporotičnog i neosteoporotičnog koštanog tkiva pokazalo je 1606 gena, ali je 352 gena prešlo uobičajeni prag dvostruke vrednosti a prvih 50 od njih je potvrđeno pomoću qPCR. Jedan od ovih gena uveden je u funkcionalne studije na kojima se trenutno radi. Svi ovi rezultati ukazali su na nekoliko bioloških puteva

GENOME WIDE ASSOCIATION STUDIES IN NEW BIOMARKERS DISCOVERY: THE CASE OF OSTEOPOROSIS

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Osteoporosis is a polygenetic and multifactorial bone disease that affects one in three women after 50 years of age. As a chronic disease it is commonly diagnosed in a progressive stage when efficient treatment is difficult to achieve. Therefore, early markers, especially genetic markers, which are detectable during whole life and are not influenced by other factors like diet or life habits, are of great importance for early diagnosis, prognosis and treatment efficacy. The largest consortium searching the osteoporosis risk alleles, the GEFOS FP 7 EU-project, performed a meta-analysis of 5 GWA studies on two traits, Bone Mineral Density (BMD) and bone fractures. GWA studies on BMD of Lumbar Spine (BMD-ls) and BMD of Femoral Neck (BMD-fn) included in total 19.195 subjects. Altogether 20 loci, potential genetic markers, reached genome-wide significance ($P<5\times10^{-8}$) for BMD, of which 13 map to regions not previously associated with BMD. These loci explain 2.9% of variance in BMD-ls and 1.9% in BMD-fn. However, GWA on fracture risk was underpowered in this setting. Therefore, the GEFOS consortium expanded to the GENOMOS consortium where our SLOPREVAL cohort participated with over 800 subjects. Estrada K. et al. (2012). Nat Genet, 44(1), 491–501. Results showed 96 top ranked SNPs which were further tested in 31.016 fracture cases and 102.444 controls. Finally, we were able to identify 6 SNPs (possible genetic markers), which were associated with fracture risk at a significance level of $p<5\times10^{-8}$: 18p11.21 (FAM210A), 7q21.3 (SLC25A13), 11q13.2 (LRP5), 4q22.1 (MEPE), 2p16.2 (SPTBN1) and 10q21.1 (DKK1). With the analysis of 63 SNPs, 5.8% of the total genetic variance in FN-BMD was explained. Along with these results, our research group also performed the gene expression profiling whole genome array analysis of primary osteoblasts obtained from osteoporotic and non-osteoporotic bone tissue. Trošt Z. et al. (2010) Bone, 46(1), 72–80. 1606 genes were differentially expressed between osteoblasts from osteoporotic and non-osteoporotic bone tissue, but 352 genes exceeded the common 2-fold threshold and the top 50 of them were confirmed using qPCR. One of these genes was introduced to functional studies which are in progress right now. All these results highlighted several biological pathways influencing osteoporosis, such as the Wnt signaling pathway, mesenchymal cell differentiation, endochondral ossification process and RANKL/RANK/OPG

koji utiču na osteoporozu, kao što su signalni put Wnt, diferencijacija mezenhimskih ćelija, proces endohondralne osifikacije i sistem RANKL/RANK/OPG a ključni geni/molekuli u ovim putevima predstavljaju potencijalne dijagnostičke i farmakogenomske markere osteoporoze.

Ključne reči: osteoporoza, aleli rizika za osteoporozu, sistem RANKL/RANK/OPG, ključni geni /molekuli

ENZIMI PARAOKSONAZE I KARDIOVASKULARNE BOLESTI

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Enzimi paraoksonaze uključuju grupu od tri enzima: PON1, PON2 I PON3. Enzimi PON1 i PON3 su smešteni na HDL lipoproteinskoj čestici i sintetišu se uglavnom u jetri, dok je PON2 prisutan u svakoj ćeliji, vezan za ćelijsku membranu i ima ulogu ćelijskog antioksidansa. Dobro je poznata sposobnost ovih enzima da razgrade oksidovane lipide, da štite od oksidativnog stresa i da inhibiraju inflamaciju, tako da bar delimično, inhibiraju razvoj ateroskleroze. Lipoprotein visoke gustine (HDL) je jedan od glavnih antiaterogenih faktora zbog svog učestvovanja u reverznom transport holesterola, inhibiciji oksidativne modifikacije lipoproteina niske gustine (LDL) i slabljenju biološke aktivnosti oksidovanog LDL. Ovakva antioksidativna i antiaterogena svojstva HDL se povezuju sa različitim proteinima na HDL čestici, posebno enzimu paraoksonazi (PON). Patološka sredina u kardiovaskularnim bolestima snižava aktivnost ovog enzima. Sa druge strane nekoliko terapeutskih mogućnosti postoji koje mogu da povećaju i sačuvaju aktivnost ovog enzima. Tokom prethodnih deset godina ispitivali smo status ovog enzima i njegovu vezu sa kardiovaskularnim bolestima kod različitih grupa pacijenata povezanih sa razvojem ateroskleroze: koronarna srčana bolest, moždani udar, krajnji stadijum bubrežne bolesti, dijabetes. U nekim grupama smo našli povećan udeo RR PON1 192 fenotipa aktivnosti, koji nije aktivan u antioksidativnoj zaštiti. Kod svih grupa pacijenata našli smo značajno nižu PON1 enzimsku aktivnost u poređenju sa zdravim ljudima sa istim PON1 fenotipom. Mi trenutno ne raspolažemo niti definitivnom metodom niti znamo pravi fiziološki supstrat ovog značajnog enzima. PON1 doprinosi antioksidativnoj i ateroprotektivnoj funkciji HDL lipoproteina, tako da je potrebno istraživati dublje ovaj intrigantni protein.

system and key genes/molecules in these pathways represent potential diagnostic and pharmacogenomic markers of osteoporosis.

Keywords: osteoporosis, osteoporosis risk alleles, RANKL/RANK/OPG system, key genes/molecules

PARAOXONASE ENZYMES AND CARDIOVASCULAR DISEASE

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Paraoxonase (PON) enzymes include group of three enzymes: PON1, PON2, and PON3. High-density lipoprotein-associated PON1 and PON3 are synthesized primarily in the liver, whereas PON2 is ubiquitously expressed in human tissues, membrane-bound, and may act as a cellular antioxidant. It is well-known that all three enzymes have a capability to decompose oxidized lipids, protect against oxidative stress and to inhibit inflammation, thus, at least in part, inhibit atherosclerosis development. High-density lipoprotein (HDL) is one of the key anti-atherogenic player due to its contribution to the reverse cholesterol transport process, inhibition the oxidative modification of low-density lipoproteins (LDL) and attenuation the biological activities of oxidized LDL. Such anti-oxidant and anti-atherogenic properties of HDL have been attributed to various proteins associated with HDL, particularly the enzyme paraoxonase (PON). Pathological milieu in cardiovascular disease leads to reduction of paraoxonase activity. At the other side, several therapeutic modalities could have power to increase and preserve this enzyme's activity. During past 10 years we have investigated PON1 enzyme status and its relationship with cardiovascular disease in different patients groups related with atherosclerosis development i.e. coronary heart disease, stroke, end stage renal disease, diabetic patients. In some groups we found increased ratio of RR PON1 192 activity phenotype, which is not very able for antioxidative protection. In all patients groups we have found significantly lower PON1 enzymatic activity compared to healthy people with the same PON1 192 phenotype. At the moment, we do not have neither definitive method, nor we know real physiological substrate for this important enzyme. The PON1 enzyme contributes to the antioxidative and atheroprotective functions of

Ključne reči: paraoksonaza, ateroskleroza, oksidativni stress, kardiovaskularne bolesti

HDL, so further and deeper investigation of this intriguing protein is necessary.

Key words: paraoxonase, atherosclerosis, oxidative stress, cardiovascular disease

PRIMENA LC-MS/MS U ANALITICI MARKERA SINTEZE I APSORPCIJE HOLESTEROLA

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Epidemijske razmere gojaznosti u savremenom svetu i posledična učestalost niza hroničnih oboljenja ukazuju na značaj rasvetljavanja mehanizama putem kojih prekomerna telesna težina učestvuje u patogenezi različitih bolesti. Među najznačajnijim patofiziološkim aspektima gojaznosti su poremećaji metabolizma lipida, a stanovište savremene nauke je da potpuni uvid u karakteristike dislipidemije nije moguće postići rutinskim određivanjem parametara lipidnog statusa, već je potrebno izvršiti detaljnu analizu procesa koji leže u osnovi izmenjenog lipidnog profila. Procesi sinteze i apsorpcije holesterola su od suštinskog značaja za održavanje homeostaze holesterola i lipoproteina, te promene u njihovoj ravnoteži značajno doprinose razvoju dislipidemije. Endogeni prekursori holesterola koriste se kao indikatori brzine i efikasnosti sinteze, dok se kao markeri apsorpcije primenjuju biljni steroli, budući da je njihova intestinalna apsorpcija analogna apsorpciji holesterola. Analiza ovih lipidnih komponenti moguća je primenom naprednih metoda razdvajanja, detekcije i kvantifikacije. U našem istraživanju razvili smo metodu tečne hromatografije-tandem masene spektrometrije (LC-MS/MS) koja je omogućila razdvajanje i kvantitativnu analizu dezmosterola i latosterola kao markera sinteze, te kampesterola, stigmasterola i beta-sitosterola kao markera apsorpcije holesterola. Koristeći reverzno-faznu tečnu hromatografiju na C-18 analitičkoj koloni i hemijsku jonizaciju pod atmosferskim pritiskom kao konstantan deo analitičkog postupka, ispitivali smo različite metodološke pristupe koji su uključivali baznu hidrolizu estara holesterola, tečno-tečno ekstrakciju i ekstrakciju čvrstom fazom, te različite odnose komponenti mobilne faze i njihove protoke, a sa ciljem da se u potpunosti iskoriste analitičke prednosti LC-MS/MS i omoguće optimalni rezultati u

APPLICATION OF LC-MS/MS FOR ANALYSIS OF CHOLESTEROL SYNTHESIS AND ABSORPTION MARKERS

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Global epidemic of obesity and subsequent prevalence of various chronic diseases emphasize the importance of understanding the mechanisms by which excess body weight participates in the development of different pathological conditions. Dyslipidaemia is certainly among the most significant pathophysiological features of obesity. Current scientific approach considers routine determination of serum lipid parameters as insufficient for complete assessment of lipid disturbances. It is now accepted that it is possible to achieve a comprehensive insight into patients' lipid profiles only by means of detailed analysis of metabolic processes which essentially contribute to the development of dyslipidaemia. Cholesterol synthesis and absorption are profoundly involved in homeostasis of cholesterol and lipoproteins, so changes in these pathways significantly contribute to the onset of dyslipidaemia. Endogenous cholesterol precursors are used as surrogate markers of cholesterol synthesis, while plant sterols are considered as adequate markers of cholesterol absorption efficiency. Analysis of these lipid compounds is accomplished by use of advanced methods of separation, detection and quantitative assessment. In our research we derived a method of liquid chromatography – tandem mass spectrometry (LC-MS/MS) for separation and quantitative analysis of desmosterol and lathosterol as markers of cholesterol synthesis, as well as campesterol, stigmasterol and β-sitosterol as markers of cholesterol absorption. We used a reversed-phase LC C-18 analytical column and atmospheric pressure chemical ionization during the procedure and further explored different methodological approaches, including alkaline hydrolysis of cholesteryl esters, liquid extraction, solid phase extraction, as well as variations in composition and flow rate of mobile phase, with an intention to entirely exploit the

ispitivanju karakteristika procesa sinteze i apsorpcije holesterola u humanim uzorcima.

Ključne reči: gojaznost, poremećaja metabolizma lipida, endogeni prekursori holesterola

advantages of LC-MS/MS and to gain the best results in the assessment of cholesterol synthesis and absorption in human samples.

Keywords: obesity, disturbances of cholesterol metabolism, endogenous cholesterol precursors,

DIJAGNOSTIČKI POTENCIJAL SIMETRIČNOG I ASIMETRIČNOG DIMETILARGININA KAO NOVIH MARKERA ENDOTELNE DISFUNKCIJE

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Endotel sintetiše nekoliko vazodilatatornih faktora, uključujući i azot monoksid. Pod endotelnom disfunkcijom (ED) se podrazumeva trajna i neadekvatna aktivacija endotela. ED je uključena u patogenezu i klinički tok svih poznatih kardiovaskularnih oboljenja (KVB) a udružena je i sa rizikom od budućeg nepovoljnog ishoda KVB. Takođe, ED aktivno menja strukturu aterosklerotskog plaka čime se povećava mogućnost njegove rupture i razvoj akutnog koronarnog sindroma. Centralna uloga endotela u nastanku i razvoju ateroskleroze dovela je do otkrića novih biohemijskih markera za procenu endotelne funkcije i oštećenja. Asimetrični dimetil-L-arginin (ADMA) i njegov stereoizomer simetrični dimetil-L-arginin (SDMA) se kontinuirano stvaraju u organizmu putem metabolizma metionina i u toku katabolizma jedarnih proteina koji sadrže ostatke metilovanih arginina. ADMA deluje kao snažan inhibitor sinteze endotelnog azot monoksida i tako sprečava njegovo vazodilatatorno dejstvo na glatku muskulaturu krvnih sudova. Uloga SDMA je još uvek nejasna i predmet je naučnog istraživanja. SDMA najverovatnije deluje kao snažan kompetitivni inhibitor transporta L-arginina kroz ćelijske membrane. Bubrezi imaju ključnu ulogu u regulaciji nivoa ovih metilarginina u krvi. Vallance i saradnici su prvi objavili da su vrednosti ADMA i SDMA povećane kod bolesnika u završnoj fazi bubrežne bolesti. U radu Memon i saradnika zaključeno je da hs-CRP, IL-6 i SDMA mogu da predvide ED kod bolesnika sa hroničnom bubrežnom insuficijencijom (HBI) i bolesnika sa transplantiranim bubregom. Ludwigshafen Risk and Cardiovascular Health Study je pokazala da je serumska koncentracija SDMA nezavisno udružena sa kardiovaskularnim i ukupnim mortalitetom kod pacijenata koji su podvrgnuti koronarnoj angiografiji. Veliki broj studija je potvrdio da je nivo ADMA u pozitivnoj korelaciji sa faktorima rizika za koronarnu arterijsku bolest kao što su

DIAGNOSTIC POTENTIAL OF SYMMETRICAL AND ASYMMETRICAL DIMETHYLARGININE AS NEW MARKERS OF ENDOTHELIAL DYSFUNCTION

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The endothelium synthesizes several vasodilating factors, including nitric oxide. Endothelial dysfunction (ED) is a permanent and inappropriate endothelial activation. ED has been involved in the pathogenesis and clinical course of all known forms of cardiovascular disease (CVD) and is associated with future risk of adverse cardiovascular events. Also, ED actively changes plaque structure, promotes plaque rupture and lead to the development of acute coronary syndromes. The central role of the endothelium in the appearance and progression of atherosclerosis has led to the discovery of novel biochemical markers to estimate endothelial function and injury. Asymmetrical dimethyl-L-arginine (ADMA) and his stereoisomer symmetrical dimethyl-L-arginine (SDMA) are continuously derived from metabolism of methionine and catabolism of nucleus proteins containing methylated arginine residues. ADMA is a powerful endogenous inhibitor of endothelial nitric oxide synthesis. At this way, ADMA suppresses vascular smooth muscle cell vasodilatation. Role of SDMA is still unclear and is object of intensive scientific research. SDMA seems to be a potent inhibitor of L-arginine transport throw cell membranes. Both dimethylarginines are primarily cleared by the kidney. Vallance et al first reported increased plasma concentrations of ADMA and SDMA in a group of patients with end-stage renal disease. Memon et all have found that hs-CRP, IL-6 and SDMA can predict ED in patients with chronic kidney disease (CKD) and kidney transplant recipients. Ludwigshafen Risk and Cardiovascular Health Study showed that serum concentration of SDMA are independently associated with increased cardiovascular and all-cause mortality in patients undergoing coronary angiography. Numerous numbers of studies confirmed that ADMA level positively correlate with risk factors for coronary artery disease such as hypercholesterolemia, hyperhomocysteinemia, insulin resistance, age and hyper-

hiperholesterolemija, hiperhomocisteinemija, insulinska rezistencija, godine starosti i hipertenzija. ADMA je već uveliko potvrđen kao biomarker ED i marker rizika u različitim patološkim stanjima. SDMA će najverovatnije biti koristan kao kombinovani marker za detekciju bolesnika u veoma ranoj fazi HBI i za procenu rizika za nastanak KVB kod ovih bolesnika. ED je reverzibilan proces. Sve je više naučnih dokaza koji ukazuju na neophodnost uključivanja procene ED kao dijagnostičke metode u kliničku praksu. Dijagnostički postupci koji se danas koriste zasnivaju se na primeni neinvazivnih tehnika za procenu funkcionalnog stanja endotela (npr. protokom posredovana vazodilatacija brahijalne arterije zavisne od endotela). Pošto je etiologija ED komplikovana, malo je verovatno da će jedan biomarker biti dovoljan da obezbedi sve potrebne informacije za otkrivanje ED u ranoj fazi. Zato je neophodno definisati set reprezentativnih biomarkera koji će se meriti simultano kako bi bili u mogućnosti da detektuju različite funkcionalne aspekte ED u što ranijoj fazi nastanka. Veliki broj cirkulišućih markera ED, inflamacije i oksidativnog stresa je proučavan tokom poslednje decenije. Neophodno je razviti i adekvatne laboratorijske metode pogodne za rutinsku analizu. Novi biomarkeri će biti korisni za predikciju kliničke manifestacije kao i za optimalnu terapiju mnogih bolesti u čijoj osnovi leži poremećaj funkcije endotela. ADMA i SDMA će verovatno biti sastavni deo algoritama za procenu rizika.

Ključne reči: endotel, asimetrični dimetil-L-arginin (ADMA), simetrični dimetil-L-arginin (SDMA)

tension. ADMA is a well-established biomarker for ED. In addition, ADMA is a risk marker in a variety of pathological conditions. SDMA might be a useful combined parameter for detecting patients in very early stages of CKD and for determining their risk for developing CVD. ED is reversible process. There is growing evidence that ED assessment should be a part of the routine medical practice. Nowadays, ED may be testing by noninvasive techniques based on endothelial function assessment (such as endothelium-dependent flow-mediated vasodilatation of the brachial artery). Since the etiology of ED is complicated, it is therefore unlikely that a single biomarker will provide accurate information for ED occurrence in early stage. Accordingly, simultaneous measurement of several biomarkers and formulation of models that have incremental value in ED screening in comparison to single biomarker analysis would be useful. A number of circulating markers of ED, vascular inflammation and oxidative stress have been studied over the past decade. Application of these new biomarkers may require developing of laboratory methods adequate for routine analysis. New biomarkers may be useful in the prediction of clinical manifestation and optimal therapeutic interventions for many diseases underlying ED. ADMA and SDMA likely will find their way into risk assessment algorithms.

Keywords: endothelial dysfunction, asymmetrical dimethyl-L-arginine (ADMA), symmetrical dimethyl-L-arginine (SDMA)

POVEZANOST ADIPONEKTINA I KORONARNE SRČANE BOLESTI

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Adiponektin, protein poreklom iz adipoznog tkiva, u organizmu ostvaruje anti-inflamatorne, anti-aterogene i anti-apoptotske efekte posredstvom dva receptora označena kao AdipoR1 i AdipoR2. Različite studije koje su za predmet svog istraživanja imale ulogu adiponektina u koronarnoj srčanoj bolesti (KSB) dale su kontradiktorne rezultate. Na osnovu dosadašnjih studija može se izvesti zaključak da o zaštitnim efektima adiponektina možemo govoriti samo kod zdravih pojedinaca, dok su kod pacijenata sa KSB povišene vrednosti adiponektina udružene sa povećanim rizikom od smrti. Skorija istraživanja ukazuju da nishodna regulacija adiponektinskih receptora može biti odgovorna za izostanak zaštitnih efekata adiponektina kod pacijenata sa kardiovaskularinim bolestima. Cilj ove studije bio je da se ispita da li su nivoi

ASSOCIATION OF ADIPONECTIN AND CORONARY ARTERY DISEASE

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Adiponectin is a protein secreted by adipose tissue, which exerts its anti-inflammatory, anti-atherogenic and anti-apoptotic effects via two receptors, AdipoR1 and AdipoR2. Different studies on roles of adiponectin in coronary artery disease (CAD) have reported controversial results. It seems that increased adiponectin is playing protective roll only in healthy subjects without cardiovascular disease, while elevated concentrations in CAD patients are not associated with protective roll but increased risk of cardiovascular diseases mortality. Recent data suggested that downregulation of adiponectin receptors could be responsible for loss of protective adiponectin function in cardiovascular diseases. The objective of this study was to investigate if circulating adiponectin and the expression of adiponectin receptors in PBMCs are

cirkulišućeg adiponektina i ekspresije adiponektinskih receptora u mononuklearnim ćelijama periferne krvi pacijenata sa KSB promenjeni u odnosu na zdrave ispitanike. U studiji je učestvovalo 69 pacijenata (35 muškaraca, 34 žene) sa simptomima KSB. Svi pacijenti su podvrgnuti koronarnoj angiografiji i svi pacijenti koji su imali stenozu veću od 50% u bar jednoj od tri glavne koronarne arterije označenu su kao pacijenti sa značajnom stenozom (26 pacijenata). 33 zdrava pacijenta činila su kontrolnu grupu (KG). Nivoi cirkulišućeg adiponektina određeni su ELISA metodom, dok je ekspresija adiponektinskih receptora određena real-time PCR metodom. Nivoi adiponektina bili su značajno viši u plazmi pacijenta nego zdravih ispitanika ($p<0,001$). U KG uočene su negativne korelacije adiponektina sa BMI ($r=-0,613$, $p<0,001$), trigliceridima ($r=-0,490$, $p=0,004$), insulinom ($r=-0,312$, $p=0,024$) i HOMA IR indeksom ($r=-0,298$, $p=0,033$). U grupi pacijenata adiponektin je negativno korelirao sa glukozom ($r=-0,305$, $p=0,022$), insulinom ($r=-0,312$, $p=0,024$) i HOMA IR indeksom ($r=-0,298$, $p=0,033$). Nivoi AdipoR1 iRNK su bili značajno povišeni u KG u odnosu na pacijente sa i bez značajne stenoze ($p<0,001$, $p<0,001$, respektivno). Snižena genska ekspresija AdipoR1 i povećani nivoi cirkulišućeg adiponektina kod pacijenata sa i bez stenoze u odnosu na zdrave ispitanike ukazuju na moguću povezanost KSB i »adiponektinske rezistencije«, te da uprkos povišenim nivoima adiponektina njegovi zaštitni efekti izostaju što bi moglo igrati važnu ulogu u razvoju ateroskleroze.

Ključne reči: adiponektin, koronarna srčana bolest, ateroskleroza

changed in patients with CAD compared to healthy subjects. This study included 69 patients (35 males and 34 females) with presenting symptoms of CAD. They all underwent elective coronary angiography and subjects with 50% or greater stenosis in at least one major coronary artery were considered as patients with significant stenosis (26 patients). Control group (CG) was comprised of 33 healthy subjects. Circulating adiponectin was measured by ELISA method, while PBMCs' AdipoR1 and AdipoR2 mRNA levels were determined by real-time PCR method. Adiponectin was significantly higher in patients compared to healthy subjects ($p<0.001$). In CG, adiponectin negatively correlated with BMI ($r=-0.613$, $p<0.001$), TG ($r=-0.490$, $p=0.004$), insulin ($r=-0.386$, $p=0.038$) and HOMA IR index ($r=-0.444$, $p=0.016$), and positively with HDL-C ($r=0.672$, $p<0.001$). Negative correlation of plasma glucose ($r=-0.305$, $p=0.022$), insulin ($r=-0.312$, $p=0.024$) and HOMA IR index ($r=-0.298$, $p=0.033$) with adiponectin was observed in patients' group. AdipoR1 mRNA levels were significantly higher in CG compared to patients with and without significant stenosis ($p<0.001$, $p<0.001$ respectfully). Decreased AdipoR1 mRNA levels and increased circulating adiponectin levels in patients with and without significant stenosis compared to healthy subjects implies that CAD may be related to »adiponectin resistance« and that despite adiponectin increased levels, its protective effects could be diminished, which possibly plays a certain role in atherosclerosis development.

Keywords: adiponectin, coronary artery disease (CAD), atherosclerosis

UTICAJ ZAGAĐIVAČA ŽIVOTNE SREDINE KADMIJUMA I POLIHLOROVANIH BIFENILA NA NIVO HORMONA ŠTITASTE ŽLEZDE – EKSPERIMENTALNA STUDIJA

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Poznato je da izloženost zagađivačima iz životne sredine može dovesti do različitih zdravstvenih poremećaja, od kojih oštećenje endokrine funkcije predstavlja jedan od najznačajnijih problema današnjice. Endokrini ometači, jedinjenja različite hemijske strukture, mogu izazvati svoje efekte brojnim mehanizmima, pri čemu neki od njih utiču na funkciju štitaste žlezde i na taj način štetno deluju na rast ali i na me-

INFLUENCE OF ENVIRONMENTAL POLLUTANTS CADMIUM AND POLYCHLORINATED BIPHENYLS ON THYROID GLAND HORMONES LEVELS – AN EXPERIMENTAL STUDY

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It is well known that environmental exposure can result in different health outcomes, endocrine disruption currently representing one of the major concerns. Endocrine-disrupting chemicals (EDCs), which may belong to a variety of chemical classes, can exert their effects through a number of different mechanisms, some of them interfering with thyroid function, thus causing adverse effects on develop-

tabolizam i fiziologiju odraslog organizma. Cilj ovog eksperimentalnog rada je bio da se ispita uticaj produžene izloženosti niskim dozama kadmijuma (Cd) i polihlorovanim bifenilima (PCB) na nivoe trijodotiroksina (T3) i tiroksina (T4), kao biomarkere tiroidne funkcije. Ove dve hemikalije su izabrane obzirom na činjenicu da su perzistentni, globalni zagađivači životne sredine koji mogu da uđu u lanac ishrane, i visoko toksične hemikalije koje predstavljaju opasnost po zdravlje ljudi. Osim toga, ispitivan je i efekat istovremene izloženosti Cd i PCB na fukciju štitaste žlezde, imajući u vidu da u realnom svetu nismo izloženi pojedinačnim hemikalijama već njihovim smešama. Rezultati pokazuju da je oralno davanje 6 različitih doza Cd (u opsegu od 0,3–10 mg Cd/kg t.m.) tokom 28 dana dovelo do dozno-zavisnog smanjenja T3, dok je statistički značajno smanjenje T4 dokazano za doze ≥1,5 mg Cd/kg t.m, što ukazuje da je T3 osetljiviji prema Cd nego T4. Primenjene doze PCB (6 doza u opsegu od 0,5–16 mg PCB/kg t.m.) su izazvale jači efekat na T4 nego na T3: pri svim primenjenim dozama je kontatovano značajno smanjenje T4 koje je bilo dozno zavisno, dok su nivoi T3 bili značajno smanjeni pri dozama ≥2 mg PCB/kg. Dobijeni rezultati ukazuju da i Cd i PCB utiču na funkciju štitaste žlezde, ali da Cd ispoljava efekat perifernim mehanizmima, dok PCB ispoljavaju direktan uticaj na štitastu žlezdu. Rezultati takođe ukazuju na sinergizam Cd i PCB na nivou štitaste žlezde pri istovremenoj izloženosti, pri primeni 9 različitih smeša ovih hemikalija, kao rezultat različitih mehanizama kojima ometaju homeostazu štitaste žlezde.

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Ključne reči: zagađivači životne sredine, hormoni štitaste žlezde

ment, but also on metabolism and physiology of adults. The aim of this experimental study was to evaluate the effects of prolonged, relatively low cadmium (Cd) exposure and exposure to polychlorinated biphenyls (PCBs), on serum triiodothyroxine (T3), and thyroxine (T4), as biomarkers of thyroid function. These two chemicals were chosen since they are persistent and global environmental pollutants that can enter the food chain and as chemicals of high toxicity that pose a threat to human health. Moreover, the effect of co-treatment of Cd and PCBs on thyroid function was also investigated, having in mind that we are not exposed to a single chemical in the real world, but to the mixtures of chemicals. The results show that oral treatment of rats with 6 different doses of Cd (ranging from 0.3 to 10 mg Cd/kg b.w.) during 28 days induced dose-dependent decrease of T3 while statistically significant reduction of T4 was observed for doses ≥1.5 mg Cd/kg b.w. revealing that T3 hormone is more sensitive to Cd than T4. Applied doses of PCBs (6 doses in the range of 0.5–16 mg PCB/kg b.w.) induced more pronounced reduction of T4 than T3: significant decrease of T4 was observed for all applied doses and was dose dependent while T3 levels were significantly reduced for doses ≥2 mg PCB/kg. These results indicate that exposure to both Cd and PCBs interferes with thyroid function, and while Cd influences mainly peripheral generation of T3, PCBs predominantly induce direct effect on thyroid gland. The results also implicate synergistic effects of co-exposure to Cd and PCBs (using 9 different mixtures of Cd and PCBs) on thyroid function, as a consequence of their effects on different levels of thyroid homeostasis.

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Keywords: environmental pollutants, thyroid gland hormones

FAMILIJARNA HIPERHOLESTEROLEMIJA: DIJAGNOSTIČKE I TERAPIJSKE MOGUĆNOSTI

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Familijarna hiperholesterolemija (FH) je nasledni poremećaj metabolizma lipoproteina koji se karakteriše povišenim nivoom ukupnog i LDL-h i najčešće normalnim ili lako povišenim nivoom triglicerida, uz

FAMILIAR HYPERCHOLESTEROLEMIA: DIAGNOSTIC AND THERAPEUTIC POSSIBILITIES

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Familiar hypercholesterolemia (FH) is hereditary disorder of lipoprotein metabolism charactarized by elevated levels of total and LDL-cholesterol and usually normal or slightly elevated triglyceride levels,

često (ali ne i obavezno) prisustvo vidljivih (ili palpabilnih) depozita holesterola u koži i tetivama (ksantomi), na kapcima oka (ksantelazme) i u kornei (arcus cornealis). Ovaj poremećaj se karakteriše izuzetno povišenim rizikom nastanka prematurne ateroskleroze (i pre 40 godine života) u odnosu na opštu populaciju. U osnovi nastanka FH je mutacija gena koji kodira sintezu proteina LDL receptora i koji se nalazi na kratkom kraku 19 hromozoma. FH se nasleđuje autozomno dominantno, heterozigotna forma se u populaciji javlja sa učestalošću 1:500 dok je homozigotna forma FH značajno ređa (učestalost 1:1000000). Međutim, iako je FH u osnovi monogenska bolest, fenotipska ekspresija, u smislu nivoa holesterola, pojave i intenziteta ateroskleroze značajno varira, čak i u osoba koje imaju isti genetski defekt. Faktori sredine, u prvom redu životne navike, ishrana, pušenje, gojaznost mogu imati značajnu ulogu u kliničkoj ekspresiji FH. Zlatni standard za postavljanje dijagnoze FH je genetska analiza, ali kako to nije rutinska procedura, za postavljanje kliničke dijagnoze FH neophodna je kombinacija podataka dobijenih iz laboratorijskih analiza, fizikalnog pregleda i porodične i lične anamneze. U pokušaju ujednačavanja arbitrarnih kriterijuma za postavljanje kliničke dijagnoze FH danas se preporučuje upotreba modela Dutch MEDPED razvijenog poslednjih godina koji predstavlja skor sistem kombinacije laboratorijskog nalaza ukupnog i LDL-h, fizikalnog nalaza, lične i porodične anamneze. Navedeni kriterijumi su jednostavni za upotrebu u kliničkoj praksi i obuhvataju sve relevantne kriterijume za dijagnozu FH. Kada se dijagnostikuje FH zahteva pravovremeno uvođenje medikamentne terapije, prvenstveno tretman statinima uz odgovarajuću dijetu, dok primena samo dijetetskog režima u ovih pacijenata nema značajnog efekta. U slučajevima težeg oblika FH primenjuje se tretman LDL afereze koji se već 9 godina uspešno sprovodi na našoj klinici.

Ključne reči: familijarna hiperholesterolemija, prematurna ateroskleroza

often (but not necesserily) with the presence of visible (or palpable) deposits of cholesterol in the skin and tendons (xanthomas), arround eyelids (xanthelasme) and the cornea (arcus cornealis). This disorder is characterized by an extremly high risk od premature atherosclerosis (even before of 40 years of age) compared to the general population. The main reason for the development of the FH is mutation of the gene encoding the protein synthesis of LDL receptors located on the short arm of chromosome 19. FH is inherited in an autosomal domminant pattern, heterozygous form occurs in general population at a frequency of 1:500 while the homozygous form of FH is significantly less common (incidence of 1:1000000). However, although FH is monogenic disease, phenotypic expression in terms of cholesterol levels and occurence and intensity of atherosclerosis varies considerably even in individuals who have the same genetic defect. Environmental factors, notably lifestyle habits, diet, smoking and obesity may play a significant role in the clinical expression of FH. The gold standard for the diagnosis of FH is a genetic analysis, but that is not a routine procedure, for setting up clinical diagnosis of FH combination of data obtained from laboratory analysis, physical examination and family and personal history are required. In attempt to harmonize the arbitrary criteria for the clinical diagnosis of FH now it is reccomended to use the Dutch MEDPED model developed in recent years which is a score system that combines laboratory results of total and LDL-ch, physical exam and personal and family medical history. The above quoted criteria are simple to use in clinical practice and include all the relevant criteria for the diagnosis of FH. When the diagnosis of FH is established the timely introduction of drug therapy is required, especially statin treatment with an appropriate diet, while the use of dietary regimen only in these patients has no significant effect. In cases of severe form of FH the LDL apheresis treatment is applied, this form of treatment is successfully implemented for 9 years now in our Clinic.

Keywords: familiar hypercholesterolemia, prematurne aterosklerosis

TERAPIJSKI MONITORING LEKOVA: KOJI LEKOVI, ZAŠTO, KADA I KAKO?

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Terapijski monitoring lekova (Therapeutic Drug Monitoring – TDM) podrazumeva merenje koncentracije leka u biološkom materijalu i kliničko tumačenje dobijenih rezultata i zahteva poznavanje farmakokinetike, vremena uzorkovanja biološkog materijala, istorije primene drugih lekova i kliničkog stanja pacijenta. TDM je koristan ako su ispunjeni sledeći kriterijumi: uzak terapijski raspon koncentracija, značajna farmakokinetička varijabilnost, proporcionalan odnos između koncentracija u plazmi i kliničkog odgovora, definisan terapijski raspon koncentracija, potencijalni problem u komplijansi pacijenta, dostupnost odgovarajućih analitičkih metoda. Primeri lekova za koje postoji potreba za TDM su: digoksin, litijum, fenitoin, ciklosporin, sirolimus, takrolimus, karbamazepin, valproinska kiselina, vankomicin, lamotrigin, amjodaron, flekainid. Uzorak biološkog materijala za TDM treba uzeti kada su koncentracije u stabilnom stanju ravnoteže (4–5 poluvremena eliminacije od početka terapije), osim u slučaju ispitivanja toksičnosti. Zbog promene koncentracija leka u toku intervala doziranja, vreme uzorkovanja biološkog materijala je veoma značajno. Najniža varijabilnost u koncentraciji u okviru intervala doziranja je neposredno pre primene naredne doze leka kada se očekuju minimalne koncentracije. Za lekove sa dugim poluvremenom eliminacije (fenobarbiton, amjodaron) uzorak biološkog materijala je moguće uzeti u toku trajanja intervala doziranja. Takođe značajan korak u obezbeđivanju pouzdanih TDM rezultata je primena pouzdane, reproduktivne i validirane bioanalitičke metode. Za tumačenje TDM rezultata potrebne su informacije o vremenu uzorkovanja biološke tečnosti, vremenu primene poslednje doze leka, režimu doziranja leka, indikaciji za TDM, istovremeno primenjenim lekovima i kliničkom stanju pacijenta. Iako je TDM podrška a ne zamena za kliničku procenu, kada se pravilno koristi predstavlja veoma korisno i značajno »sredstvo« u tumačenju kliničkih situacija.

Ključne reči: terapijski monitoring lekova, farmakokinetika, tumačenje TDM

THERAPEUTIC DRUG MONITORING: WHICH DRUGS, WHY, WHEN AND HOW TO DO IT?

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Therapeutic drug monitoring (TDM) involves measuring drug concentrations in various biological fluids and clinical interpretation of the result. This requires knowledge of the pharmacokinetics, sampling time, drug history and the clinical condition of the patient. TDM is useful if the following criteria are met: narrow therapeutic range, significant pharmacokinetic variability, a reasonable relationship between plasma concentrations and clinical effects, established target concentration range, potential patient compliance problem, availability of appropriate analytical techniques. Drugs suitable for TDM are: digoxin, lithium, phenytoin, cyclosporine, sirolimus, tacrolimus, carbamazepine, sodium valproate, vancomycin, lamotrigine, amiodarone, flecainide. TDM samples should be taken at steady state (4–5 half-lives after starting therapy) unless there are concerns about toxicity. As the drug concentration changes during the dosing interval the timing of the collection of the sample is important. The best sampling time is in the pre-dose or through phase just prior to a maintenance dose, when a drug is administered by multiple oral doses. The least variable point in the dosing interval is just before the administration of the next dose and this pre-dose or trough concentration is usually measured. For drugs with long half-lives (phenobarbitone and amiodarone) samples can be collected at any point of the dosage interval. Another important element in providing valuable TDM data is reliable analytical methodology. The information required for TDM data interpretation includes the time of the sample collection, the time of the last dose, the dosage regimen, indication for TDM, drug history and clinical condition of patient. Although TDM is complementary to and not a substitute for clinical judgement when used properly TDM is very useful and valuable tool in clinical settings.

Keywords: therapeutic drug monitoring, pharmakokinetics, TDM data interpretation

PRAĆENJE BIOHEMIJSKIH PARAMETARA U PROCENI BEZBEDNOSTI IMUNOSUPRESIVNE TERAPIJE

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Savremeni imunosupresivni protokoli efikasno preveniraju odbacivanje presađenog organa. Upravljanje rizikom, individualizacija i optimizacija terapije kod dugoročne izloženosti imunosupresivnim lekovima podrazumeva klinički, terapijski i biohemijski monitoring transplantiranih pacijenta. Pronalaženje novih biomarkera omogućilo bi rano otkrivanje molekularnih mehanizama koje su u osnovi disfunkcije ciljnih organa i optimizaciju terapije bez ispoljavanja njihovih neželjenih efekata. Savremeni imunosupresivni protokol podrazumeva primenu kalcineurinskog inhibitora (ciklosporin, takrolimus), mikofenolne kiseline i kortikosteroida. Ovi lekovi se karakterišu farmakokinetičkom varijabilnošću i ispoljavaju ozbiljne neželjene efekte na nivou bubrega, jetre i nervnog sistema. Cilj ovog rada je da se ukaže na značaj povećanja bezbednosti terapije imunosupresivnim lekovima pomoću redovnog biohemijskog monitoringa i pronalaženja novih biomarkera oštećenja ciljnih organa. U skladu sa dobrom kliničkom praksom, transplantiranim pacijentima se istovremeno u definisanim vremenskim intervalima određuje nivo imunosupresupresiva u krvi i izloženost organizma leku, a biohemijskim monitoringom se vrši procena funkcije transplantiranog organa i bezbedonosnog profila terapije. Na osnovu dobijenih rezultata vrši se individualizacija imunosupresivne terapije kreiranjem jedinstvenog optimalnog doznog režima za svakog pacijenta. Pored zlatnog standarda za procenu brzine glomerularne filtracije-klirensa kreatinina, postoje pokušaji da se u rutinsku kliničku praksu uvedu novi specifični biomarkeri ranog oštećenja bubrega i odbacivanja transplanta (NGAL, IL18, KIM1). Kod stabilnih pacijenata sa presađenim bubregom ukupni holesterol, LDL i vaskularni adhezioni molekul (VCAM) potvrđeni su kao jaki i nezavisni prediktori bubrežne disfunkcije. Povećana aktivnost cistitin C dovodi se u vezu sa oštećenjem bubrega, dok u kombinaciji sa nivoom ukupnog bilirubina može ukazati na insuficijenciju jetre. Novi obećavajući markeri ranog otkrivanja oštećenja jetre uzrokovanih lekovima su microRNA, miR-122. Protein koji se proizvodi u jetri, kalistin, takođe može biti značajan u proceni zdravstvenog stanja jetre. Pojava neurotoksičnosti pri primeni imunosupresiva varira zavisno od transplantiranog organa i

MONITORING OF BIOCHEMICAL PARAMETERS IN THE ASSESSMENT OF IMMUNOSUPPRESSIVE THERAPY SAFETY

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Contemporary immunosuppressive protocols prevent successfully graft rejection. Clinical, therapeutic and biochemical monitoring of transplant patients are essential for dosage optimization and risk management in long-term immunosuppressive drugs exposition. Finding of new biomarkers will provide early identification of molecular mechanisms that are the basis of target organs dysfunction as well as the optimization of pharmacotherapy with a view to reduce adverse effects. Contemporary immunosuppressive protocols include implementation of calcineurine inhibitor (tacrolimus or cyclosporine A), micophenolic acid and corticosteroid. These drugs are characterized by pharmacokinetic variability and serious adverse effects on kidney, liver and nervous system. The objective of this study is to show the importance of routine biochemical monitoring and investigation of new biomarkers of target organs damage and all aiming at increasing level of safety immunosuppressive therapy. In accordance to good clinical practice, transplant recipients have their simultaneous and regular determination of immunosuppressive drug concentration and exposure as well as biochemical monitoring of the transplanted organ function and safety profile of the therapy. The obtained results will allow the individualization of immunosuppressive therapy through creation of an optimal dosage regimen for each patient. Besides the golden standard for glomerular filtration rate assessment-clearance creatinine, there are attempts for introduction in routine clinical practice new specific and early biomarkers of kidney damage and transplant rejection (NGAL, IL18, and KIM1). Total cholesterol level, such as LDL and VCAM are strong and independent predictors of kidney dysfunction in stable renal transplant patients. Increased activity of cystitin C is connected with kidney insufficiency, while its combination with a level of total plasma billirubin may have a role in early diagnosis of hepato insufficiency. A microRNA, miR-122, has been discovered as a promising biomarker for the early prediction of drug induced liver injury. Kallistatin, that a plasma protein produced by the liver, can be a useful and reliable diagnostic indicator of hepatic health status. The incidence of neurotoxicity from immunosuppressive drugs varies by the organ

odabranog leka. U cilju ranog otkrivanja neurotoksičnih efekata intenzivno se ispituju novi biomarkeri (microRNAs, translokator protein, ubikvitin C-terminalna hidrolaza L1, protein udružen sa mikrotubulima 2) koji bi značajno pomogli optimizaciji terapije i smanjenju toksičnih efekata. Uvođenje novih biomarkera koji ukazuju na molekulske mehanizme organskih oštećenja značajno unapređuje upravljanje rizikom od pojave neželjenih događaja pri primeni kompleksne i neophodne imunosupresivne terapije kod transplantiranih pacijenata.

Ključne reči: biomarkeri, upravljanje rizikom, imunosupresivna terapija

transplanted and used drug. Regarding the difficulties in early neurotoxicity detection, it is crucial to investigate the possibility of implementation new biomarkers (microRNAs, translocator protein, ubiquitin C-terminal hydrolase's L1 or microtubule associated protein 2), which will significantly improve the optimization of therapy and reducing adverse effects. The introduction of new biomarkers that predict efficacy and safety for a given drug therapy become increasingly important for treatment strategy during the necessary and complex transplant patients immunosuppressive therapy.

Key words: biomarkers, risk management, immunosuppressive therapy

PRIMENA HPLC/MS/MS/TEHNIKE U KLINIČKO HEMIJSKOJ DIJAGNOSTICI

Duško Mirković

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Tokom protekle decenije je došlo do nagle ekspanzije u primeni HPLC tehnike sa masenom spektroskopskom detekcijom, i to u obliku takozvane »tandem mass« HPLC tehnike. Ovo se odnosi i na primenu iste u kliničko hemijskoj praksi. U ovom trenutku najvažnije oblasti primene u kliničkoj dijagnostici su u »TDM«-u, sa posebnim akcentom na određivanju svih imunosupresiva, analitici biogenih amina, prvenstveno kateholamina i metanefrina, svih hormonskih vrsta sa steroidnim jezgrom (mineralo i glukokortikoidi, androgeni i estrogeni), vitamina D i svih derivata istog, uključujući i karakteristične epimerne oblike, metilmaloničnu kiseline i drugo. Posebno poglavlje u primeni iste se odnosi na kliničko toksikološku praksu. U svim slučajevima HPLC MS/MS nudi analitičku specifičnost superiorniju u odnosu na kompetitivna joj imunoodređivanja različitog tipa, odnosno veću propusnu moć i u odnosu na pomenuta imuno određivanja, kao i klasičnu HPLC tehniku. Međutim, primena HPLC MS/MS i dalje podrazumeva veći stepen analitičke stručnosti, što u mnogim kliničko hemijskim laboratorijama više nije problem. Sa analitičkog aspekta metode koje se koriste u kliničkim laboratorijama u svetu kao rutinske, se odnose na kvantitativnu analizu analita relativno male molekulske mase. Kao izazov u nastupajućem bliskom periodu je uvođenje i određivanja proteinskih analita, sa čime se počelo u nekim laboratorijama u svetu. Radi lakšeg razumevanja materije u predavanju će na početku biti u najkraćem iznet podsetnik o

APPLICATION HPLC/MS/MS TECHNIQUES IN CLINICAL CHEMISTRY DIAGNOSTICS

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Over the past decade there has been a rapid expansion in the application of HPLC techniques with mass spectroscopic detection, in the form of so-called »tandem mass« HPLC techniques. This also applies the use of the same technique in clinical chemistry practice. At this point the most important fields of application in clinical diagnostics is in the »TDM« stay, with special emphasis on the determination of immunosuppressant, analytics of biogenic amines, primarily catecholamines and metanephrines, all kinds of hormones, with steroid nucleus (minerals and glucocorticoids, androgens and oestrogens) vitamin D, and all derivatives thereof, including the epimeric forms thereof, determination of methyl malonic acid, and so on. A special chapter in applying the same applies to the practice of clinical toxicology. In all cases HPLC MS/MS provides the analytical specificity superior to her competitive immunoassays of various types, and higher throughput in relation to the above-mentioned immune determination, as well as classical HPLC technique. However, the use of HPLC MS/MS still implies a higher degree of analytical expertise, which in many clinical chemistry laboratories is no longer a problem. From the analytical aspect of the methods used in clinical laboratories in the world as a routine relating to the quantitative analysis of analytes relatively low molecular weight. As a challenge in the upcoming short term, the introduction and determination of protein analytes, with which began in someone laboratories in the world. In

fiziko-hemijskim principima masene spektroskopije, da bi nakon istog na karakterističnim primerima bila elaborirana primena kvantitativne analize kliničko hemijskih parametara iz gore navedenih grupa, sa posebnim akcentom na one parametre koji se određuju imuno tehnikama, odnosno klasičnom HPLC tehnikom.

Ključne reči: HPLC tehnika, klinička hemija, klinička dijagnostika

order to facilitate understanding of the matter in the lecture will initially be set forth in the shortest reminder of the physical-chemical principles of mass spectroscopy, and after the same characteristic examples was elaborated application of quantitative analysis of clinical chemical parameters of the above groups, with special emphasis on those parameters which are determine immune techniques or classical HPLC.

Keywords: HPLC techniques, clinical hemistry, clinical diagnostics

25 GODINA PRIMENE PCR METODOLOGIJE U MEDICINSKOJ DIJAGNOSTICI – IDENTIFIKACIJA MIKROBA I HUMANA GENOMIKA

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U osnovi dijagnostičkih metoda molekularne genetike najčešće se nalazi lančana reakcija DNK polimeraze – PCR. Ovom enzimskom reakcijom se umnožavaju delovi gena ili genoma koji se ispituju kako bi se brže i jednostavnije analizirali. PCR metoda zahteva niz specifičnih uslova koji su neophodni za realizaciju veoma složenih koraka. Poseban problem u radu sa PCR-om su lažni negativi, koji se rešavaju obaveznom upotrebom različitih internih i eksternih kontrola i lažni pozitivi, koji se izbegavaju pomoću niza fizičkih i biohemijskih metoda kao što je podeljen rad u tri prostorije, upotreba UV zračenja i specifičnih enzimatskih sistema za degradaciju viška DNK molekula. Dijagnostički PCR testovi su razvijeni u cilju identifikacije mikroba kada imunološke metode ne daju jasan rezultat kao što je razlikovanje prošle od tekuće infekcije i određivanje genotipova i/ili rezistentnih sojeva različitih patogena za potrebe uspostavljanja adekvatne terapije. Druga oblast primene PCR-a u kliničkoj praksi je identifikacija mutacija koje dovode do pojave bolesti i detekcija genskih polimorfizama koji povećavaju rizik za pojavu bolesti. Pored toga, PCR identifikacija humanih genskih polimorfizama našla je primenu kod izbora vrste i doze leka tzv. farmakogenomika. PCR sve više dopunjava pa i potpuno zamenjuje metode citogenetike kao što je određivanje hromozomskih duplikacija, delecija i translokacija.

Ključne reči: molekularna genetika, PCR metoda, genski polimorfizmi

25 YEARS OF PCR METHODOLOGY IMPLEMENTATION IN MEDICAL PRACTICE – PATHOGEN DETECTION AND HUMAN GENOMICS

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The main molecular genetic method for in vitro diagnostic is DNA polymerase chain reaction – PCR. This is enzymatic reaction for amplification of genes or genome parts with aim to make faster and easier analyzes. PCR method requires a number of specific conditions necessary for the realization such a complex reaction. A particular problems in working with PCR are false negative, which are solved by construction various internal and external control and false positives, which are avoided by using a series of physical and biochemical methods, such as working in into three divided rooms, the use of UV radiation and specific enzyme system for the degradation of the excess of DNA molecules. The diagnostic PCR assays were developed in many different area of medical practice. In identification of microbes PCR is the method of choice when immunological approaches do not provide a clear result, for example: differentiation between the past and current infection and determination of genotypes or resistant strains for the purposes of establishing adequate therapy. Other areas of PCR diagnostic applications are the identification of mutations that lead to diseases and detection of genetic polymorphism that increases the risk of disorder appearance. In addition, PCR identification of human gene polymorphism has been applied in ordering of a particular type and dose of medication – pharmacogenomics. PCR is increasingly supplementing or even completely replaces cytogenetic methods such as the determination of chromosomal duplications, deletions and translocations.

Keywords: molecular genetic method, PCR method, genetic polymorphism

PRIMENA PREPORUKA SVETSKE ZDRAVSTVENE ORGANIZACIJE (SZO) U STANDARDIZACIJI PROCEDURE ZA IZRADU SPERMOGRAMA

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Pravilno izvođenje analize spermograma zahteva implementaciju procedure koju propisuje SZO. Standardizacija procedure je potrebna da bi se obezbedila tačna procena kvaliteta sperme, usvojili referentni intervali preporučeni u petom izdanju preporuka SZO (WHO laboratory manual for the examination and processing of human semen, fifth edition, 2010) i harmonizovala interpretacija rezultata. Standardizacijom analize spermograma jasno je definisan značaj primene preanalitičke, analitičke i postanalitičke faze u izradi spermograma. Peto izdanje preporuka SZO sastoji se iz tri dela: analize sperme, pripreme sperme i kontrole kvaliteta. Analiza sperme daje informacije o količini (broj spermatozoida) i kvalitetu spermatozoida (pokretljivost i morfologija). U delu koji se odnosi na pripremu sperme za analizu detaljno su opisane metode i reagensi koji se za to koriste. Da bi se dobili pouzdani rezultati relevantni za dijagnostiku i lečenje neophodno je da svaka laboratorija u kojoj se ova analiza izvodi primenjuje kontrolu kvaliteta, koja je detaljno opisana u petom izdanju. Analiza spermograma predstavlja surogat merenja muškog fertiliteta. Po preporukama SZO izmenjene referentne vrednosti, mogu da pomognu u prognozi fertiliteta ili dijagnozi infertiliteta muškaraca.

Ključne reči: spermogram, preporuke, SZO

IMPLEMENTATION OF THE RECOMMENDATIONS OF THE WORLD HEALTH ORGANIZATION (WHO) IN STANDARDIZATION PROCEDURES FOR MAKING SPERMOGRAM

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The proper execution of semen analysis requires implementation of the procedure proposed by the WHO. Standardization of the procedure is needed to provide an accurate assessment of the quality of sperm, to adopt reference intervals recommended in the fifth edition of the WHO recommendations (WHO laboratory manual for the examination and processing of human semen, fifth edition, 2010) and to harmonize interpretation of the results. Standardization of semen analysis clearly defines the importance of applying the preanalytical, analytical and postanalitical phase in the development of sperm. The fifth edition of the WHO recommendations consists of three parts: semen analysis, sperm preparation and quality assurance. Semen analysis provides information about the quantity (number), and quality (motility and morphology) of the sperm. In the part which relates to the preparation of sperm for analysis are described methods and reagents for this use. In order to obtain reliable results relevant to diagnosis and treatment it is essential that each laboratory where this analysis is performed applies quality control, which is described in detail in the fifth edition. Analysis of semen represents a surrogate measurement of male fertility. According to recommendation of the WHO, modified references values in the sperm analysis could provide more accurate prognosis of fertility or diagnosis of infertility.

Keywords: spermogram, recommendations, WHO

ISTOVREMENO SPROVOĐENJE FARMAKOGENETSKIH ISPITIVANJA, BIOHEMIJSKOG I TERAPIJSKOG MONITORINGA PREDSTAVLJA OSNOVU PERSONALIZOVANE MEDICINE

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Lekovi, koji se karakterišu malim terapijskim indeksom, izraženom farmakokinetičkom varijabilnošću u i/ili ozbiljnim neželjenim efektima, podležu rutinskom terapijskom, kliničkom i/ili biohemijskom monitoringu u cilju postizanja optimalnih terapijskih ishoda. Istovremeno određivanje biohemijskih i kliničkih parametara kao i koncentracije ovih lekova u biološkim materijalima, unapredili su efikasnost i bezbednost lečenja mnogih bolesti. Pokazano je da genetski faktori imaju udeo od 20–95% u varijabilnosti farmakokinetičkih parametara i kliničkih efekata lekova. Genotipizacija određenih metaboličkih enzima, transportnih i ciljnih proteina može doprineti pravilnom izboru leka, doznom režimu i odrediti učestalost uzorkovanja biološkog materijala radi terapijskog i/ili biohemijskog monitoringa, što predstavlja osnovu personalizovane medicine. Cilj ovog rada je da ukaže na značaj uvođenja farmakogenetskih ispitivanja u kliničku praksu zbog njihovog doprinosa terapijskom i biohemijskom monitoringu u ostvarivanju efikasne i bezbedne farmakoterapije. U cilju postizanja optimalne imunosupresivne terapije, pacijenti sa transplantiranim bubregom na terapiji takrolimusom rutinski podležu određivanju koncentracije leka u punoj krvi i proceni funkcije bubrega, merenjem serumske koncentracije kreatinina i uree. Genotipizacija citohrom P450 (CYP) 3A5 izoenzima, prediktora farmakokinetičke varijabilnosti takrolimusa, i njegovo razmatranje u vezi sa izborom inicijalne doze leka, doprinelo bi individualizaciji terapije u prvim danima nakon transplantacije, kada se terapijskim monitoringom ne može postići adekvatna kontrola izloženosti leka. Studije su pokazale povezanost između varijabilnosti u farmakokinetičkim parametrima i kliničkim efektima lekova i varijabilnosti gena koji kodiraju njihove metaboličke enzime, transportne i ciljne proteine, poput CYP2C9 i VKORC1 gena i varfarina, CYP2C19 gena i klopidogrela, CYP2D6 gena i tamoksifena, HLA–B gena i alopurinola, abakavira, karbamazepina, SLCO1B1 gena i simvastatina, DYPD gena i fluoropirimidinskih antineoplastika i IFNL3 gena i peginterferona-alfa. Razmatranje ove povezanosti u okviru kliničke prakse može omogućiti ranu selekciju pacijenata prema riziku od neefikasnosti terapije i

SIMULTANEOUS IMPLEMENTATION OF PHARMACOGENETICS TESTING, BIOCHEMICAL AND THERAPEUTIC MONITORING REPRESENTS THE BACKBONE OF PERSONALIZED MEDICINE

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Drugs, characterized by a narrow therapeutic index, marked pharmacokinetic variability and/or serious adverse effects, have routine therapeutic, clinical and/or biochemical monitoring with a view to achieve optimal therapeutic outcomes. Simultaneous determination of biochemical and clinical parameters as well as the concentrations of these drugs in biological materials, has enhanced the efficiency and safety of the treatment of many diseases. It has been shown that genetic factors have a share of 20–95% in the variability of pharmacokinetic parameters and clinical effects of drugs. Genotyping of certain metabolic enzymes, transport and target proteins may contribute to the proper selection of drug, dosage regimen, and to determine the frequency of biological material sampling for therapeutic and/or biochemical monitoring, which is the basis of personalized medicine. The aim of this work is to indicate the importance of pharmacogenetics testing in clinical practice due to their contribution to the therapeutic and biochemical monitoring in achieving effective and safe pharmacotherapy. The renal transplant recipients on tacrolimus treatment have routinely determination of drug concentrations in whole blood and assessment of renal function by measuring serum concentrations of creatinine and urea in order to achieve optimal immunosuppressive therapy. Genotyping of cytochrome P450 (CYP) 3A5 isoenzyme, predictor of tacrolimus pharmacokinetic variability, and its consideration regarding the choice of drug's initial dose, would contribute to individualization of a therapy in the first days after transplantation, when adequate control of drug exposure cannot be achieved using therapeutic monitoring. Studies showed the association between drugs' variability in pharmacokinetics and clinical effects and variability of genes encoding their metabolic enzymes, transport and target proteins, such as CYP2C9 and VKORC1 genes and warfarin, CYP2C19 gene and clopidogrel, CYP2D6 gene and tamoxifen, HLA-B gene and allopurinol, abacavir and carbamazepine, SLCO1B1 gene and simvastatin, DYPD gene and fluoropyrimidines and IFNL3 gene and peginterferon-alfa. Considering this association in the framework of clinical

pojave neželjenih efekata, s tim da farmakogenetska ispitivanja nisu zamena za postojeći terapijski i biohemijski monitoring, ali mogu značajno unaprediti individualizaciju terapije.

Ključne reči: farmakogenetika, personalizovana medicina, terapijski monitoring leka, biohemijski monitoring

practice may provide early selection of the patients to the risk of therapy ineffectiveness and occurrence of the adverse effects. Still, pharmacogenetics testing is not a substitute for existing therapeutic and biochemical monitoring, but can significantly improve the individualization of a therapy.

Key words: pharmacogenetics, personalized medicine, therapeutic drug monitoring, biochemical monitoring

OKSIDATIVNI STRES I BOLESTI SADAŠNJICE

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Današnji način života koji podrazumeva velika mentalna opterećenja, nedovoljne količine odmora i sna, jednoličnu (brzu) ishranu i uživanje prekomerne količine alkohola, kafe i cigareta, uveliko doprinosi povećanju oksidativnog stresa u našem organizmu. Emocionalni stres, UV zračenje, toksične materije iz okruženja, dim cigarete i drugi faktori, takođe generišu slobodne radikale. Tokom oksidativne fosforilacije u mitohondrijama, ćelija proizvodi energiju i sintetišu se slobodni radikali. Pri nižim koncentracijama, slobodni radikali funkcionišu kao značajni medijatori ćelijske signalizacije i imunološkog odgovora-procesa koji su izuzetno važni za opstanak ćelije i organizma u celini. U fiziološkim uslovima, slobodni radikali se brzo neutralizuju dejstvom sistema antioksidantne zaštite, koji je u ljudskom organizmu dobro organizovan i funkcionalno podeljen u više nivoa. Kada, iz nekog razloga, nadvlada sinteza slobodnih radikala ili oslabi mehanizam antioksidantne zaštite, nastupa stanje koje zovemo oksidativni stres. Oksidativni stres se smatra »krivcem« za veliki broj patoloških stanja i bolesti. Mnogi naučni radovi ukazuju, da je oksidativni stres uključen u nastanak i progresiju većine zdravstvenih problema savremenog čoveka, preko nekoliko mehanizama kao što su: inaktivacija značajnih metaboličkih enzima i oštećenje ćelijskih komponenata, oksidativnom modifikacijom proteina, lipida, glukoze i nukleinskih kiselina. Sve te promene utiču na razvoj velikog broja bolesti kao što su: bolesti kardiovaskularnog sistema, očne i neurodegenerativne bolesti, ateroskleroza, dijabetes, bolesti pluća, bubrega, jetre, pankreasa i reproduktivnih organa, kancera itd. Oksidativni stres ubrzava starenje i bolesti koje su vezane za starenje. Održavanje sistema antioksidativne zaštite u humanom organizmu ne predstavlja samo uklanjanje slobodnih radikala, već igra značajnu ulogu u očuvanju dobrog zdravlja i prevenciji hro-

OXIDATIVE STRESS AND CONTEMPORARY DISEASES

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Today's way of life involves great mental burden, insufficient rest and sleep, fast food and excessive amounts of alcohol, coffee and cigarettes, greatly contributes to increased oxidative stress in the body. Emotional stress, UV radiation, toxic substances from the environment, cigarette smoke and other factors additionally generating free radicals. During oxidative phosphorylation in the mitochondria, the cell produces energy and free radicals are synthesized. At lower concentrations, free radicals function as important mediators of cell signaling and immune response processes that are extremely important for the survival of the cell and the whole organism. In physiological conditions, free radicals are quickly neutralized by the antioxidant defense system, which is well organized in the human organism, and functionally divided into several levels. When, for some reason, the synthesis of free radicals overwhelms or/and the mechanism of antioxidant protection become weak, the condition, called oxidative stress occurs. Oxidative stress is considered responsible for a large number of pathological conditions and diseases. Many scientific studies showed that oxidative stress was involved in the onset and progression of many health problems of modern man, through several mechanisms such as: the inactivation of some metabolic enzymes and damage of cellular components, by oxidative modification of proteins, lipids, nucleic acids and glucose. All of these changes are implicated in the development of a large number of diseases such as: the cardiovascular diseases, eye disorders, neurodegenerative diseases, atherosclerosis, diabetes, lung and kidney disorders, liver and pancreatic diseases, cancer, ageing and ageing related diseases. Maintaining the system of antioxidative defense in the human body is not only the removal of free radicals, but also plays an important role in pre-

ničnih i degenerativnih bolesti. Mnoga pitanja, vezana za antioksidantnu suplementaciju u prevenciji hroničnih bolesti su i dalje otvorena i nerešena i zahtevaju dodatna istraživanja. U međuvremenu, izbegavanje izvora slobodnih radikala (cigarete, alkohol, emocionalni stres. masna hrana), upražnjavanje neke fizičke aktivnosti i korišćenje hrane bogate antioksidansima (voće, povrće, razni začini), treba da postane novi stil života savremenog čoveka, kako bi povećali kvalitet života i usporili/sprečili razvoj hroničnih bolesti.

Ključne reči: oksidativni stres, slolbodni radikali, antiooksidativna zaštita

OKSIDATIVNI STRES KAO FAKTOR RIZIKA ZA RAZVOJ OČNIH BOLESTI

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Danas je opšte prihvaćeno da oksidacioni stres učestvuje u nastajanju nekih bolesti, ali i u samom procesu starenja. S obzirom na svoju funkciju, oko je izloženo ambijentalnom zračenju tokom celog života. Pojedini delovi sunčevog spektra poseduju oštećujući potencijal. Otud, oksidativni stres u oku ima neke specifičnosti, jer sunčevo zračenje učestvuje u patogenezi nekih bolesti u sklopu fotooksidacionog stresa. Takođe, tokom evolucije, oko dnevnih organizama razvilo je i posebne zaštitne, antioksidacione mehanizme. Veliki niz istraživanja ukazuje da je oksidativni stres bitan faktor rizika za brojne ozbiljne očne bolesti kao što su senilna degeneracija makule, neke vrste glaucoma i katarakta. Međutim, pronađeni su brojni dokazi o ulozi oksidacionog stresa u patogenezi i nekih drugih očnih bolesti: prematurne retinopatije, dijabetičke retinopatije, uveitisa, suvog oka i nekih bolesti rožnjače.

Ključne reči: oksidativni stres, očne bolesti, antioksidacioni mehanizmi

serving the good health and the prevention of chronic and degenerative diseases. Many questions related to antioxidant supplementation in the prevention of chronic diseases are still open and unresolved and require further research. In the meantime, avoiding the sources of free radicals (cigarettes, alcohol, emotional stress, fatty foods), practicing some physical activity and utilization of foods rich in antioxidants (fruits, vegetables, various spices), should become a new lifestyle of modern man, in order to increase the quality of life and slow or prevent the development of chronic diseases.

Keywords: oxidative, stress, free radicals, antioxidative protection

OXIDATIVE STRESS AS A RISK FACTOR FOR THE DEVELOPMENT OF EYE DISEASES

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It has been accepted that oxidative stress is involved in many acute and chronic diseases and even in normal aging. With regard to its function, the eye is exposed to the ambient radiation through whole life. Some parts of the solar spectrum have a damaging potential. Hence, oxidative stress in the eye has some specifics, because solar radiation participates in oxidative damage of various structures of the eye as photooxidative stress. As a consequence, during evolution, the eye of diurnal species has developed some special antioxidative defense mechanisms. Laboratory and epidemiological studies have implicated oxidative stress in the pathogenesis of the majority of common serious eye diseases such as cataract, primary and some secondary open angle glaucoma and age-related macular degeneration. However, there are numerous evidences about oxidative stress role in pathogenesis of some other ocular diseases: retinopathy of prematurity, uveitis, dry eye syndrome, diabetic retinopathy and corneal diseases.

Keywords: oxidative stress, eye diseases, antioxidative defense

PREDIJABETES I OKSIDATIVNI STRES

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Rani poremećaji glikoregulacije, povišena glikemija našte (IFG) i intolerancija na glikozu (IGT), udruženi su sa gojaznošću, hiperinsulinemijom I hiperlipoproteinemijom i značajni su faktori rizika za dijabetes mellitus tip 2 (DM 2), aterosklerozu i za za kardiovaskularne bolesti. Cilj ovog rada bio je analiziranje korelacije insulinske senzitivnosti i sekrecije, glikoregulacije, lipidnog statusa i parametara antioksidativne aktivnosti gojaznih pacijenata, IFG, IGT pacijenata i novo otkrivenog dijabetes melitusa tip 2. U ispitivanje je uključeno 80 gojaznih osoba (BMI>25 kg/m^2), preko 45 godina starosti kod kojih je oralni glukoza tolerans test (OGTT) otkrio prisustvo gojaznosti (18,7 %), IFG (16,2%), IGT (28,7%)i novo otkriven DM2 (36,2%). Insulinska senzitivnost određivana je preko insulin / glikemija (I/G) odnosa i HOMA IR, insulinska sekrecija određivana je preko HOMA b, dok je lipidni status ispitivan preko ukupnog holesterola, HDL-holesterola, LDL-holesterola, triglicerida spektrofotometrijski i Apo i Apo B, Lp(a) imunohemijskom metodom. Određivali smo superoksid dismutazu u eritrocitima /E-SOD/, eritrocitnu glutation peroksidazu /E-GPX/, i totalni antioksidantni status /TAS/ kao markere antioksidativne zaštite. Glikozilovani hemoglobin HbA1C određivan je kao parameter glikoregulacije spektrofotometrijskom metodom. Najveća insulinska sekrecija dobijena je u IGT pacijenata u poređenju sa IFG I DM 2 dok HOMA IR pokazuje progresiju od najranijeg poremecaja glikoregulaacije IFG (3,18±2,43) u IGT (4,8±3,6) i DM 2 (5,3±3,0). Povećanje insulinske rezistencije prati kretanje mokraćne kiseline kao i aterogeni parametri LDL/HDL i Apo B/Apo Al odnos. U grupi gojaznih pacijenata dobijena je negativna korelacija TAS sa LDL/HDL odnosom (p<0,05); TAS sa Apo B/Apo A1 (p<0,05) dok je u grupi IFG dobijena korelacija GPX sa Apo B/Apo Al (p<0,001). Sa progresijom poremećaja glikoregulacije u grupi IGT dobijena je pozitivna korelacija HOMA ß sa Apo B (p<0,05) i negativna korelacija SOD sa HbAlC (p< 0,05). U novootkrivenom dijabetesu Apo A pokazuje negativnu korelaciju sa parametrima insulinske rezistencije HOMA IR (p<0,01) i mokraćnom kiselinom (p<0,05) kao i HbA1C (p<0,05) dok Apo B/Apo Al pokazuje pozitivnu korelaciju sa HbAlC. Naši rezultati pokazuju da rani poremećaji glikoregulacije udruženi sa gojaznošću, insulinskom rezistencijom, hiperlipoproteinemijom, višom lipidnom peroksidacijom /oksidativni stres/ i nižom antioksidativnom zaštitom mogu imati značajan uticaj na ubrzanu aterosklerozu. Šest meseci nakom Mediteranske dijete bogate u složenim ugljenim hidratima, dijetnim vlaknima, mononezasićenim masnim kiselinama i omega-3-masnim kiselinama došlo je do povećanja antioksidantne zaštite, p>0.05 u IGT i DM2. Rezultati jasno ukazuju na ko-

PREDIABETES AND OXIDATIVE STRESS

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Early glucoregulation disorders, IFG and IGT, associated with obesity, hyperinsulinemia and hyperlipoproteinemia are important risk factors for diabetes mellitus type 2 (DM type 2), atherosclerosis and cardiovascular diseases. The aim of the study was to analyse correlation of insulin sensitivity and secretion, glycoregulation, lipid status, and parameters of antioxidative activity in following groups of patients: with obesity (I), IFG (II), IGT (III) and newly diagnosed DM type 2 (IV). The study included 80 obese individuals (BMI>25 kg/m^2), 45 years of age or older, in whom oral glucose tolerance test (OGTT), revealed the IFG, group II – 16.2%, IGT, group III – 28.7% and newly diagnosed DM type 2, group IV – 36.2%, while OGTT was normal in obesity group I – 18.7 %. The insulin sensitivity was determined by the insulin/glycaemia ratio and HOMA IR, insulin secretion was determined by HOMA b, while the lipid status was determined by total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, insulin resistance was associated with increase of acidum uricum level as atherogenic parameters LDL/HDL and ApoB/ApoAl ratio. We estimated Erythrocyte Dismutase (SOD), Erythrocyte Glutathione Peroxidase (GPX), and Total Antioxidative Status (TAS) as marker of antioxidative defence. In group I (obesity patients) were found negative correlations of TAS and LDL/HDL ratio (p<0.05); TAS and ApoB/ApoA1 (p<0.05) while in group II was found correlation GPX and ApoB/Apo Al ratio (p<0.001). In group III were found positive correlation HOMA β and ApoB (p<0.05) and negative correlation SOD and HbAlC (p<0.05). In group IV ApoA1 showed negative correlation with parameters of insulin resistance HOMA IR (p<0.01) and acidum uricum (p<0.05) like HbA1C (p<0.05) while ApoB/ApoAl showed positive correlation with HbA1C. In patients with obesity and IFG antioxidative activity correlates with insulin resistance and lipid status while progression of disease in IGT correlates with glucoregulation parameters. Our results confirm that early glucoregulation disorders are associated with obesity, insulin resistance, hyperlipoproteinemia, higher lipid peroxidation (oxidative stress) while lower antioxidative activity may have important influence on accelerated atherosclerosis. Six months later Mediterranean diet rich in complex carbohydrates, dietary fibers, monounsaturated fats and omega-3 fats resulted in increase of antioxidative protection, p>0.05 in IGT and DM2. The results clearly indicate beneficial effects of application of Mediterranean diet on obesity, insulin sensitivity, glycoregulation and antioxidative protection in primary prevention of DM2.

risne efekte primenjene Mediteranske dijete na gojaznost, insulinsku senzitivnost, glikoregulaciju i antioksidantnu zaštitu u primarnoj prevenciji DM 2.

Ključne reči: predijabetes, oksidativni stres, biohemijski markeri

GENSKI POLIMORFIZAM GLUTATION TRANSFERAZA KLASE OMEGA KOD BOLESNIKA SA TERMINALNOM BUBREŽNOM SLABOŠĆU

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Terminalna bubrežna slabost (TBS) je stanje koje karakteriše oksidativni stres, što pokazuje povećan stepen oksidativnog oštećenja biološki važnih makromolekula. Pored uloge u detoksikaciji, citosolne glutation transferaze (GST) poseduju i antioksidantnu aktivnost. Članovi GST klase omega, GSTO1 i GSTO2, katališu nekoliko reakcija koje nisu tipične za ostale GST, kao što su reakcija uklanjanja glutationa i dehidroaskorbat reduktazna aktivnost. Polimorfizam gena postoji kod GSTO1 i GSTO2 klase, što rezultuje izmenjenom aktivnošću ovih enzima. Cilj ove studije bio je da se ispita povezanost polimorfizma GSTO1(rs 4925) i GSTO2 (rs156697) gena sa oksidativnim fenotipom bolesnika sa TBS. Studija je izvedena po tipu case-control study koju je činilo 199 bolesnika sa potvđenom dijagnozom TBS i 199 ispitanika uparenih po polu i starosti koji su činili kontrolnu grupu. Polimorfizam GSTO gena je određivan analizom polimorfizma dužine restrikcionih fragmenata po PCR-RFLP metodi (polymerase chain reaction–restriction fragment length polymorphism, PCR–RFLP). Kao markeri oksidativnog oštećenja proteina i lipida spektrofotometrijski su određivani malondialdehid (MDA) (metoda po Dousset-u) i proteinske tiol grupe (metoda po Jocelyn-u), dok su karbonilne proteinske grupe, nitrotirozin i MDA adukti određivani enzimskim imunoesejima (OxiSelectTM ELISA kits, Cell Biolabs). Dobijeni rezultati pokazuju da su osobe sa varijatnim GSTO2 GG genotipom bili pod 2,45 većim rizikom za razvoj TBS u odnosu na osobe sa referentnim GSTO2 AA genotipom (OR=2,45; 95%CI=1,18–5,07; p=0,016). Rezultati pokazuju da ne postoji povezanost različitih varijanti GSTO1 gena i oksidativnog oštećenja proteina i lipida (p>0,001). Međutim, kod nosioca varijantnog genotipa GSTO2 GG uočeno je statistički značajno povećanje koncentracije tiol grupa (p=0,005), dok je koncentracija MDA bila statistički značajno snižena (p=0,041) u odnosu na nosioce referentnog genotipa GSTO2 AA. Polimorfizam gena za GSTO2 je povezan sa povećanim rizikom za razvoj terminalne slabosti bubrega, kao i sa oksidativnim oštećenjem biološki važnih makromolekula ovih bolesnika.

Ključne reči: terminalna bubrežna bolest, glutation transferaza, polimorfizam

Keywords: prediabetes, oxidative stress, biochemical markers

GENETIC POLYMORPHISMS OF GLUTATHIONE TRANSFERASES OMEGA CLASS IN PATIENTS WITH END STAGE RENAL DISEASE

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End-stage renal disease (ESRD) is a condition characterized by oxidative stress, as indicated by increased levels of oxidative damage of important biological macromolecules. Cytosolic family of glutathione S-transferases (GSTs) are involved in detoxi cation of various toxic compounds, as well as antioxidant protection. GSTO1 and GSTO2 possess, unlike other GSTs, dehydroascorbat reductase and deglutathionylation activities. Gene polymorphism exists in both, GSTO1 and GSTO2 class, which alters the expression of these enzymes activities. The aim of this study was to investigate the association between GSTO1 (rs 4925) and GSTO2 (rs156697) polymorphism and oxidative phenotype of ESRD patients. Case-control study consisted of 199 ESRD patients and 199 age-and gender-matched control subjects. GSTO polymorphism was determined by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism, PCR-RFLP) method. Protein thiol groups and malondialdehyde (MDA) were determined spectrophotometrically by method of Jocelyn and Dousset, respectively. Carbonyl protein derivatives, nitrotyrosine and MDA adducts were measured by enzyme immunoassay (OxiSelect TM ELISA kits, Cell Biolabs). The obtained results indicate that individuals carrying variant GSTO2 GG genotype were at 2.45-fold higher risk of ESRD development compared to wild type GSTO2 AA genotype carrying individuals (OR=2.45; 95%CI=1.18–5.07; p=0.016). The results show that there was no association between different GSTO1 gene variants and protein and lipid oxidative damage markers (p>0.001). However, carriers of the variant GSTO2 GG genotype had statistically significant increased concentration of the thiol group (p=0.005), while the concentration of MDA was significantly decreased (p=0.041) compared to the carriers of referent GSTO2 AA genotype. GSTO2 polymorphism is associated with increased risk of the ERSD development, as well as oxidative damage of important biological macromolecules in these patients.

Keywords: end-stage renal disease (ESRD), glutathione S-transferases (GSTs), gene polymorphism

SNIŽENA ANTIOKSIDANTNA ZAŠTITA I GOJAZNOST KAO FAKTORI RIZIKA ZA RAZVOJ KARDIOVASKULARNIH BOLESTI KOD MLADIH

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Gojaznost, diabetes mellitus i starenje dele nekoliko istih etiopatogenetskih mehanizama koji doprinose endotelijalnim i vaskularnim oštećenjima. Ono što je veoma važno je da gojaznost povećano utiče da se kod adolescenata u mladim godinama razvije arterijska hipertenzija, kardiovaskularne bolesti i diabetes mellitus. Gojaznost indukuje prevremeno srčano starenje kod mladih i prekomerno uhranjena deca zaista razviju abnormalni vaskularni zid. Cilj naših istraživanja je bio da se jednom sveobuhvatnom analizom studenata, kroz anketu, antropometrijska merenja i laboratorijske analize antioksidativnih enzima, stekne uvid u njihovo zdravstveno stanje, kao i navike u ishrani i načinu života i utvrde novi ciljevi za kardiovaskularnu prevenciju. Ispitivanja su obuhvatila 510 studenata novosadskog Univerziteta, oba pola (199 muškaraca i 311 žena), starosne dobi 22,25±2,07 godina koji su popunili anketu sa opštim i ličnim podacima, podacima lične i porodične anamneze, i dali odgovore na mnoga pitanja koja se odnose na životne navike. Na osnovu analize odgovora u anketi i prema indexu telesne mase (BMI) nižim ili višim od 25 kg/m^2 i obimu struka (OS) nižim ili višim od 94 cm za muškarce (80 cm za žene) formirane su dve grupe studenata, kontrolna (74 studenta) i rizična (164 studenta) sa kojima su sprovedena dalja laboratorijska istraživanja antioksidantnog statusa. Aktivnosti svih antioksidativnih enzima značajno su niže kod studenta u rizičnoj grupi u poređenju sa kontrolnom grupom. Aktivnost dva važna enzima, superoksid dismutaze (SOD-1) i glutation reduktaze (GR) su u rizičnoj grupi čak ispod donje granice referentnih vrednosti. Značajne korelacije su pokazane za antioksidantne i antropometrijske parametre u rizičnoj grupi studenata. BMI značajno korelira sa GR ($p<0,001$) i TAS ($p<0,001$), dok je OS pokazao signifikantnu negativnu korelaciju sa svim antioksidativnim parametrima ($p<0,05$). U vezi sa promenama životnog stila i promocijom zdravog načina života, negativna signifikantna korelacija ustanovljena kod konzumiranja cigareta i aktivnosti glutation reduktaze, superoksid dizmutaze, glutation peroksidaze i TAS-a ($p<0,01$), dok je pokazana signifikantna pozitivna korelacija fizičke aktivnosti sa glutation peroksidazom i totalnim antioksidativnim statusom ($p<0,05$). Za aktivnost TAS i SOD-1 kod nutritivnog

REDUCED ANTIOXIDANT DEFENSE AND OBESITY AS RISK FACTORS FOR THE DEVELOPMENT OF CARDIOVASCULAR DISEASE IN YOUNG

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Obesity, diabetes and aging share a number of the same etiopathologies that contributes to endothelial and vascular injury. One of the most concerned thing, is that obesity increasingly affects school children, who at young age develop arterial hypertension, cardiovascular disease and diabetes mellitus. Obesity induces premature cardiac aging in young patients and overweight children really developed abnormal vascular wall. The aim of this study was to gain an insight into student's health, nutrition habits and general lifestyle with a comprehensive analysis of the student health conducting the survey, specific anthropometric measurements, and laboratory analyses of antioxidative enzymes and to established novel targets for the cardiovascular prevention. In this study were included 510 students from the University of Novi Sad, both sexes (199 men and 311 women), mean age of 22.25 ± 2.07 years. According to the body-mass index (BMI) lower and higher than 25 kg/m^2 and waist circumference (WC) lower and higher than 94 cm (80 cm for females) the selected group of 238 students was divided into 2 subgroups: the control group of 74 students and the risk group of 164 students and the laboratory examinations of the antioxidant protection were performed. The activities of the antioxidant enzymes were significantly lower among students in the high risk group -obese students with increased risk for cardiovascular disease (CVD) compared with the control group. Activity of two important enzymes, superoxide dismutase (SOD-1) and glutathione reductase (GR) are even lower of reference values in risk group. Significant correlations were obtained between antioxidant and anthropometric parameters in risk students. BMI correlated significantly with GR ($p<0.001$) and TAS (p 0.001) and WC showed significantly negative correlation with all antioxidant parameters ($p<0.05$). In relationship to lifestyle changes and promotions of health way of life, the results showed significantly positive correlation between physical activity and glutathione peroxidase (GSH-Px) and total antioxidative status (TAS) ($p<0.05$) and negative correlation for smoking and activity of GR, SOD-1, GSH-Px and TA ($p<0.01$). Activity of TAS and SOD showed significantly positive correlation of nutritive status as weekly consummation of fish and drinking red wine

statusa utvrđena je signifikantna pozitivna korelacija za nedeljno konzumiranje ribe i crnog vina ($p<0,05$), kao i za suplementaciju omega-3-masnim kiselinama u rizičnoj grupi studenata. Dobijeni rezulatati korelacija aktivnosti antioksidativnih enzima i životnih navika pokazuju da postoje međusobni uzajamni odnosi izvesnih navika i da se oni pojavljuju kao pouzdani prediktori rizika za masovne nezarazne bolesti u odnosu na ostale praćene parametre iz anketnog lista u rizičnoj studentskoj populaciji. Podaci mogu da pruže dobru osnovu za preduzimanje primordijalnih i primarnih mera prevencije kroz promenu i promociju zdravog stila života i modifikovanja faktora rizika. Promenom stila života (»eat less and exercise more«) moguće je smanjiti faktore rizika za oboljevanje od kardiovaskularnih bolesti i drugih masovnih nezaraznih bolesti.

Ključne reči: gojaznost, oksidativni stres, životne navike, kardiovaskularni faktori rizika, primarna prevencija, studentska populacija

($p<0.05$) and as well as supplementation of omega -3-fatty acids in the risk student population. The obtained correlations indicate that among activities of antioxidant enzymes and lifestyle changes, exist a mutual connection which are reliable risk predictors for no communicable diseases compared to the other investigated parameters from the Survey list in the risk student population. These data can provide a good basis for taking the primordial and primary prevention measures through the change and promotion of a healthy lifestyle (»eat less and exercise more«) and the modifications of the risk factors for cardiovascular diseases.

Keywords: obesity, oxidative stress, lifestyles changes, cardiovascular risk factors, primary prevention, students population

MOLEKULARNI BIOMARKERI TERAPIJSKOG ODGOVORA U MULTIPLOJ SKLEROZI

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Multipla skleroza (MS) se karakteriše izrazitom heterogenošću kliničke prezentacije i varijabilnim terapijskim odgovorom na primenjivanu terapiju. Terapijski armamentarijum lekova koji mogu da menjaju tok bolesti u MS je značajno proširen poslednjih godina, a očekuje se registracija još nekoliko novih lekova. Međutim, individualizacija tretmana još uvek predstavlja značajan izazov u MS. Istraživanja farmakogenomike MS su u ekspanziji, ali još uvek pouzdani biomarker terapijskog odgovora u MS nije identifikovan. Iako, u ovom trenutku, nije moguće pouzdano predvideti terapijski odgovor pre započinjanja primene terapija koje mogu da modifikuju tok bolesti u MS, pojedini molekularni biomarkeri ipak imaju određen prediktivni značaj. Pored toga, klinički značaj imaju i biomarkeri kojima se, kod bolesnika kod kojih je lečenje pomenutim lekovima već započeto, može identifikovati ona subpopulacija bolesnika koja je u riziku od izostanka terapijskog odgovora ili pojave ozbiljnih neželjenih efekata terapije. U ovom trenutku, kombinovanjem kliničkih biomarkera, biomarkera na magnetnoj rezonanci, i molekularnih biomarkera, se mogu identifikovati bolesnici kod kojih terapijski odgovor izostaje. Farmakokinetski i farmakodinamski biomarkeri, dodatno, imaju značaja pri donošenju odluke o primenjivanoj dozi lekova i praćenju njihovih

MOLECULAR BIOMARKERS OF TREATMENT RESPONSE IN MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is characterised by a high degree of heterogeneity in clinical features and therapeutic response. The disease-modifying treatment armamentarium in MS has increased tremendously over the last years and more treatments are close to registration. However, to tailor the therapy to the needs of the individual patient still remains an important challenge and anticipating individual treatment response is even more challenging. Research on the pharmacogenomics of MS is increasing but a useful biomarker for clinical practice has so far not emerged. Although it is not possible to predict response to MS treatments at present, molecular biomarkers are currently considered to have some predictive value. Additionally, treatment-response biomarkers are measured in patients receiving MS treatments to identify individuals who are at risk for treatment failure or serious adverse drug reactions and, therefore, are eligible for a treatment change. Currently, a combination of clinical features, magnetic resonance imaging parameters and some molecular biomarkers such as neutralizing antibodies, are usually used to classify treatment responders and non-responders. Pharmacokinetic and pharmacodynamics biomarkers can be also used to guide dose selection, dose modifications and safety monitoring. Although a great deal of

neželjenih efekata. Iako je ogroman napredak učinjen na polju istraživanja biomarkera u MS, i dalje postoji veliki jaz između biomarkera čiji se značaj samo pretpostavlja, validiranih biomarkera i klinički značajnih biomarkera. Očekuje se da dodatna istraživanja u ovoj oblasti značajno doprinesu optimizaciji individualizovanog tretmana u MS.

Kjučne reči: multipla skleroza, molekularni biomarkeri, biomarkeri terapijskog odgovora

progress has been made in the area of biomarkers in MS, gaps are present between candidate biomarkers, validated biomarkers and clinically useful biomarkers. It is now essential to verify the utility and accuracy of these markers in large, prospectively sampled patient cohorts in an effort to bring personalised medicine to patients.

Keywords: multipole sclerosis, molecular biomarkers, treatment-response biomarkers

POREĐENJE ELF TESTA SA FIBRO TESTOM KAO NE INVAZIVNIM LABORATORIJSKIM MARKERIMA FIBROZE JETRE

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ELF (Enhanced Liver Fibrosis) test predstavlja algoritam koji sačinjavaju tri markera fibroze (hijaluronska kiselina, aminoterminalni propeptid kolagena tip II i tkivni inhibitor matriksne metaloproteinaze – 1). Fibro test (FT) se do sada najčešće koristio kao serumski marker fibroze i njega predstavlja algoritam za pet analitičkih parametara (alfa2-makroglobulin, Apolipoprotein A1, haptoglobin, GGT I bilirubin). Revijski prikazi rezultata su pokazali komparabilne rezultate za oba pojedinačna izračunata markera. Cilj ovog ispitivanja se nije odnosio samo na međusobnu korelacionu analizu, već i na tzv. »cost-benefit« analizu oba markera. U ovom ispitivanju ELF test je određivan retrospektivno u pacijenata sa hroničnim bolestima jetre, kojma je urađena biopsija jetre i određen Fibro test a histološki nalaz je predstavljao referentnu metodu. Histološki nalazi su klasifikovani prema metodi METAVIR (F0-F4) za bolesnike sa hroničnim hepatitisom i primarnom bilijarnom cirozom. Dvadesetčetiri uzoraka seruma pomenutih bolesnika je analizirano za parametre FT, nefelometrijskom metodom na (SIEMENS DADE Behring BNII) analizatoru a parametri ELF testa su određivani na (SIEMENS ADVIA Centaur XP) sistemu. Dobijeni rezultati oba testa su izračunavani prema adekvatnim matematičkim algoritamskim formulama. Oba testa su pokazala zadovoljavajuću korelaciju. Spearman-ov koeficijent korelacije (rho) između Fibro testa i ELF testa je iznosio 0,63 ($p<0,001$). Dijagnostička tačnost (AUROC) za dijagnozu značajne fibroze (F≥2) za ELF i FibroTest je iznosila 0,53(95%–CI: 0,08–0,97) i 0,79(95%–CI:

COMPARISON OF ELF TEST WITH FIBROTEST AS THE NON-INVASIVE LABORATORY MARKERS OF LIVER FIBROSIS

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The Enhanced Liver Fibrosis (ELF) test consists of an algorithm of three fibrosis markers (hyaluronic acid, amino-terminal propeptide-of-type-IIIcollagen, tissue-inhibitor of matrixmetaloproteinase-1). FibroTest (FT) is the most frequently used serum fibrosis marker and consists of an algorithm of five fibrosis markers (alfa2 macro-globulin, apolipoproteinA1, haptoglobin, GGT, bilirubin). Systematic review has shown comparable results for both individual markers. The aim of this evaluation is not only based to the correlation analysis but also to the cost-benefit analyses of both markers. In our study, the ELF-test was analysed retrospectively in patients with chronic liver disease, who received a liver biopsy and the FibroTest using histology as the reference method. Histology was classified according to METAVIR (F0-F4) for patients with chronic hepatitis and primary biliary cirrhosis (PBC), respectively. Twenty-four sera of mentioned patients were analysed for FT parameters by Nephelometry (SIEMENS DADE BNII) method and the ELF parameters were tested by (SIEMENS ADVIA Centaur XP) system. Obtained results for both tests were calculated according to adequate mathematical algorithm formulas. Both tests showed well correlation. Spearman's correlation coefficient (rho) between Fibro Test and ELF was 0.63 ($p<0.001$).The diagnostic accuracy (AUROC) for the diagnosis of significant fibrosis (F≥2) for ELF and Fibro Test was 0.53(95%–CI: 0.08–0.97) and 0.79(95%–CI: 0.46–0.99), respectively. Fibro score had greater sensitivity but lower specificity and vice

0,46–0,99), respektivno. Fibroskor je imao veću osetljivost ali manju speciifičnost i obratno. FT i ELF testovi se mogu primenjivati jer imaju komparabilnu dijagnostičku tačnost za neinvazivni stadijum fibroze jetre. Preciznije poređenje bi moglo da se sprovede kada bio uključen znatno veći broj pacijenata za testiranje ELF i FT testova. Opredeljenje za jedan od pomenutih testova zavisi takođe i od postojećih laboratorijskih analitičkih platformi odnosno opreme.

Ključne reči: ELF (Enhanced Liver Fibrosis) test, Fibro test (FT)

versa. FT and ELF can be performed with comparable diagnostic accuracy for the non-invasive staging of liver fibrosis. More precise comparison could be done if a higher number of patients for ELF and FT testing should be included. Cost-benefit analysis showed an advantage of FT which was about ten times less expensive than ELF. Decision to one or another test also depends to the existing laboratory analytical platform.

Keywords: the Enhanced Liver Fibrosis (ELF) test, fibrosis markers, chronic liver disease

SAVREMENA LABORATORIJSKA DIJAGNOSTIKA CELIJAKIJE

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Celijakija je imunski posredovana enteropatija izazvana ingestijom glutena koji sadrže žita (uključujući pšenicu, raž i ječam) kod genetski osetljivih osoba. Bolest je povezana sa HLA-DQ2 u 90 do 95 odsto slučajeva i sa HLA-DQ8 u 5 do 10 odsto slučajeva i neprekidno se održava ukoliko se gluten stalno unosi. To je interakcija između gena (kako HLA tako i drugih) i okruženja (tj glutena) koja dovodi do oštecenja creva tipičnog za ovu bolest. Procenjuje se da je prevalencija celijakije u opštoj populaciji između 0,3% i 1,2%. Velike epidemiološke studije su pokazale da se samo 10–20% slučajeva celijakije identifikuje na osnovu kliničkog nalaza i da su laboratorijski testovi od ključnog značaja za identifikaciju subjekata sa blagim ili atipičnim simptomima. Nove dijagnostičke smernice su revolucionarne u dva glavna aspekta: dokazana je presudna uloga seroloških testova u dijagnostičkom postupku kod simptomatskih pacijenata, dok je detekcija HLA DQ2 /DQ8 alela neophodna u dijagnostici asimptomatskih osoba koji pripadaju rizičnim grupama. Serološka dijagnostika celijakije zasniva se na detekciji klase IgA antitela protiv tkivne transglutaminaze (anti-tTG) i antiendomizijalnih antitela (EMA). Kod pacijenata sa IgA deficijencijom neophodno je određivanje IgG klase anti-tTG ili antitela protiv deaminiranih glijadinskih peptida (anti-DGP). Međutim, ni IgG anti-tTG ni IgG protiv EMA nisu specifična kao IgA antitela. Ukoliko su testovi negativni neophodna je biopsija radi postavljanja dijagnoze. Kada su koncentracije anti-tTG antitela IgA klase veoma visoke (više od 10x u odnosu na gornju granicu normalnih vrednosti) preporučuje se da se proveri IgA-EMA i da se uradi tipizacija za HLA DQ2 / HLA-DQ8. Kada su EMA pozitivna i

CONTEMPORARY LABORATORY DIAGNOSIS OF CELIAC DISEASE

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Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten-containing grains (including wheat, rye, and barley) in genetically susceptible persons. The disease is associated with HLA-DQ2 in 90 to 95 percent of cases and with HLA-DQ8 in 5 to 10 percent of cases and is self-perpetuating in the continued presence of gluten. It is the interplay between genes (both HLA and other types) and environment (i.e., gluten) that leads to the intestinal damage that is typical of the disease. The prevalence of celiac disease in the general population is estimated to be between 0.3% and 1.2%. Large-scale epidemiological studies have shown that only 10–20% of cases of CD are identified on the basis of clinical findings and that laboratory tests are crucial to identify subjects with subtle or atypical symptoms. The new diagnostic guidelines are revolutionary in two major respects: the crucial role of serological tests in the diagnostic process of symptomatic subjects, and the detection of HLA DQ2/DQ8 alleles in diagnosing asymptomatic subjects belonging to at-risk groups. The serological diagnosis of CD is based on the detection of class IgA anti-tissue transglutaminase (anti-tTG) and antiendomysial antibodies (EMA). In patients with IgA deficiency, anti-tTG or anti-delaminated gliadin peptide (anti-DGP) antibody assays of the IgG class are used. However, neither IgG anti-tTG nor IgG anti-EMA is as specific as IgA antibodies. Biopsy may still be clinically indicated if these tests are negative. When IgA anti-tTG antibody levels are very high and greater than 10x upper limit of normal for assay it is recommended to check IgA-EMA and determine HLA-DQ2/HLA-DQ8 typing. If EMA are positive and

pacijenti imaju ili DRQ ili DRQ8 hapolotip, dijagnoza je potvrđena bez potrebe za biopsijom dvanaestopalačnog creva. Pravilan izbor i klinička upotreba ovih dijagnostičkih preporuka može omogućiti tačnu dijagnozu i rano prepoznavanje kako simptomatskih tako i asimptomatskih slučajeva.

Ključne reči: celijakija, gluten, serološka dijagnostika

patient either DRQ2 or DRQ8 haplotype, the diagnosis is confirmed without the need for a duodenal biopsy. The correct choice and clinical use of these diagnostic tools may enable accurate diagnosis and early recognition of symptomatic as well as silent CD cases.

Keywords: celiac disease, gluten, serological diagnosis