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Princip ovog testa, koji istovremeno dokazuje prisustvo sve četiri navedene mutacije, je zasnovan na PCR umnožavanu i reverznoj hibridizaciji sa alel-specifičnim oligonukleotidima. Za jednog ispitanika je dokazano da je heterozigotni nosilac sve četiri mutacije. Za četiri bolesnika je potvrđeno heterozigotno prisustvo faktor V Leiden kombinovano sa heterozigotnim prisustvom MTHFR mutacije, i to sa A1298C mutacijom kod tri, a sa C677T kod jednog ispitanika. Testiranje je kod 5 bolesnika dokazalo kombinovano heterozigotno prisustvo obe ispitivane mutacije na MTHFR genu, uz odsustvo mutacija faktor V Leiden i faktor II G20210A. Heterozigotno prisustvo faktor V Leiden mutacije je kod jednog ispitanika bio jedini genetski faktor rizika za trombofiliju. Pojedinačno prisustvo MTHFR mutacija, kao jedinog faktora rizika, je utvrđeno kod 4 bolesnika: jednog homozigotnog i dva heterozigotna nosioca C677T, odnosno jednog heterozigotnog nosioca A1298C mutacije. Navedeni preliminarni rezultati označavaju ThromboType® plus test kao pouzdan za primenu u kliničkim laboratorijama. Dalja ispitivanja, uključivanjem većeg broja bolesnika i korelacijom sa nalazima funkcionalnih testova hemostaze, će povećati efikasnost testiranja i omogućiti identifikaciju i procenu kliničkog značaja novih genetskih faktora rizika za trombofiliju.

group of 18 patients with thrombophilia. This test, which simultaneously detects the above-mentioned mutations, is based upon a combination of PCR amplification and reverse hybridization with allele-specific oligonucleotides. One patient was identified as the heterozygous carrier of all four mutations. The heterozygous presence of factor V Leiden mutation combined with heterozygous presence of MTHFR mutations was confirmed in four patients, precisely with C677T in three and A1298C in one of them. Results showed that five patients were heterozygous carriers of both MTHFR mutations, with no factor V Leiden and factor II G20210A mutations detected. In five participants, only one genetic risk factor was confirmed. One was heterozygous carrier of factor V Leiden mutation. The individual presence of MTHFR mutations was confirmed as homozygosity for C677T in one patient, while four participants were heterozygous carriers of the same mutation. Additionally, in one patient the only mutation identified was MTHFR A1298C in the heterozygous form. These preliminary results confirm reliability of application of ThromboType® plus test in clinical laboratories. Further studies will, through enrollment of more patients and correlation with functional tests, improve testing efficiency and enable identification and clinical evaluation of novel genetic risk factors for thrombophilia.

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### UTICAJ SMANJENOG UNOSA HRANE NA ACTH ĆELIJE PERIPUBERTALNIH ŽENKI PACOVA

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Smanjen unos hrane kod ženki pacova aktivira sistem uključen u odgovor na stres (hipotalamo-hipofizno-nadbubrežni sistem). Dobro je poznato da različiti tipovi hipofiznih ćelija (adrenokortikotropne- ACTH, prolaktin-prodajujuće i somatotropne ćelije) učestvuju u odgovoru na stres. Cilj ove studije je bio da se ispituju efekti smanjenog unosa hrane na imunohistomorfometrijske karakteristike hipofiznih ACTH ćelija kao i nivo ACTH hormona u krvi. Peripubertalne (38 dana stare) ženke Wistar pacova su gajene pojedinačno u kavezu, pod standardnim ambijentalnim uslovima (12:12 h svetlo-tama, 22±2°C) sa hranom i vodom *ad libitum*. Ženke eksperimentalne grupe su hranjene 50% manjom količinom hrane nego kontrolna grupa ženki, tokom šest nedelja. ACTH ćelije su obeležene korišćenjem peroksidaza-antiperoksidaza imunohistohemijske procedure te je sprovedena stereološka analiza. Nivo ACTH u krvi određen je radioimuno testom (ACTH-

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### DIETARY RESTRICTION AFFECTS ACTH CELLS OF PERIPUBERTAL FEMALE RATS

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Dietary restriction (DR) in female rats presumably activates the stress (hypothalamo-pituitary-adrenal) system. It's well known that various pituitary cell types are involved in the reaction to stress, i.e. adrenocorticotrophic hormone (ACTH), prolactin and growth hormone cells. The aim of this study was to examine the effects of DR on the immunohistomorphometric features of pituitary ACTH cells and blood levels of ACTH. Peripubertal (38 days old) female Wistar rats were housed one per cage, under standard environmental conditions (12 h light/dark cycle, 22 ± 2 °C) with food and water *ad libitum*. Experimental females were fed with 50% of the total amount of food consumed by the controls, during 6 weeks. The ACTH cells were stained using the peroxidase-antiperoxidase immunohistochemical procedure and stereological analyses were conducted. The blood ACTH was measured by radioimmunoassay (ACTH-IMMULITE kit). In the experimental group body weight was

IMMULITE kit). U eksperimentalnoj grupi ženki prosečna telesna masa je smanjena ( $p < 0,05$ ) za 48,8%, dok je relativna masa hipofize povećana ( $p < 0,05$ ) za 76,9% u poređenju sa odgovarajućim parametrima kod kontrola. Volumen ACTH ćelija, kao i njihova volumenska gustina su povećani ( $p < 0,05$ ) kod eksperimentalnih ženki za 17,6%, odnosno 12,5%. Nivo ACTH u krvi je povećan ( $p < 0,05$ ) u eksperimentalnoj grupi za 13,4%. Naši rezultati ukazuju da smanjen unos hrane kod peripubertalnih ženki pacova stimuliše sistem uključen u odgovor na stres, što potvrđuju povećani morfofunkcionalni parametri ACTH ćelija.

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### ULOGA CITOLOŠKOG PREGLEDA LIKVORA U DIJAGNOSTIFIKOVANJU MULTIPLE SKLEROZE

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Multipla skleroza (MS) je upalna autoimuna demijelinizacijska bolest centralnog nervnog sistema koja se karakteriše multifokalnom inflamatornom destrukcijom mijelina, oštećenjem aksona i gubitakom oligodendrocita. Dijagnoza se postavlja magnetnom rezonancom, sveobuhvatnim anamnestičkim, kliničkim praktičnim i laboratorijskim analizama. Pored elektroforetskog, izoelektričnog fokusiranja likvora kojim se utvrđuje prisustvo oligoklonalnih traka IgG u CSF-u, analizira se i broj, vrsta ćelija u CSF-u, kao i nivo ukupnih protein u likvoru. Cilj rada je bio da se utvrdi koji procenat pacijenata kojima je elektroforezom i izoelektrio-fokusiranjem likvora utvrđeno prisustvo oligoklonalnih traka IgG i potvrđena dijagnoza MS, ima patološki nalaz ćelija u likvoru i povišen nivo proteina. Svim pacijentima (182 pacijenata obuhvaćena istraživanjem) je citološki pregledan likvor i određen je nivo proteina u njemu. Iz originalne epruvete pre nego što je materijal centrifugiran 5 minuta na 2000 obrtaja, 100  $\mu$ L likvora je obojeno pomoću 20  $\mu$ L Genciane violet. Nakon 10 minuta likvor je postavljen u Fush-Rosenthal ovu komoru za brojanje ćelija i mikroskopiran na elektronskom mikroskopu na uveličanju 40x. Ukupni proteini u likvoru su određivani na biohemijskom analizatoru Vitros 350 (Johnson & Johnsons Company), po principu suve hemije sa pirokatehol violet. Dobijeni rezultati su prema nalazu ćelija razvrstani u dve grupe. Prvu grupu su činili pacijenti koji su imali povećan broj ćelija ( $\geq 5$  ćelija). Drugu grupu su činili pacijenti sa ćelijama u granici normale ( $< 5$  ćelija). Od svih pacijenata obuhvaćenih istraživanjem, 25,3% je imalo patološki nalaz ćelija, a 74,7% normalan nalaz ćelija. U grupi koja je imala patološki nalaz ćelija, 43,5%

( $p < 0,05$ ) decreased by 48.8%, while the relative pituitary weight was ( $p < 0,05$ ) increased by 76.9% in comparison with corresponding parameters in controls. The volume of ACTH cells, as well as their volume density was significantly increased ( $p < 0,05$ ) in the experimental group, by 17.6% and 12.5% respectively. The blood ACTH level was increased ( $p < 0,05$ ) in experimental group by 13.4%. Our findings show that the DR in peripubertal female rats stimulates the stress system, which was confirmed by increased morphofunctional parameters of ACTH cells.

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### THE ROLE OF CITOLOGICAL CSF EXAMINATION IN DIAGNOSIS OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is an autoimmune inflammatory demyelinating disease of the central nervous system and is characterized by multifocal inflammatory myelin destruction, damage and loss of axon oligodendrocyte. The diagnosis is set by magnetic resonance imaging, comprehensive anamnesis, clinical practice and laboratory analysis. In addition to isoelectric focusing (electrophoresis) where the presence of IgG in oligoclonal bands in CSF is determined, analysis of the number and type of cells in the CSF and the total level of protein are done. The aim of this study was to determine what percentage of patients had pathological findings of CSF cells and proteins (with whom the presence of IgG oligoclonal bands was determined and who were confirmed the MS diagnosis). Cell examination of the liquid and the level of protein in it were determined to all the patients (182 patients were included in the study). Before the material was centrifuged for 5 min. at 2000 rpm, 100  $\mu$ L of fluid from the original test tube was painted using 20  $\mu$ L Genciane violet. After 10 min. the fluid was placed in the Fush-Rosenthal cell counting chamber and microscopied on electronic microscope at 40x magnification. Total proteins in the liquor were determined on a biochemical analyzer Vitros 350 (Johnson & Johnsons Company), on the principle of dry chemistry with pirokatehol violet. The results are divided into two groups according to cell findings. The first group consisted of patients who had an increased number of cells ( $\geq 5$  cell). The second group consisted of patients with cells in normal range ( $< 5$  cells). Of all patients surveyed, 25.3% had pathological findings of cells, and 74.7% had normal cell finding. In the group that had a