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KONCENTRACIJA EOZINOFILNOG KATJONSKOG PROTEINA KOD BOLESNIKA SA BRONHIJALNOM ASTMOM I NJEGOVA KORELACIJA S TEŽINOM I EGZACERBACIJOM BOLESTI

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Aktivisani eozinofilni leukocit u astmi sekretuje brojne medijatore među kojima i eozinofilni katjonski protein (ECP). Cilj ovog rada je bio da se izmeri serumska koncentracija ECP kod 46 astmatičara u stabilnom stanju i pogoršanju i korelira koncentracija ECP kod bolesnika u pogoršanju bolesti sa težinom kliničke slike. Geometrijska sredina ECP u ispitivanoj grupi bolesnika je bila 7,5 μg/L, dok je kod 15 zdravih kontrola iznosila 3,05 μg/L. Nađeno je da postoji statistički visoko značajna korelacija koncentracije serumskog ECP sa aktivnošću bolesti (r = 0,897) i težinom kliničke slike (r = 0,790). Bolesnici sa stabilnom astmom imali su značajnu korelaciju koncentracije serumskog ECP sa težinom bolesti (r = 0,600). Bolesnici sa pogoršanjem astme imaju značajno više koncentracije serumskog ECP nego bolesnici sa stabilnom astmom. Koncentracije serumskog ECP kod bolesnika s pogoršanjem astme su proporcionalne težini bolesti.

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SERUM EOSINOPHIL CATIONIC PROTEIN CONCENTRATION IN PATIENTS WITH BRONCHIAL ASTHMA AND ITS CORRELATION WITH SEVERITY AND EXACERBATION OF THE DISEASE

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Activated eosinophilic leukocytes in asthma secrete numerous mediators, among which is ECP as well. The aim of our study was to measure the serum ECP concentrations in 46 asthmatic patients with exacerabating and stable asthma, and to correlate the serum ECP concentrations with severity and exacerbation of the disease. Geometric mean of ECP in serum in our group of patients was 7.5 μg/L, while it was 3.05 μg/L in 15 healthy subjects (controls). Highly significant correlation of serum ECP concentrations with the activity of the disease (R = 0.897) and the severity of clinical picture (R = 0.790) was found. Patients with stable asthma had significant correlation of ECP and severity of disease (R = 0.600). Patients with exacerbating asthma have significantly higher serum ECP concentrations than patients with stable asthma. Serum ECP concentrations in patients with exacerbating asthma correlate with the severity of the disease.

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ZNAČAJ BIOHEMIJSKIH MARKERA NA HROMOZOMOPATIJE U PRVOM TRIMESTRU TRUDNOĆE

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Kroz prospektivnu analizu ispitana je osetljivost »pregnancy associated plasma protein-A« (PAPP-A) i »free-beta human horionic gonadotropin« (free-beta

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INFLUENCE OF BIOCHEMICAL MARKERS ON CHROMOSOMOPATHY IN FIRST THREE MONTHS OF PREGNANCY

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The study deals with the examination of sensitivity of pregnancy associated with plasma protein-A (PAPP-A) and free-beta human horionic gonadothro-

HCG), fetoplacentarnih proizvoda u maternalnom serumu prvog trimestra trudnoće, za otkrivanje fetalnih hromozomopatija. Analiza je obavljena na 1200 trudnica podeljenih u šest grupa od 8–14 nedelje trudnoće. Određivanje nivoa PAPP-A i free-beta HCG je rađeno na imunološkom analizatoru DPC Immulite na principu hemioluminiscencije. Koncentracija analita u serumu, koja je proporcionalna broju fotona, je preko »softvera« iskazana u MOM (multiple of the median) i korigovana u odnosu na dob, težinu trudnice, ultrazvučne parametre fetalnog rasta i gestacijsku nedelju, a konačan nalaz pokazuje rizik na hromozomopatije. Trudnoće normalnog ishoda imale su raspon 0,27 MOM – 4,95 MOM za PAPP-A. Raspon za free-beta-HCG je u normalnim trudnoćama 0,21 MOM – 3,85 MOM. U našem uzorku bilo je 19 (1,58%) hromozomopatija koje su dokazane prenatalnom kariotipizacijom horiona i amniona ili retrospektivnom obradom fetalnog tkiva nakon spontanog pobačaja. Sve fetalne hromozomopatije su imale bar jedan biohemijski marker, a 17 hromozomopatija oba markera izvan utvrđenog raspona normalnih trudnoća. U hromozomskim aberacijama PAPP-A je bio u rasponu 0,03 – 0,24 MOM, dok je free-beta HCG u 17 slučajeva bio 0,13 MOM – 6,70 MOM, a u dva slučaja u rasponu normalnih trudnoća, ali veći od 3,20 MOM. »Softver« rizik je u svim hromozomopatijama bio manji od 1:50. Od 19 trudnoća sa hromozomskom greškom, 16 majki su bile mlađe od 30 godina. Biohemijski markeri prvog trimestra trudnoće, pokazali su visoku osetljivost ovih markera na hromozomopatije. Preporučuje se da se oni koriste u kombinaciji sa ultrazvučnim nalazom kao populacioni »skrining« kojim bi se zaštitile trudnice mlade dobi, a kod starijih od 35 godina bi se mogao smanjiti broj invazivnih prenatalnih intervencija.

pin (free-beta HCG), foetoplacentar products in maternal serum in the first three months of pregnancy in order to detect foetal chromosomopathy. The analysis was carried out on over 1200 pregnant women devided in six groups from 8–14 weeks of pregnancy. The level of PAPP-A and free-beta HCG was determined on the immunologic chemioluminiscence analyzer DPC Immulite. The concentration of serum markers that is proportional to the number of photons, was expressed by MOM software (multiple of the median) corrected according to the age and weight of a pregnant woman, ultrasonic parameters of foetal growth and weeks of gestation, while the final finding indicated the risk of chromosomopathy. In pregnancies with normal outcome the range was 0.27 MOM – 4.95 MOM for PAPP-A. The range for free-beta HCG was 0.21 MOM – 3.85 MOM in normal pregnancies. In our samples we found 19 (1.58%) patients with chromosomopathies that were proved by prenatal caryotypisation of chorions and amnions or by retrospective processing of foetal tissue after spontaneous miscarriage. All foetal chromosomopathies had at least one biochemical marker, while 17 chromosomopathies had both markers beyond the determined range for normal pregnancies. In chromosome aberrations PAPP-A was ranged from 0.03 to 0.24 MOM, while free-beta HCG in 17 cases was between 0.13 and 6.70 MOM, and in two cases as that in normal pregnancy, but somewhat greater than 3.20 MOM. The software risk was smaller than 1:50 in all chromosomopathies. Of 19 pregnancies with chromosomopathic aberration, 16 mothers were younger than 30 years. Biochemical markers in the first three-month-period of pregnancy were hifth degree of sensitivity, and they are recommended to be performed in combination with ultrasonic markers for nuchal translucent procedure, as population screening that would protect young pregnant women, and in that over 35 years of age could decrease the number of invasive prenatal interventions.

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PROMENE RASTVORLJIVOSTI PARAPROTEINA KOD PACIJENATA SA MALIGNIM MONOKLONSKIM GAMAPATIJAMA

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Izmenjen molekulski integritet, abnormalna interakcija i visoka koncentracija paraproteina mogu dovesti do upadljivih promena u njihovoj rastvorljivosti, što često ima patološke konsekvence. Promene rasvorljivosti paraproteina dovode do promena u krvi (sindrom hiperviskoznosti) i deponovanja paraprote-

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CHANGES IN SOLUBILITY OF PARAPROTEINS IN PATIENTS WITH MALIGNANT MONOCLONAL GAMMAPATHIES

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Changes in molecular integrity, abnormal interaction and high concentracion of paraproteins can provoke significant changes in their solubility and have pathological consequenes. Changes in solubility of paraproteins can provoke changes in blood (hyperviscosity syndrome) and deposition of paraproteins in

ina u tkiva i organe. Ispitivani su serumi 52 bolesnika sa malignim monoklonskim gamapatijama (MMG). Svojstva krioglobulina odnosno euglobulina dokazana su analizom precipitata koji su se stvorili nakon stajanja seruma na +4 °C odnosno nakon ukapavanja seruma u destilovanu vodu. Nakon toga, rađena je elektroforeza i imunoelektroforeza krioprecipitata i Sia precipitata. Otkrivene su 63 paraproteinske frakcije od čega se 10 razlikovalo u pogledu svoje rastvorljivosti. Tri su imala osobine krioglobulina (4,76%), a 8 osobine euglobulina (12,69%). Osobinu da se reverzibilno talože na temperaturi nižoj od telesne ispoljio je 1 IgG i 2 IgM paraproteina, a osobinu da se ireverzibilno talože u destilovanoj vodi ispoljila su 7 IgM i 1 IgA paraprotein. Jedan od krioprecipitabilnih proteina imao je i svojstvo euglobulina. Dva paraproteina su imala aktivnost auto-antitela. Mehanizmi odgovorni za krioprecipitaciju nisu do kraja razjašnjeni. Svaki krioglobulin ima jedinstvene osobina i u znatnoj meri zavisi od strukturnih osobina varijabilog domena. Svojstvo euglobulina zavisi od njihovog ugljenohidratnog profila ili strukturnih anomalija molekula paraproteina.

tissues and organs. The study deals with the analysis of monoclonal immunoglobulins in sera of 52 patients with Malignant Monoclonal Gammapathies (MMG). The characteristics of cryoglobulins and euglobulins were evidenced by analysis of precipitates formed after standing of sera at temperature +4 °C and after dropping of sera into distilled water. The electrophoresis and immunoelectrophoresis of cryo- and Sia precipitates were performed. Sixty three paraprotein fractions were established and within this number 10 were different regarding their solubility. Three of them had cryoglobulinaemic (4.76%) and eight euglobulinaemic (12.69%) properties. One IgG and 2 IgM paraproteins had reversible precipitation at temperature lower than body temperature. Seven IgM and 1 IgA paraprotein had of irreversible precipitation in distilled water. One of cryoglobulins had also euglobulin properties. Two paraproteins had auto-antibody activity. The mechanism responsible for cryoglobulinaemic properties has not yet been explained. Every cryoglobulin has its unique property and depends on caharcteristic variable structure. Euglobulinaemic properties depend on their carbohydrate profile or structural anomalies of paraproteins molecules.

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ODREĐIVANJE *NEU* ONKOPROTEINA U SERUMU BOLESNICA SA KARCINOMOM DOJKE

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Neu onkoprotein, molekulske mase 185 kDa je proizvod *neu* onkogena i često se naziva i p185 protein. Postoji strukturna sličnost ovog proteina sa receptorom za epidermalni faktor rasta. Dosta podataka u literaturi govori da je povećana ekspresija *neu* onkogena i njegovog proteinskog produkta u direktnoj korelaciji sa progresijom i prognozom malignih oboljenja. U ovom radu je dat prikaz određivanja *neu* onkoproteina u serumu pacijentkinja sa karcinomom dojke. Onkoprotein je određivan kod 30 pacijentkinja sa karcinomom dojke i 10 zdravih davalaca korišćenjem ELISA testova »Oncogene science«. Statistička obrada podataka je vršena primenom analize varijanse i Student-t testa. U grupi zdravih davalaca dobijena je srednja vrednost $\bar{x}$ = 2105,55 HNU/mL, a u grupi bolesnica $\bar{x}$ = 3058,33 HNU/mL i ne postoji značajna statistička razlika između srednjih vrednosti ove dve grupe. Značajna statistička razlika postoji između dobijenih rezultata bolesnica sa metastazama i bolesnica bez metastaza (p=0,015). Takođe postoji značajna statistička razlika između stadijuma bolesti

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DETERMINATION OF *NEU* ONCOPROTEIN IN SERA OF PATIENTS WITH BREAST CARCINOMA

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Neu oncoprotein (molecular mass of 185 kDa) is a product of *neu* oncogene, frequently named as p185 protein. There is a stuctural resemblance between this protein and the receptor of epidermal factor of growth. Many data reported that increased expression of *neu* oncogene and of its protein products are in direct correlation with the progression and prognosis of malignant diseases. This paper deals with the procedure of *neu* oncoprotein determination in sera of the patients with breast cancer. Determination was performed in 30 patients hospitalized in our Institute and in 10 healthy donors, by means of ELISA tests »Oncogene science«. Statistical data processing was done by the analysis of variance and Student's-t test. In healthy group of donors the average value of $\bar{x}$ = 2105.55 HNU/mL was obtained, while neu oncoprotein value in patients was $\bar{x}$ = 3058.33 HNU/mL; there was no significant difference in average values between these two groups. Significant difference was found between the results obtained in patients with metastases and patients without metastases

0–IV, gde su zdravi davaoci označeni kao 0-ti stadijum (p=0,030) i između vrednosti ranih stadijuma I i II i IV stadijuma (p=0,023). S obzirom na mali broj uzoraka i povišene vrednosti *neu* onkoproteina u bolesnica sa uznapredovalim stadijumima bolesti, rezultati će biti procenjivani nakon dugogodišnjeg praćenja i poređenja ukupnog preživljavanja i slobodnog intervala kod ovih bolesnica.

(P = 0.015) a rather significant statistical difference was also evident between the 0–IV stages of the disease, where healthy donors were marked as 0 stage (P = 0.030) and among the obtained values in early stages I, II and IV (P = 0.023). As this study included a small number of samples and increased values of *neu* oncoprotein in patients with advanced stages of the disease, the results will be evaluated after a long-term follow-up and the concequent comparason between the total number of survivals and free intervals in these patients.

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POREĐENJE VREDNOSTI PROSTATIČNOG SPECIFIČNOG ANTIGENA I ODNOSA SLOBODNI/UKUPNI PROSTATIČNI SPECIFIČNI ANTIGEN KOD PACIJENATA SA KANCEROM PROSTATE I BENIGNOM HIPERPLAZIJOM PROSTATE

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Prostatični specifični antigen (PSA) je glikoprotein (30 kDa) koji pripada familiji kalikreina. On se uglavnom sintetiše u epitelu prostate i sekretuje u semenu tečnost. Takođe se nalazi i u urinu i u krvi. Vrednosti PSA se povećavaju kod bolesti prostate kao što su benigna hiperplazija prostate (BHP) ili kancer prostate. PSA se u krvi nalazi u tri osnovna oblika: slobodan PSA, PSA u kompleksu sa 1-antihimotripsinom i PSA u kompleksu sa 2-makroglobulinom. U cilju poboljšanja diferencijalne dijagnostike BHP i kancera prostate, izračunat je procenat slobodnog PSA (količnik koncentracije slobodnog PSA i ukupnog PSA). Vrednosti slobodnog PSA i procenat slobodnog PSA određivani su kod 72 pacijenta (56 sa BHP i 16 pacijenata sa kancerom prostate). Korišćeni su komercijalni testovi Vidas FPSA i Vidas TPSA, a određivanje je vršeno na aparatu Vidas (Vidas, BioMerieux®). Princip određivanja zasniva se na kombinaciji dvostepenog sendvič imuno-određivanja i fluorescentne detekcije, ELFA (»enzyme linked fluoroscent assay«) tehnikom. Pacijenti sa kancerom prostate imali su statistički značajno veće vrednosti ukupnog PSA i manji procenat slobodnog PSA u odnosu na pacijente sa BHP (p<0,01). Kod pacijenata sa vrednostima ukupnog PSA između 4–10 ng/mL, procenat slobodnog PSA kretao se od 0,05 do 0,69 i bio je statistički značajno veći kod pacijenata sa BHP u odnosu na pacijente sa kancerom prostate (p<0,05). Samo 20% pacijenata

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COMPARISON OF TOTAL PROSTATE SPECIFIC ANTIGEN WITH FREE/TOTAL PROSTATE SPECIFIC ANTIGENS IN PATIENTS WITH PROSTATE CANCER AND BENIGN PROSTATIC HYPERPLASIA

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Prostate-specific antigen (PSA) is a glycoprotein (30 kDa) which belongs to the kalikrein family. It is principally produced by the glandular epithelium of the prostate, and is secreted in the seminal fluid. It is also present in urine and blood. PSA levels rise in prostatic pathologies such as benign prostatic hyperplasia (BPH) or prostate cancer. PSA is present in blood in three main forms: PSA bound to alpha-1-antichymotripsin, free PSA and PSA bound to alpha-2-macroglobulin. Calculation of the percentage of free PSA, determined by dividing the free PSA (FPSA) concentration by that of total PSA (TPSA) has been suggested as a way of improving the differentiation of BHP and prostate cancer. FPSA and TPSA were measured in 72 patients (56 with BPH and 16 with prostate cancer). Vidas FPSA and Vidas TPSA, automated quantitative tests for use on the Vidas instrument (Vidas, BioMerieux®) were used for quantitative measurement of FPSA and TPSA in human serum. The assay principle combines a two step enzyme immunoassay sandwich method with a final fluorescent detection – ELFA technique (enzyme linked fluorescent asssay). Patients with prostate cancer had significantly higher TPSA, and lower free to total PSA ratio than patients with BPH (P<0.01). In patients with total PSA values of 4–10 ng/mL, the percentage of free PSA ranged from 0.05–0.69 and it was significantly higher in patients with BHP than in patients with prostate can-

sa kancerom prostate, kao i svi pacijenti sa BHP imali su odnos slobodnog i ukupnog PSA veći od 0,18. Na osnovu dobijenih rezultata može se zaključiti da određivanje odnosa slobodnog i ukupnog PSA kod pacijenata sa BHP i kancerom prostate čije su vrednosti ukupnog PSA između 4 i 10 ng/mL može kliničarima biti od velike koristi.

cer ($P<0.05$). Only 20% of patients with prostate cancer and all patients with BHP had free to total PSA ratio higher than 0.18. We concluded that the free/ total PSA determination in patients with BHP and prostate cancer, whose TPSA is within the range of 4–10 ng/mL, gave very usefull information to clinicians.

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OPTIMIZACIJA ELEKTROFORETSKE METODE ZA FENOTIPIZACIJU HAPTOGLOBINA

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Haptoglobin je tetramerni glikoprotein strukture $(\alpha\text{-}\beta)_2$, pri čemu je α lanac polimorfan, pa se javljaju tri glavna fenotipa Hp 1-1, Hp 2-1 i Hp 2-2. Između fenotipova postoje funkcionalne razlike, zbog čega se fenotip haptoglobina smatra faktorom rizika za nastanak niza patoloških stanja (npr. ateroskleroza, maligniteti, autoimmune bolesti). Za fenotipizaciju su najpogodnije elektroforetske metode jer su ostale (»imunobloting«, izoelektrofokusiranje i imunofiksacija) dugotrajne, komplikovane i skupe. Cilj ovog rada bio je da se izvrši optimizacija elektroforetske metode za fenotipizaciju haptoglobina na poliakrilamidnom gelu, koju je uveo Grunbaum, u smislu laboratorijskog pripremanja gelova većih dimenzija i određivanja optimalnih uslova za razdvajanje na njima. 5 μL smeše EDTA plazme, rastvora hemoglobina (12 g/L) i 2% rastvora KCN (8:1:1, v: v: v) nanošeno je na poliakrilamidni gel (T = 6%, C = 5%, pH = 8,6), a elektroforeza je trajala 2 sata (U = 240–270 V, I = 26–43 mA, pH = 8). Za bojenje je korišćen alkoholni rastvor o-dianizidina, uz dodatak vodonik peroksida. Utvrđivanje fenotipa su vršila dva lica, a u slučaju neslaganja fenotipizacija je ponavljana. Analizom 205 uzoraka zdravih osoba sa teritorije Srbije, dobijena je sledeća raspodela fenotipova: 17,6% Hp 1– 1,41% Hp 2–1 i 41,4% Hp 2–2, što je u skladu sa Hardy-Weinberg-ovom ravnotežom. Primenom ovako optimizirane elektroforetske metode za fenotipizaciju haptoglobina može se olakšati procena rizika za nastanak određenih patoloških stanja.

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AN OPTIMIZATION OF AN ELECTROPHORETIC METHOD FOR HAPTOGLOBIN PHENOTYPING

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Haptoglobin is a glycoprotein with a tetrachain structure $(\alpha\text{-}\beta)_2$, characterized by three common phenotypes Hp 1-1, Hp 2-1 and Hp 2-2, due to the α-chain polymorphism. There are functional differences between them, haptoglobin phenotype is considered to be a risk factor for development of various pathologic conditions (e.g. atherosclerosis, malignancies, and autoimmune diseases). The electrophoretic methods are commonly used for phenotype determination because other methods (immunoblotting, isoelectric focusing and immunofixation) are time consuming, complex and expensive. The aim of this work was to optimize the electrophoretic method for haptoglobin phenotyping on polyacrylamide gel, introduced by Grunbaum, in terms of laboratory preparation of gels with larger dimensions and determination of optimal separation conditions for their use. EDTA plasma was mixed with hemoglobin solution (12 g/L) and 2% KCN solution (8:1:1, v: v: v) and 5 μL of mixture was applied on a polyacrylamide gel (T = 6%, C = 5%, pH = 8.6) and electrophoresis was performed for 2 h (U = 240–270 V, I = 26–43 mA, pH = 8). The staining solution was a mixture of ethanol solution of o-dianisidine and hydrogen peroxide. Two persons determinated phenotype and in a case of disagreement the sample was retyped. The phenotype distribution in a sample of 205 healthy subjects of Serbian origin was Hp (1–1), 17.6%; Hp (2–1), 41%; and Hp (2–2), 41.4%, what was in agreement with Hardy-Weinberg equilibrium. The evaluation of a risk for developing of certain pathologic condition might be improved by the use of this optimized electrophoretic method for haptoglobin phenotyping.

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PROMENE AKTIVNOSTI KSANTIN OKSIDAZE U PLAZMI I LIKVORU BOLESNIKA S OBOLJENJIMA MOZGA RAZLIČITE ETIOLOGIJE

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Ksantin oksidaza (XO) je značajan izvor reaktivnih metabolita kiseonika koji imaju posebno učešće u patogenezi moždane ishemije-reperfuzije, diseminovanog demijelinizacionog oboljanja CNS – multiple skleroze (MS) i upale mekih moždanih ovojnica – meningitisa. Humana XO katalizuje poslednja dva koraka u razgradnji purinskih baza, oksidaciju hipoksantina u ksantin i dalje u mokraćnu kiselinu. Istraživanje je obavljeno u uzorcima krvi (plazma) i likvoru bolesnika sa bakterijskim (BM = 19) i virusnim meningitisom (VM = 11), sa akutnim ishemičnim cerebrovaskularnim insultom (AICVI = 30) i sa multiplom sklerozom u fazi kliničkog pogoršanja i povećanja imunološke aktivnosti u intratekalnom prostoru (MS = 30). Kontrolnu grupu je činilo 12 bolesnika ispitivanih pod dijagnozama meningizam i lezija intervertebralnih diskusa u lumbosakralnoj regiji. Aktivnost XO je određivana spektrofotometrijskom metodom autora Kizaki i Sakurade. Značajno povećanje aktivnosti je dobijeno u likvoru bolesnika sa BM i AICVI u odnosu na kontrolu ($p<0,001$) i VM u odnosu na kontrolu ($p<0,01$), što ukazuje na intenzivne procese degradacije ATP-a sa stvaranjem purinskih supstrata u moždanom tkivu, dok u plazmi nisu nađene značajne promene aktivnosti XO ni u jednoj od ispitivanih grupa bolesnika. Upala moždanica, kao i druga inflamatorna stanja, može dovesti do poremećaja mikrovaskulature koji rezultira delimičnom ili potpunom ishemijom tkiva. Opisani rezultati sugerišu da brzo uspostavljanje recirkulacije nakon antiedematozne i antiinflamatorne terapije rezultira reperfuzijom tkiva i oslobađanjem slobodnih radikala kiseonika uz učešće enzima ksantin oksidaze.

B17

CHANGES IN ACTIVITY OF XANTHINE OXIDASE IN PLASMA AND CSF OF PATIENTS WITH BRAIN DISEASES OF VARIOUS AETIOLOGY

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Xanthine oxidase (XO) is an important cytoplasmic source of reactive oxygen species, and has been implicated in the pathogenesis of cerebral ischaemia-reperfusion, disseminated demyelinating CNS disease – multiple sclerosis (MS) and inflammation of the pia mater-meningitis. Human XO catalyzes the terminal two steps of purine degradation pathway, the oxidation of hypoxanthine to xanthine and later to uric acid. The study was performed on blood samples (plasma) and cerebrospinal fluid of patients with bacterial meningitis (BM = 19), viral meningitis (VM = 11), acute ischaemic cerebrovascular insult (AICVI = 30), and those with multiple sclerosis in the phase of clinical exacerbation and increase in immune activity in intrathecal space (MS = 30). The control group consisted of 12 patients with documented meningism and intravertebral disk lesions in the lumbo-sacral region. Xanthine oxidase activity was determined with spectrophotometric method by Kizaki and Sakurada. The activity of XO was significantly increased in CSF of patients with BM and AICVI compared to controls ($P<0.001$) and those with VM in comparison to controls ($P<0.01$), indicating intensive processes of ATP decomposition with purine substrate formed in the brain tissue, while in plasma significant XO changes were not observed in the groups studied. Meningeal inflammation, as well as other inflammatory conditions, may lead to disturbed microvascular structure resulting in partial or complete ischaemia of tissues. The obtained findings suggest that prompt recirculation after antioedematous and anti-inflammatory therapy results in tissue reperfusion and release of oxygen free radicals with increased XO enzyme activity.

B18

ZNAČAJ ODREĐIVANJA AKTIVNOSTI ALT I CK NA KLINIČKI TOK I ISHOD LEČENJA PACIJENATA SA TEŠKOM KRANIOCEREBRALNOM POVREDOM

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Od 01. 09. 2003. godine do 31. 01. 2004. godine, primljeno je u neurohiruršku službu u Urgentnom centru Kliničkog centra Srbije 27 pacijenata sa teškim kraniocerebralnim povredama kojima je određivana aktivnost enzima ALT i CK u serumu i likvoru, drugog i četvrtog dana po prijemu kako bi se utvrdilo da li postoji korelacija sa kliničkim tokom i ishodom lečenja. Aktivnost ovih enzima u likvoru određivana je metodama za određivanje u serumu na biohemijskom analizatoru ILab 600. Vrednosti ovih enzima u serumu, po prvom uzimanju bile su blago povišene u odnosu na referentne vrednosti, a kod određivanja enzimske aktivnosti u likvoru, pošto ne postoje, zvanične, referentne vrednosti, mogli smo da pravimo, samo, komparaciju rezultata posle prvog i drugog uzimanja uzorka likvora. Od 27 pacijenata egzitiralo je troje, a kod preostalih 24 značajno je sniženje vrednosti enzima i u serumu i u likvoru, što nije bio slučaj sa troje koje je egzitiralo. U zaključku se može reći da određivanje enzimske aktivnosti u likvoru nije neophodno, a u serumu ima pozitivan prediktorni značaj, u smislu povoljnog ishoda lečenja pacijenata sa teškom kraniocerebralnom povredom.

B18

IMPORTANCE OF DETERMINATION OF ALT AND LDH ACTIVITY IN SERUM AND LIQUOR FOR THE CLINICAL COURSE AND OUTCOME IN PATIENTS WITH SEVERE TRAUMATIC BRAIN INJURY

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From 1st September 2003 to 31st January 2004 in the Neurosurgical Department of the Trauma Centre of the Clinical Centre of Serbia 27 patients with severe traumatic brain injury were admitted. We analyzed the enzymatic activity of ALT and CK in serum and liquor of these patients second and fourth day of the admittance, and corelated these findings with their clinical course and outcome. Activities of these enzymes were obtained by standardized methods on biochemical analyzer ILab 600. On the second day, levels of these enzymes in serum was slightly elevated in comparison with referent values. In liquor for these enzymes there are still no official referent values, so we compared the found values on the second and fourth day. Three patients who had no significant changes in the level of these enzymes, died. In 24 survivors, the decrease in ALT and LDH values in serum and liquor were significant. In conclusion, we can say that the determination of the level of enzymatic activity in liquor is not necessary as its level in serum is of positive predictor importance for outcome in patients with severe traumatic brain injury.

B19

KARAKTERISTIKE LIKVORA KOD PACIJENATA SA POVREDAMA LOBANJE I MOZGA

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Dnevna produkcija likvora iznosi oko 500 mL i kod normalnih osoba se nalazi oko 150 mL. Glavno mesto stvaranja likvora u organizmu je horoidni pleksus lateralnih komora. Likvor obezbeđuje mehaničku potporu mozgu, pomaže u uklanjanju metaboličkih proizvoda

B19

CHARACTERISTICS OF CEREBROSPINAL FLUID IN PATIENTS WITH BRAIN INJURY

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Cerebrospinal fluid is formed at a rate of approximately 500 mL/day, and in normal adults it occupies a volume of about 150 mL. The major site of production is the choroids plexus of the lateral ventricles. CSF provides mechanical support to the brain, helps

ili otpadnih materija iz mozga, transportuje biološki aktivna jedinjenja i ima važnu ulogu u održavanju hemijskog okruženja mozga. Rastvorljive supstance ulaze u likvor putem aktivnog transporta, olakšane ili pasivne difuzije. Cilj ovog ispitivanja bio je da se utvrde aktivnosti enzima, koncentracije elektrolita i ukupnih proteina u likvoru pacijenata koji su imali povredu lobanje i mozga i bili životno ugroženi, s obzirom da su podaci o vrednostima navedenih parametara kod ove vrste pacijenata nepotpuni. Analizirano je 60 uzoraka likvora dobijenih lumbalnom punkcijom. Svi uzorci bili su crveno obojeni. Nakon centrifugiranja u supernatantu su određene aktivnosti sledećih enzima: aspartat aminotransferaze (AST), alanin aminotransferaze (ALT), kreatin kinaze (CK) i laktat dehidrogenaze (LDH), kao i koncentracije natrijuma, kalijuma, kalcijuma, fosfora, magnezijuma i ukupnih proteina. Aktivnosti enzima, kao i koncentracije kalcijuma, fosfata i magnezijuma određene su na biohemijskom analizatoru ILab 600 (Instrumentation Laboratory, Lexington, USA) korišćenjem odgovarajućih komercijalnih testova. Koncentracije natrijuma i kalijuma u likvoru izmerene su na plamenom fotometru (IL 943, Instrumentation Laboratory, Lexington, USA), a koncentracije proteina u likvoru određene komercijalnim testom firme Chema (Chema Diagnostica, Italy) na filterfotometru EOS 880 (CGA, Italy). Dobijeni su sledeći rezultati (srednja vrednost standardna devijacija): AST (U/L): 20 ±11, ALT (U/L): 3 ± 3, LDH (U/L): 139 ± 155, CK (U/L): 25 ± 34, ukupni proteini (g/L): 1,58 ± 1,32, kalcijum (mmol/L): 1,18 ± 0,13, fosfat (mmol/L): 0,42 ± 0,17, natrijum (mmol/L): 145 ± 4, kalijum (mmol/L): 2,65 ± 0,26, magnezijum (mmol/L): 1,13 ± 0,16.

in removing metabolic products or waste from the brain, transports biologically active compounds, plays an important role in maintaining the chemical environment of the brain. Solutes enter CSF by a variety of processes including active transport, facilitated diffusion and passive diffusion. The aim of this examination was to monitor electrolyte concentrations and enzyme activity in CSF of patients with trauma and brain injury with respect to a very poor literature data. Sixty CSF samples were obtained by lumbar spinal puncture. All samples were coloured red. After centrifugation, the activity of aspartat aminotranspherase (AST) and alanin aminotranspherase (ALT), creatine kinase (CK), lactate dehydrogenase (LDH), and concentrations of sodium, potassium, calcium, phosphate, magnesium, and total protein were determined in a supernatant. Enyzme activities, and calcium, phosphate, magnesium concentrations were determined on biochemical analyzer ILab 600 (Instrumentation Laboratory, Lexington, USA) with appropriate commercial kits. Sodium and potassium concentrations were measured by flame photometer (IL 943, Instrumentation Laboratory, Lexington, USA), and concentration of total protein in CSF samples were determined by commercial kit (Chema Diagnostica, Italy) on filterphotometer (EOS 880, CGA, Italy). The following results (mean SD) were obtained: AST (U/L): 20 ± 11, ALT (U/L): 3 ± 3, LDH (U/L): 139 ± 155, CK (U/L): 25 ± 34, TP (g/L): 1.58 ± 1.32, calcium (mmol/L): 1.18 ± 0.13, phosphate (mmol/L): 0.42 ± 0.17, sodium (mmol/L): 145 ± 4, potassium (mmol/L): 2.65 ± 0.26, magnesium (mmol/L): 1.13 ± 0.16.

B20

GAMA-GLUTAMIL TRANSFERAZA: POTENCIJALNI MARKER POSTOJANJA METASTAZA KARCINOMA BUBREŽNOG PARENHIMA

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Određivanje aktivnosti alkalne fosfataze (AF) se često koristi za preoperativnu procenu pacijenata sa karcinomima bubrežnog parenhima, a povećane vrednosti ukazuju na postojanje metastaza u kostima

B20

GAMMA-GLUTAMYL TRANSFERASE: A POTENTIAL MARKER OF METASTATIC RENAL CELL CARCINOMA

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Alkaline phosphatase (AP) has long been used for the preoperative evaluation of patients with renal cell carcinoma (RCC), and an elevated value usually prompts a search for bone and/or liver metastases.

i/ili jetri. γ-glutamil transferaza (GGT) je enzim jetre koji se normalno ne nalazi u kostima, a povećane aktivnosti u serumu su nađene u mnogim bolestima jetre. Osim toga, GGT se nalazi i u proksimalnim tubulima bubrega i u karcinomima bubrežnog parenhima. Međutim, GGT do sada nije korišćen kao marker za procenu postojanja metastaza kod pacijenata sa karcinomima bubrežnog parenhima. U ovom radu je određivana aktivnost GGT i AF u serumu kod 30 pacijenata sa klinički dijagnostikovanim metastazama karcinoma bubrežnog parenhima (starost 61,2 ± 8,1) i upoređivana sa aktivnostima koje su dobijene kod 26 pacijenata sa lokalizovanim karcinomom (starost 62,2 ± 9,4). Pacijenti sa metastazama su podeljeni u 4 grupe na osnovu lokalizacije metastaza (samo u jetri, samo u kostima, u jetri i kostima, u plućima i/ili mozgu). Aktivnost gama-glutamil transferaze je određivana spektrofotometrijski uz korišćenje gama-glutamil-3-karboksi-4-nitroanilida kao supstrata. Statistička analiza je vršena pomoću *Chi*-kvadrat testa i Studentovog t-testa. Aktivnosti GGT i AF u serumu su bile značajno veće kod pacijenata sa klinički dijagnostikovanim metastazama u odnosu na grupu pacijenata bez metastaza (110 ± 96 prema 38 ± 33 U/L, 252 ± 133 prema 90 ± 28 U/L). Učestalost povećane aktivnosti AF i GGT bila je značajno veća u grupi pacijenata sa metastazama (AF: 25 od 30; GGT: 20 od 30) u odnosu na grupu pacijenata bez metastaza (AF: 2 od 26; GGT: 3 od 26, $p < 0,01$). Kod pacijenta sa mestazama samo u jetri zabeleženo je povećanje aktivnosti samo GGT (10%), AF (20%) ili oba enzima (70%). U grupi pacijenata sa metastazama samo u kostima, 37% je imalo povećane aktivnosti AF, 45% oba enzima, dok su kod 18% pacijenata aktivnosti oba enzima bile u fiziološkim granicama. Kod svih pacijenata u grupi sa metastazama u jeti i kostima aktivnosti oba enzima su bile povećane. Specifičnost i pozitivna prognostička vrednost GGT su vrlo slične onima kod AF (88 i 87 prema 92 i 92), dok su senzitivnost i negativna prognostička vrednost niže (67 i 70 prema 83 i 83). Na osnovu ovih rezultata može se zaključiti da GGT pored AF može biti uključen u preoperativnu procenu pacijenata sa metastazama karcinoma bubrežnog parenhima.

Gamma glutamyl transferase (GGT) is a relatively new frequently measured liver enzyme. It is elevated in many forms of liver diseases and is not present in normal bone. In addition, GGT is located on the proximal convoluted tubules of the kidney and most RCC. Although GGT is not a useful tumour marker of low stage RCC, it has not been extensively evaluated as a marker of metastatic RCC. In this study, we evaluated serum GGT and AP activities as indicators of metastatic RCC. We determined serum GGT and AP activities in 30 patients with metastatic RCC (aged 61.2 ± 8.1 years) and compared them with those of 26 patients with clinically localized RCC (age 62.2 ± 9.4 years). Patients with metastatic RCC were divided into four groups based on the site(s) of metastases, including liver only, bone only, liver and bone, and lung /or brain (metastatic disease, but not liver or bone). GGT activity was measured spectrophotometrically by using γ-glutamyl-3-carboxy-4-nitroanilide as a substrate and glycilglycine as an acceptor forming 5-aminobenzoate. Chi square test and Student's t-test were used for statistical analysis. Overall serum GGT and AP activities were significantly higher in patients with metastatic RCC in comparison to those with localized RCC (110 ± 96 vs. 38 ± 33 U/L and 252 ± 133 vs. 90 ± 28 U/L, respectively). GGT activity was elevated in a significantly greater number of patients in metastatic group (20/30) than in localized RCC group (3/26, $P< 0.01$). AP was also increased in significantly more patients with metastatic (25/30) than localized RCC group (2/26, $P<0.01$). In patients with liver metastases only, all patients exhibited an increase in the activity of either GGT (10%), AP (20%) or both GGT and AP (70%). Among patients with bone metastases only, 37% had increased AP, 45% had increased both GGT and AP, while the activities of both enzymes were normal in 18% of patients. All patients in the group with metastases in liver and bone had increased activities of both GGT and AP. In the patient group with lung/brain metastases the activities of AP and GGT were either both increased (67%) or normal (33%). Specificity and positive predictive values of GGT were similar to those of AP (88 and 87 vs. 92 and 92, respectively), while sensitivity and negative prognostic values were lower (67 and 70 vs. 83 and 83, respectively). Based on results presented in this study GGT has similar quality as a potential marker of metastatic RCC as AP. Therefore, we conclude that GGT should be included together with AP in the preoperative metastatic evaluation of patients with RCC.

B21

NIVOI MALONDIALDEHIDA, KSANTIN OKSIDAZE, C-REAKTIVNOG PROTEINA I GLUKOZE U AMNIONSKOJ TEČNOSTI PRI PORODAJU

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Amnionska tečnost se vrlo često koristi za uvid u biohemijski profil amnionske šupljine. Koncentracija glukoze u amnionskoj tečnosti ima visoku prediktivnu vrednost kod brzih dijagnoza intraamnionskih infekcija. Dokaz za ovo je činjenica da je katabolizam glukoze sa aktiviranim neutrofilima primarni mehanizam uključen u ove procese. Neutrofili dobijeni iz inficirane amnionske tečnosti koriste glukozu u odnosu dva do tri puta više od neutrofila kod neinficiranih pacijenata. Glavni cilj ovog rada bio je da se proceni koncentracija malondialdehida (MDA) i aktivnost ksantin oksidaze (XOD), kao najodgovornijih za stvaranje slobodnih radikala kiseonika u amnionskoj tečnosti dobijenoj od trudne žene na porođaju. Takođe je određivan i C-reaktivni protein kao protein koji stvara jetra kada postoji neko žarište u organizmu. 26 pacijenata je bilo podeljeno u dve grupe uzimajući u obzir koncentraciju glukoze (0,78 mmol/L). Glukoza je određivana standardnom metodom na analizatoru Axon. Intenzitet lipidne peroksidacije je određen kao MDA koncentracija reakcijom tiobarbiturne kiseline; aktivnost ksantin oksidaze je određena spektofotometrijskim merenjem koncentracije mokraćne kiseline, dok je CRP određen imunohemijskom metodom na aparatu Turbitimer, firme Dade Bering. Znatno viši nivo MDA (560 ± 233, p<0,001), smanjena XOD aktivnost (U/L) (50,1 ± 22,4, p<0,001) i CRP (mg/L) (17,85 ± 5,3) su dobijene u inficiranoj amnionskoj tečnosti u poređenju sa MDA (102,56 ± 53,26), XOD (142 ± 38,2) i CRP (4,4 ± 0,84) u neinficiranoj amnionskoj tečnosti u kojoj je koncentracija glukoze bila viša od 0,78 mmol/L. Povišena koncentracija MDA i smanjena XOD aktivnost u amnionskoj tečnosti je verovatno posledica neutrofilne lipoperoksidne hiperprodukcije. Dobijeni rezultati kao što je niski nivo glukoze u amnionskoj tečnosti i visoki nivo CRP, smanjena aktivnost XOD kao i visok nivo MDA ukazuju na to da mogu biti specifični markeri intraamnionske infekcije na porođaju.

B21

MALONDIALEHYDE CONCENTRATION, XANTHINE OXIDASE ACTIVITY, C-REACTIVE PROTEIN AND GLUCOSE LEVEL AS SPECIFIC MARKERS IN AMNIOTIC FLUID AT DELIVERY

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Amniotic fluid is often used to access the biochemical profile of the amniotic cavity. The glucose concentration of amniotic fluid has a predictive value in rapid diagnosis of intra-amniotic infection. It is established that glucose catabolism, by activated neutrophils, is the primary mechanism involved in these processes. Neutrophils obtained from infected amiotic fluid consume glucose two or three times more than neutrophils from non-infected patients. The aim of this study was to evaluate the concenration of malondialdehyde (MDA) and the activity of xanthine oxidase (XOD), mostly responsible for generation of oxygene free radicals, in amniotic fluid obtained from pregnat women at delivery. C-reactive protein (CRP) is a protein produced by the liver when there is some inflammation in the body. Twenty six patients were divided in two groups on the basis of the concentration of glucose (0.78 mmol/L). Glucose was estimated routinely on standard Axon analyzer. Intensity of lipid peroxidation was determined as MDA concentration by thiobarbituratic acid reaction; xanthine oxidase activity was determined by spectrophotometric method of uric acid concetracion, while CRP was determined by imunochemical reaction with the Turbitimer, Dade Behring. The significantly higher MDA level (560 ± 233, P<0.001), reduced XOD activity (U/L) (50.1 ± 22.4, P<0.001), and CRP (mg/L) (17.85 ± 5.3) were detected in amniotic fluid of infected patients in comparison to MDA (102.56 ± 53.26), and XOD (142 ± 38.2) and CRP (4.4 ± 0.84) levels in non-infected patients. The elevated concentration of MDA and reduced XOD activity in amniotic fluid may be the conseqeunce of neutrophil lipoperoxide hyper production. The obtained results indicate that low amniotic fluid level of glucose and high levels of C-reactive protein, decreased XOD activity as well as high MDA level could be specific markers of intra-amniotic infections at delivery.

B22

MERENJE AKTIVNOSTI ENZIMA AST, ALT I GLDH U UZORCIMA KARCINOMA KOLONA

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Osnovni cilj ovog rada je bila procena značaja kvantitativne analize aktivnosti enzima AST (aspartat aminotransferaze), ALT (alanin aminotransferaze) i GLDH (glutamat dehidrogenaze) u biohemijskoj dijagnostici karcinoma debelog creva. Ispitivanjem je obuhvaćeno 20 uzoraka karcinoma debelog creva i okolne sluzokože udaljene 10 cm od malignog tkiva (uzorak normalnog tkiva). Pacijenti kod kojih je vršena analiza uzoraka tumora nisu preoperativno bili podvrgnuti radio ili hemioterapiji, a svaki uzorak je patohistološki verifikovan. Rezultati merenja aktivnosti enzima AST, ALT i GLDH ukazuju da je njihova aktivnost bila značajno povišena u uzorcima malignih tumora u odnosu na sluzokožu udaljenu 10 cm od malignog lokusa. Aktivnost enzima AST i GLDH bila je dva puta veća u uzorcima karcinoma kolona u odnosu na normalnu sluzokožu, dok je izmerena enzimska aktivnost ALT u malignom tkivu bila tri puta povišena u odnosu na normalno tkivo. S obzirom da je aktivnost izmerenih enzima u ispitivanim uzorcima karcinoma debelog creva bila značajno povećana u odnosu na sluzokožu udaljenu 10 cm od malignog lokusa, ovi rezultati mogu ukazivati na specifične biohemijske karakteristike malignih tkiva. U isto vreme dobijeni rezultati mogu predstavljati dodatnu informaciju u evaluaciji dužine resekcije tkiva u blizini maligniteta kolona.

B22

ENZYMATIC ACTIVITY OF AST, ALT AND GLDH IN COLON CARCINOMA SAMPLES

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The main goal of our study was to estimate the significance of quantitative analysis of AST (aspartate aminotransferase), ALT (alanine aminotransferase) and GLDH (glutamate dehydrogenase) enzymes and biochemical diagnosis of colon carcinoma. In this study 20 samples of colon carcinoma and surrounding normal mucose (10 cm distant from malignant tumour) were analysed. The patients who were included in this study were not exposed to radio or chemiotherapy before surgery. All the samples were pathohystologically verified. The results of AST, ALT and GLDH activity showed that the activity of these enzymes was significantly higher in malignant tumour samples compared to samples of normal mucosa. AST and GLDH activity was two times higher in samples of colon carcinoma compared to normal mucosa, whereas ALT enzymatic activity was three times higher compared to normal surrounding tissue. Taking into consideration that the activity of all measured enzymes in carcinoma samples analysed in our study was significantly higher compared to mucosa 10 cm distant from malignant loci, our results may indicate the existence of specific biochemical properties of malignant tissues. At the same time, our results may be useful as an additional information for the evaluation of the length of colon resection in the close proximity of colon carcinoma.

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PROTEKTIVNI EFEKTI URSODEOKSIHOLNE KISELINE I METIONINA NA OŠTEĆENJE JETRE IZAZVANO OPSTRUKCIJOM EKSTRAHEPATIČNOG BILIJARNOG KANALA KOD PACOVA

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Akumulacija toksičnih žučnih kiselina u holestazi dovodi do destabilizacije hepatične plazma membrane. U radu su ispitivani protektivni efekti metionina i

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PROTECTIVE EFFECT OF URSODEOXYCHOLIC ACID ON OXIDATIVE STRESS INDUCED BY LIGATURE OF EXTRA-HEPATIC BILIARY DUCT IN RATS: COMPARISON WITH THE EFFECT OF METHIONINE

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The accumulation of toxic bile acid in cholestasis causes destabilisation of the liver plasma membrane. In the present examination, we studied the protective

ursodeoksiholne kiseline (UDHK) na stabilnost plazma membrane hepatocita u holestazi izazvanoj podvezivanjem ekstrahepatičnog bilijarnog kanala kod muških Wistar pacova. Životinje su podeljene u sedam grupa: I – je kontrolna; II – šam operisane životinje; III – pacovi tretirani metioninom; IV – grupa tretirana sa UDHK (25 mg/kg t.t. *per os*) V – životinje sa holestazom; VI – životinje sa holestazom tretirane metioninom; VII – životinje sa holestazom tretirane sa UDHK. Stabilnost membrane hepatocita evaluirana je merenjem aktivnosti plazma i hepatičnih enzima 5'-nukleotidaze, alkalne fosfataze, γ-glutamil-transferaze. Aktivnost enzima određivana je standardnim spektrofotometrijskim metodama. Eksperimentalno izavana holestaza podvezivanjem bilijarnog kanala dovodi do značajnog povećanja aktivnosti ispitivanih enzima ($p<0,001$), zbog čega se oni ubrajaju u markere holestaze. Takođe je poznato da je oksidativni stres jedan od mehanizama oštećenja jetre u holestazi, što se potvrđuje značajnim porastom koncentracije malondialdehida (MDA) ($p<0,001$), proizvoda lipidne peroksidacije. Oba protektora, metionin i UDHK su se pokazala efikasna u stabilizaciji hepatične membrane, mada su protektivni efekti UDHK u odnosu na metionin bili značajniji ($p<0,01$). UDHK prevenira porast MDA i značajno snižava porast aktivnosti ispitivanih enzima, budući da snižava nivo hidrofobnih žučnih kiselina. Imajući u vidu prethodno prikazane protektivne efekte UDHK, ova istraživanja ukazuju na mogućnost njene primene u terapiji bilijarne holestaze kao i prednost u odnosu na metionin.

effect of methionine and ursodeoxycholic acid (UDCH) on hepatocyte membrane stability in cholestasis produced by common bile duct ligation (CBDL) in adult male Wistar rats. The animals were divided in 7 groups: I – control; II – sham operated; III – rats treated with methionine; IV – rats treated with UDCH (25 mg/kg b.w. *per os*); V – rats with CBDL; VI – cholestatic rats treated with methionine; VII – cholestatic rats treated with UDCH. Hepatocyte membrane stability was evaluated by the changes in the activity of plasma and hepatic tissue enzymes (5'-nucleotidase, alkaline phosphatase, γ-glutamyl-transferase). CDBL caused highly significant increase in plasma and liver homogenate enzyme activities ($P<0.001$). This procedure produced a notable increase in the MDA level ($P<0.001$). Both methionine and UDCH administration were effective as hepatoprotective substances, although the protective effects of ursodeoxycholic acid were superior ($P<0.01$); it prevented the increase of lipid peroxidation products (MDA) and reduced significantly the increase in enzyme activities, since UDCH reduces hydrofobic bile acid level in hepatocytes and stabilizes the membrane. UDCH is far more potent and provides superior protection as compared to methionine. Considering the decrease in oxidative stress and the intensity of cholestasis, these findings have significant clinical implications on UDCH as a possible therapeutic agent in biliary cholestasis.

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ODNOS IZMEĐU KATABOLIZMA POLIAMINA U JETRI I PROTEKTIVNOG EFEKTA L-METIONINA U EKSPERIMENTALNOJ HOLESTAZI

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Holestazno oštećenje jetre posledica je intrahepatičke akumulacije toksičnih žučnih kiselina. Hidrofobne žučne kiseline izazivaju: povećanje fluidnosti i propustljivosti ćelijske membrane, apoptozu hepatocita i smanjenje sadržaja poliamina u jetri. S-adenozil-L-metionin (SAM) je aktivna forma L-metionina (L-met). On je »metil donor« za sintezu DNK, RNK, proteina, fosfolipida i prekursor za sintezu glutationa i taurina. SAM učestvuje u sintezi poliamina, koji su neophodni za rast i diferencijaciju ćelija, stabilizaciju ćelijske membrane. Cilj ovog rada bio je ispitivanje mogućeg odnosa između katabolizma poliamina (aktivnost poliamino oksidaze, PAO i diamino oksidaze,

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RELATIONSHIP BETWEEN LIVER POLYAMINE CATABOLISM AND PROTECTIVE L-METHIONINE EFFECT IN EXPERIMENTAL CHOLESTASIS

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Cholestatic liver diseases result from the intrahepatic accumulation of toxic bile acids (BA). Hydrofobic BA induced: increased cell-membrane fluidity and permeability, apoptosis of hepatocytes and decreased polyamines contents. S-adenosyl-L-methionine (SAM) is an active form of L-methionine (L-Met). It is »methyl donor« for the synthesis of DNA, RNA, proteins and phospholipids, and the precursor for glutathione and thaurine synthesis. SAM catalyzes the synthesis of polyamines (putrescine, spermidine and spermine), which are essential for cell growth, differentiation and membrane stabilization. The aim of the study was to examine a possible relationship between polyamine

DAO) i protektivnog efekta L-met u jetri pacova sa holestazom. Wistar pacovi su bili podeljeni u 4 grupe: I – »šam« operisani pacovi, II – pacovi sa podvezanim *ductus choledochusom* (BDL pacovi), III – BDL pacovi tretirani sa L-met (150 mg/kg telesne mase, per os). Životinje su žrtvovane nakon 7 dana. Aktivnost PAO (1,08 ± 0,02 vs. 2,1 + 0,39 U/mg proteina) i DAO (1,1 ± 0,17 vs. 1,85 ± 0,16 U/mg proteina) u jetri BDL pacova je značajno smanjena u odnosu na »šam« operisane ($p<0,001$). Aktivnost PAO and DAO u III groupi je značajno povećana u odnosu na II grupu (1,53 ± 0,12 i 1,58 ± 0,07 U/mg proteina, $p<0,01$). Povećana aktivnost alkalne fosfataze i gama glutamil transferaze (enzima markera holestaze) u serumu BDL pacova (340,5 ± 45 i 19,9 ± 3,3 U/L), znatno je smanjena administriranjem L-met (276 ± 34 i 12,9 ± 1,8 U/L), $p<0,01$. U uslovima holestaze dolazi do smanjenja katabolizma poliamina u jetri pacova. Davanjem L-met pacovima sa podvezanim *ductus choledochusom*, povećava se nivo poliamina i normalizuje aktivnost PAO i DAO. Sve ovo ukazuje na važnost poliamina u stabilizaciji membrane hepatocita i njegovoj protektivnoj ulozi na jetru pacova sa holestazom.

catabolism (polyamino oxidase, PAO and diamino oxidase, DAO activities) and L-Met protective effect in cholestatic liver injury. Wistar rats were divided into 4 groups: I – sham operated, II – bile duct ligated (BDL) rats, III – BDL rats treated with L-Met (150 mg/kgBW, per os). The animals were killed after 7 days of treatment. Activity of PAO (1.08 ± 0.02 vs. 2.1 ± 0.39 U/mg proteins) and DAO (1.1 ± 0.17 vs. 1.85 ± 0.16 U/mg proteins) was significantly decreased in the liver of BDL rats compared with controls ($P< 0.001$). PAO and DAO activity in III group was significantly higher than in II group (1.53 ± 0.12 and 1.58 ± 0.07 U/mg proteins, $P<0.01$). Increasing serum activity of cholestatic markers (alkaline phosphatase and gamma glutamil transferase) in BDL rats (340.5 ± 45 and 19.9 ± 3.3 U/L) was decreased by oral administration of L-Met (276 ± 34 and 12.9 ± 1.8 U/L), $P<0.01$. Cholestasis decreased polyamine catabolism in liver. Administration of L-Met increases polyamine production in BDL and results in normalization of DAO and PAO activity. This indicates the important role of polyamines in hepatocytes membrane stabilization and points to their protective role in BDL rats.

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UTICAJ PUŠENJA NA VREDNOSTI ANTIOKSIDANTNIH ENZIMA, SOD I GPX KOD ZDRAVIH ISPITANIKA

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Dokazano je da pušenje utiče na patogenezu mnogih bolesti kao što su: ishemijska bolest srca, emfizem pluća, opstruktivne bolesti pluća i neoplastične promene. U dimu cigarete identifikovano je preko 1000 jedinjenja. Neka od njih deluju kao oksidansi ili kao pro-oksidansi a neka deluju kao slobodni radikali ili pak kao redukujući agensi. U ovom radu su ispitani antioksidantni enzimi superoksid dismutaza (SOD) i glutation peroksidaza (GPX) u eritrocitima zdravih pušača sa ciljem da se utvrdi uticaj pušenja na aktivnost ovih enzima. Aktivnost enzima je određivana komercijalnim testovima; Ransod (za SOD) i Ransel (za GPX) firme Randox Laboratories u eritrocitima 42 zdrava ispitanika, i to kod 21 aktivnog pušača i 21 nepušača. Statističkom obradom podataka dobijeno je da su vrednosti SOD i GPX značajno povišene kod pušača (SOD = 1037 ± 100,29 U/gHb, GPX = 30,25 ± 3,56 U/gHb) u odnosu na nepušače (SOD = 908,5 ± 73,7 U/gHb i GPX = 28,1 ± 3,16 U/gHb) za $p < 0,05$. Ovim je dokazano da pušenje izaziva povećan oksidativni stres kod pušača koji se manifestuje povećanjem aktivnosti antioksidativnih enzima.

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EFFECT OF CIGARETTE SMOKING ON VALUES OF ANTIOXIDATIVE ENZYMES, SOD AND GPX IN HEALTHY SUBJECTS

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It has been documented that cigarette smoking has a certain effect on many diseases such as ischaemic heart disease, lung emphysema, obstructive lung disease and neoplastic disorders. Over 1000 supstances have been indentified in cigarette smoke. Some of theme are acting like oxidants or pro-oxidants, while the others like free radicals and reducing agents. In this study, the activity of antioxidative enzymes: superoxide dismutase (SOD) and glutathione peroxidase (GPX) in red blood cells of healthy cigarette smokers were tested, in order to determine the effect of cigarette smoking on the activity of these enzymes. The commercial kits Ransod for SOD and Ransel for GPX from Randox Laboratories were used, to analyze the enzymes activity in red blood cells of 21 active smokers and 21 non smokers. Statistical data processing yielded significantly higher values of tested parameters in active smokers (SOD = 1037 ± 100.29 U/gHb; GPX = 30.25 ± 3.56 U/gHb) in comparison to non smokers (SOD = 908.5 ± 73.7 U/gHb; GPX = 28.1 ± 3.16 U/gHb) ($P < 0.05$). These findings suggest that cigarette smoking increses oxidative stress which is manifested by an increasing activity of antioxidative enzymes.

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DIJAGNOZA OŠTEĆENJA JETRE ALKOHOLOM U PRIMARNOJ ZDRAVSTVENOJ ZAŠTITI

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Najzastupljenija »laka droga« pored duvana u nas je alkohol. Česta konzumacija alkohola uzročnik je mnogih masovnih nezaraznih bolesti, gde primarno dolazi do značajnog oštećenja jetre. U ovom radu su prikazane mogućnosti kao i rezultati laboratorijskog ispitivanja jetre, primenom biohemijskih testova, a u objektivnim mogućnostima manjih ustanova primarne zdravstvene zaštite. Ispitano je 54 pacijenata, od kojih je 39 imalo uputnu dijagnozu hroničnog etilizma, a 15 je činilo kontrolnu grupu. Srednje vrednosti u grupi pacijenata sa dijagnozom hroničnog etilizma su iznosile za: MCV = 115 fL, AST = 40 U/L, ALT = 54 U/L, ukupni bilirubin = 23,2 μmol/L, ukupne proteine = 76,3 g/L, mokraćnu kiselinu = 454 μmol/L, triglyceride = 2,94 mmol/L, holesterol = 4,6 mmol/L, γGT = 68 U/L i ALP = 268 U/L. Ovi nespecifični testovi daju odličnu mogućnost sagledavanja stanja jetre kod hroničnih konzumenata alkohola i predstavljaju dobar osnov za početak jedne duge i mukotrpne borbe protiv ovog socijalno – medicinskog problema. Predlažemo da se naprave medicinski timovi u koje bi bili uključeni lekari opšte medicine, psihijatri, psiholozi kao i klinički biohemičari koji bi inicirali problem u dijagnostičkom smislu, a u kasnijem stadijumu upućivali i na mnoge specifične testove i markere alkoholnog oštećenja jetre koji se rade u kliničkim centrima.

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DIAGNOSIS OF LIVER DAMAGE BY ALCOHOL IN PRIMARY HEALTH CARE

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Alcohol besides tobacco is a »light drug« which people use mostly in our country. The frequent consumption of alcohol is the cause of many mass and noninfectious diseases. However, the primary effect is a significant damage of the liver. In this paper we present the possibilities and the results of laboratory biochemical tests of the liver and especially in small primary health care institutions. The study concerned 54 patients, of whom 39 patiens were diagnosed as chronic ethyl patients and 15 patients made a control group. On the basis of the performed tests, the following results were obtained: MCV ($\bar{x}$ = 115 fL), AST ($\bar{x}$ = 40 U/L), ALT ($\bar{x}$ = 54 U/L), total bilirubin ($\bar{x}$ = 23.2 μmol/L), total protein ($\bar{x}$ = 76.3 g/L), uric acid ($\bar{x}$ = 454 μmol/L), triglycerides ($\bar{x}$ = 2.94 mmol/L), cholesterol ($\bar{x}$ = 4.6 mmol/L), γGT ($\bar{x}$ = 68 U/L), ALP ($\bar{x}$ = 268 U/L). These tests, although nonspecific, are excellent for insight the state of the liver in chronic alcoholics, and present a good basis of the beginning of one longlasting and painstaking struggle against this serious social and medical problem. We suggestion, the formation of medical teams of physicians, psychiatrists, psychologists and clinical biochemists.