2022/25/Приложение 2. Обмяна на медта – нови симетрии

Спешна медицина

**Национално списание по спешна медицина,
орган на Българското дружество по спешна медицина**

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по спешна медицина

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Уважаеми колеги,

За мен е удоволствие да представя настоящото Приложение 2 на списание „Спешна медицина“ – Обмяна на медта – нови симетрии, в което за първи път фокусът е поставен върху разработки в областта на лабораторната медицина.

Темата на приложението е свързана, от една страна, с рядкото генетично заболяване болест на Уилсон, а от друга – с болестта на Алцхаймер, която има социалнозначим ефект. Връзката между тях е в споделянето на общи патобиохимични механизми, които са част от нарушена, в по-голяма или по-малка степен, обмяна на микроелемента мед.

Основният фокус е поставен върху ролята и метаболизма на медта и взаимовръзката между всички микроелементи в организма, протеините, за тяхната регулация и генетичните основи при нарушен баланс. В детайли са разгледани лабораторните и клиничните аспекти в обмяната на медта. Представени са данни за етапите в общия процес на лабораторния анализ, класическите изисквания при диагноза и проследяване на пациенти с болест на Уилсон, както и сравнително новата металохипотеза за развитието на невродегенеративните заболявания.

Двойнственият характер на микроелементите, специфичният им и високоспециализиран анализ, и тяхното есенциално значение ги правят все по-интересни за медицинската наука.

Избрани са общо 15 доклада – 11 от международни и три от национални научни форуми, както и едно резюме към изследователски проект. Подредени са по хронологичен ред.

д-р Ирена Иванова

СЪДЪРЖАНИЕ

ПРИЛОЖЕНИЕ 2. ОБМЯНА НА МЕДТА – НОВИ СИМЕТРИИ

COMPARISON BETWEEN 6-HOUR AND 24-HOUR CUPRIURIA IN MONITORING OF THERAPY WITH D-PENICILLAMINE IN PATIENTS WITH WILSON DISEASE

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2nd EFLM-BD European Conference on Preanalytical Phase; March 01-02 2013, Zagreb Biochemia Medica 2013; 23 (1):A18.

Background: Adequacy of treatment in patients with Wilson Disease (WD) was monitored by measuring 24-hour urinary copper excretion while on treatment with D-penicillamine. The major effect of the drug is to promote the urinary excretion of copper. Values between 3-8 μmol/24 h (200-500 μg) per day cupriuria means adequate therapy. The problems of measuring 24-hour copper excretion include: incomplete urine collection and copper contamination of the collection device. Therefore, by reducing time for testing we will also reduce errors in pre-analytical stage of examination.

Materials and methods: The aim of the study was to assess and compare the 6-hour and 24-hour copper urinary excretion in patients with WD treated with D-penicillamine. The data used were from 42 measurements in 32 patients with proven WD in University Hospital Sv. Ivan Rilski, Medical University – Sofia. The average dose D-penicillamine was 800-1000 mg per day. Urine was collected in two portions – 6-hour and 18-hour. The concentration of copper in two portions were calculated to gat 24-hour cupriuria. Copper analysis was made with atomic absorption spectrophotometry in Analyst 400 device.

Results: The copper urine excretion in 6-hour, 18-hour and inn 24-hour urine was respectively x = 4.12 ± 2.26 μmol/6 h, x = 5.75 ± 3.42 μmol/18 h and x – 9.89 ± 4.7 μmol/24 h. Copper excretion in 6-hour urine was 41.7% of 24-hour cupriuria.

Conclusion: About half of copper urine excretion was up to 6-hour diuresis. Reducing the time of urine collection could reduce the errors in pre-analytical stage of examination. It is necessary the 6-hour urine copper excretion to be standardized and validated to use it as criteria of adequacy of therapy.

Key words: cupriuria, Wilson Disease, D-penicillamine

SERUM TOTAL HOMOCYSTEINE IN ADULT BULGARIANS: HEALTHY STUDY GROUP

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2nd RAML Congress with International Participatio; Jun 05-08 2013, Brasov;
under the auspices of IFCC and EFLM;
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Homocysteine (Hcy) is an independent risk factor for cardio- and neuro-vascular and neuropsychiatric disorders. It is well known that Hcy blood concentration depends on genetic predisposition, diet and renal function.

The aim of the study were to describe the distribution of serum total Hcy concentrations in a healthy group of people and to test for differences in Hcy concentrations among sex, age, Vitamin B12, Folic acid and lipid status.

Materials and methods: The study included 60 healthy persons – 30 women and 30 men with average age 42.27 ± 13,64 (20-80 years). The patients were with normal renal function-creatinine x = 65,12 ± 14,28 μmol/l; good status of Folic acid, x = 13,26 ± 4,9 ng/ml (5,6-26,3 ng/ml) and of Vitamin B12, x = 287 ± 177,15 pg/ml (49-1073 pg/ml).Total Hcy, Vitamin B12 and Folic acid were detected by competitive chemiluminiscence immunoassay in Immulite 1000 using gel tubes. Questionnaire forms and statistical analysis – SPSS 19 were used.

Results: Mean level of total Hcy was 10,54 ± 5,48 μmol/l (from 1,34 to 25,40 μmol/l). It was up to 15 μmol/l in 83,33% of whole group samples (n = 50) and up to 8 μmol/l in 31,66% in 19 persons. Detected total Hcy was higher in men with mean values 11,06 ± 6,07 μmol/l than in women with mean values 10,03 ± 4,88 μmol/l. No statistical correlation was established between total Hcy levels and age, vitamin B12, Folic acid and serum lipids in healthy study group.

Conclusions: It was established the tendency that total Hcy was higher in men than in women without statistical significance (p = 0.473). In the study group there was no established correlation between studied tests.

NEPHROTIC SYNDROME AFTER TREATMENT WITH D-PENICILLAMINE

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2nd RAML Congress with International Participation; Jun 05-008 2013, Brasov;
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Revista Romana de Medicina de Laborator, Suplement Ia Vol. 21, Nr. 2/4, Iunie 2013, S27

D-penicillamine is a classic drug for treatment of Wilson's disease. It's major effect is to promote the urinary copper excretion. Treatment with D-penicillamine is linked to wide range of side effects. The most serious of them are kidney damage and hematopoietic disorders. We aim to present a 31 year old man identified on routine screening tests in 2008 with increased value of ASAT – 184 U/L. He had no history of fever, jaundice, abdominal pain or gastrointestinal bleeding. Clinical, laboratory and histological tests were carried out. In 2011 Wilson's disease was diagnosed. Then treatment with D-penicillamine strated – 1.5 g/day. In 2013 the patient appeared with edema syndrome and he was diagnosed kidney damage. Serum creatinine was measured – 134 μmol/l, urea – 10.25 mmol/l, albumin – 15.8 g/l and proteinuria – 4.38 g/24h. Nephrotic syndrome was diagnosed on standard criteria. Kidney biopsy proved focal proliferative glomerulonephritis. After stopping D-penisillamine and initiating corticosteroid therapy the patient's condition improved. The average period of treatment with D-penicillamine before developing of nephrotic syndrome was nearly 2 years. It is necessary the urinary copper excretion to be monitored as a criteria for adequate treatment with D-penicillamine. Kidney function and signs of proteinuria have to be followed in cases with chelating therapy. Drug caused damage has to be searched in every patient with unexpected and unexplained renal manifestation not related with the underlying disease.

THE INFLUENCE OF THE TYPE OF BIOLOGICAL MATERIAL ON THE VALUES OF TOTAL HOMOCYSTEINE

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September 25-28 2013, Budva
Balkan Journal of Clinical Laboratory, XXI, 13, 1, PF27

As a biological material for determination of total homocystein (tHcy) manufacturer recommended heparinized and EDTA plasma as a samples of choice, but serum is also suitable for use. Blood collection tubes from different types may yield differing values, depending on materials and additives, including gel or physical barriers, clot activators or anticoagulants.

We aimed to compare levels of tHcy measured in K_2EDTA plasma and serum using Becton Dickinson tubes.

The study included 46 paired samples analysed parallel. All patients, with average age 48 ± 11 years old (20-78), were randomly selected with normal renal function – mean creatinine 69.85 ± 14.73 μmol/l (32-104). In 10.9% of individuals, only women, anemia was established – haemoglobin under 120 g/l.

Blood was collected by standard venepuncture after fasting overnight. K_2EDTA tubes, and gel separated tubes were used. After blood taking both plasma and serum were immediately centrifuged (for 15 min at room temperature at 15.000 x g) and examined by competitive immunoassay with direct chemiluminescence detection on immunoanalyzer IMMULITE® 1000 Siemens.

Levels of tHcy in K_2EDTA plasma and serum were statistically different ($p = 0.005$) and higher in serum: x = 14.35 ± 4.41 μmol/l (8.13-31.00) than in plasma: x = 13.39 ± 3.98 μmol/l (6.93-27.00), but we established a high correlation between them – Pearson coefficient 0.86. Under 15.00 μmol/l tHcy were 69.9% of plasma samples and 60.9% of serum ones.

The values of tHcy correlated well depending on the type of biological material, but in examination and interpretation of tHcy it is recommended each laboratory to established its own pre-analytical rules and reference limits.

SERUM COPPER AND ZINC CONCENTRATIONS IN HEALTHY INDIVIDUALS OF BULGARIAN POPULATION

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IFCC WorldLab Istanbul 2014; June 22-26 2014, IstanbulClin Chem Lab Med 2014; 52, Special Suppl, p. S1707

Background: Copper (Cu) and zinc (Zn) play key role in hematopoiesis, nerve transmission, pigmentation, oxide-reductive reactions. Body content of Cu and Zn strongly depends on intake of food, water and supplements. The aim of this study is to evaluate serum Cu and Zn concentrations of healthy individuals from Bulgarian population.

Methods: The study comprises 140 healthy individuals with average age 44.88 ± 13.23 y, with no data for acute inflammation (C-reactive protein 2.33 ± 1.84 mg/l) and for impaired glucose tolerance (fasting serum glucose 5.68 ± 1.08 mmol/l). Evaluation of health status also includes serum creatinine (69.94 ± 12.74 μmol/l) and parameters for protein balance (total protein 70.60 ± 4.78 g/l; albumin 45.89 ± 3.34 g/l). Serum Cu and Zn concentrations are analyzed by flame atomic absorption spectrophotometry. The other biochemical analyses are done by analyzer Cobas Integra 400. All data are statistically processed by statistical packet SPSS19. Serum Cu and Zn for all individuals are expressed as mean value ± SD: Cu 14.50 ± 2.96 μmol/l and Zn 12.77 ± 3.00 μmol/l respectively.

Results: Significant statistical difference ($p < 0.01$) has been observed between males and females with higher serum concentrations in females for both Cu and Zn (serum Cu for females 15.40 ± 3.11 μmol/l and serum Cu for males 13.59 ± 2.51 μmol/l; for serum Zn: females 13.09 ± 2.25 μmol/l vs. 12.45 ± 3.58 μmol/l for males). In 68.6% of males and in 71.4% of females serum Cu is up to lower limit of reference interval. In 75.7% of males and in 85.7% of females serum Zn is up to lower limit of reference interval.

Conclusion: A tendency for lowering of serum Cu and Zn in healthy individuals and higher concentrations for females than for males have been observed in comparison to earlier evaluations. This could be explained by changed dietary habits, different numbers of individuals in the studied groups, increased use of supplements and some medications. Cu deficiency (27.85%) could be more prevalence than Zn deficiency (21.45%).

STUDY OF COPPER STABILITY IN 24-HOUR URINE BY FLAME ATOMIC ABSORPTION

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EuroMedLab Paris 2015; June 21-25 2015, Paris
Clin Chem Lab Med 2015; 53, Special Suppl, p. S736

Background – aim: Serum and urine copper measurements could be used for monitoring of nutritional adequacy and evaluation of copper balance in clinical disorders of homeostasis. We aimed to study the stability of urine copper, measured by flame atomic absorption, in healthy individuals and in patients on D-penicillamine (DPA) therapy.

Methods: Urine 24-hour samples of 14 healthy individuals and 22 DPA treated patients were analyzed. The intake of DPA was 1000 mg twice daily. Urine samples were stored in polyethylene vessels for 2, 3 and 14 days at two temperatures: 15-25°C and 2-8°C. No preservatives were added. Copper levels were measured by flame atomic absorption spectrophotometer AAnalyst 400, Perkin Elmer. The mean percentage deviation (d%) was calculated and compared to the Acceptable Change Limit (ACL) as d% > ACL represented probable difference in copper concentration. Establishment of ACL was derived from analytical imprecision CV of in house routine QC data accumulated over a 3-month period.

Results: Stability was tested against initial copper urine concentration, measured up to 2 hours after 24-hour urine collection had been completed, with a mean value 0.5 μmol/L for the healthy group and 6.5 μmol/L for DPA group. In the healthy group, the lowest d% was observed only for the 2nd day of storage for both temperatures and except the 3rd day at 2-8°C, d% for all the other tested conditions did not exceed ACL. For all tested storage conditions in DPA group, d% did not exceed ACL. In healthy individuals prolonged time and low storage temperature seemed to stimulate the leaking of copper ions from the walls of plastic caps followed by adsorption on the wall surface. In urine of patients on DPA therapy, copper stability for two temperature regimens was up to 2 weeks. Optimal delay of 2 days before analysis of urine 24 h samples for healthy individuals were observed. Greater stability up to 2 weeks for room temperature and refrigeration was typical for urine samples of patients on DPA. It might be due to the fact that urine copper complex with DPA is more stable that the urine copper complexes with peptides, amino-acids and low-weight molecular proteins.

Conclusion: Generally, storage of urine samples up to 2 weeks in both temperature regimens with no added preservatives is acceptable for copper analysis by flame atomic absorption.

SERUM COPPER AND ZINC IN PATIENTS WITH CHRONIC HEPATITIS C

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EuroMedLab Paris 2015; June 21-25 2015, Paris
Clin Chem Lab Med 2015; 53, Special Suppl, p. S1235

Background – aim: Trace elements are of a great importance for many physiological processes in human body. Their abnormal distribution may contribute to hepatic damage and from the other hand, liver disorders may lead to their disbalance. Disturbed trace-element status in patients with chronic hepatitis C (HCV) is related to stronger oxidative stress and inflammation thus concerning the therapy. Recently in the literature, the data about disordered copper (Cu) and zinc (Zn) homeostasis in HCV are contradictory. The aim of this study was to compare Cu and Zn serum concentrations in patients with chronic hepatitis C and healthy controls.

Methods: The study included 20 patients with HCV and 40 age and gender matched controls of healthy Bulgarian individuals. Copper/zinc ratio (Cu/Zn ratio) was also studied. Blood was drawn 7:30-9:30 am by standard collection procedure followed 12-hour fasting pause overnight. Serum samples were separated and immediately stored at -2/8°C until analysis. Serum copper and zinc were measured by flame atomic absorption spectrophotometer AAnalyst 400, Perkin Elmer. The results were expressed as mean ± SD and statistically processed by Student's t-test as $p < 0,05$ was considered significant.

Results: Statistically significant differences ($p < 0,001$) between serum metal levels and Cu/Zn ratio of healthy controls and patients with HCV were found. Serum Cu and Zn and Cu/Zn ratio of the healthy individuals were 15,7 ± 3,0 μmol/L; 13,4 ± 1,9 μmol/L and 1,2 ± 0,3 respectively. The same parameters for the patients with HCV were 19,9 ± 4,1 μmol/L; 10,7 ± 3,3 μmol/L and 2,0 ± 0,7 respectively. Significantly higher serum copper, lower serum zinc and increased Cu/Zn ratio were observed for HCV patients in comparison to healthy group.

Conclusions: In summary, our data imply that serum levels of copper and zinc and copper/zinc ratio might serve as biomarkers for viral hepatic damage.

BD BARRICOR® LH PLASMA TUBES FOR COPPER (CU) AND ZINC (ZN) DETERMINATION

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4th EFLM-BD European Conference on Preanalytical Phase; March 24-25 2017, Amsterdam
Clin Chem Lab Med 2017; 55(4):eA2

Background: In 2016 Becton Dickenson launched BarricorTM LH plasma blood collection tubes with mechanical separator. Especially for trace analyses the following BD tubes are in use: K_2 EDTA TE and Serum TE. Contamination is a major problem in trace analyses. So, standardization of preanalytical phase requires testing of each kind of tubes in the labs, even for particular LOT. The main goal of the study is comparison between BD BarricorTM LH tubes and the other specialized TE-BD tubes for Cu and Zn.

Materials and methods: Blood was drawn simultaneously from 32 patients into 3 types BD tubes: BarricorTM LH Plasma (REF 365032), K_2EDTA 10.8 mg Trace Element (REF 368381) and Serum Trace Element (REF 368380). All vacutainers were further processed in similar manner. Standards, controls and patient samples were measured for Cu and Zn by flame atomic absorption (FAAS)-AAnalyst 400, Perkin Elmer, USA.

Results: Results (mean ± SD in μmol/L) for Cu: BarricorTM LH Plasma – 20.1 ± 4.0; K_2EDTA 10.8 mg Trace Element – 19.8 ± 3.9 and Serum Trace Element – 20.5 ± 4.4, and for Zn: BarricorTM LH Plasma – 10.4 ± 2.2; K_2EDTA 10.8 mg Trace Element – 10.5 ± 2.1 and Serum Trace Element – 10.6 ± 2.3. Zn concentration in heparin plasma tubes did not differ significantly from that in the other two TE types: $p > 0.05$. Also no statistical difference was observed between serum (TE) and plasma (LH, K_2EDTA-TE) zinc. Significant statistical difference between copper in 3 tested tube types were established with the highest levels measured in BD Serum Trace Element Tubes. Significant difference between plasma Cu in LH and K_2EDTA tubes was found ($p < 0.001$) and also between Cu in LH plasma and TE serum ($p = 0.006$). The difference between Cu in all 3 tested tube types as absolute concentration was less than 1 μmol/L Cu.

Conclusion: It could be recommended for each laboratory to test the preferred vacutainers for determination of Cu and Zn especially when low concentrations in plasma or serum have been suspected.

Key words: copper, zinc, serum, plasma

STUDY ON CERULOPLASMIN ACTIVITY IN HEALTHY BULGARIANS

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EuroMedLab Athens 2017; June 11-15 2017, Athens
Clin Chem Lab Med 2017; 55, Special Suppl, p. S998

Background: Ceruloplasmin (Cp) is the major carrier of copper (Cu) in the body. Typically, it is measured for diagnosis of rare diseases such as Wilson disease (WD), Menke’s syndrome and aceruloplasminemia. Cp could be determined both as protein concentration (iCp) and enzymatic activity (eCp). Routine immunological testing is loaded with some methodological limitations, so evaluation of enzymatic Cp activity might be useful approach.

Methods: The study comprised 41 healthy Bulgarians (male:female = 16:25) with average age of 43 ± 13 years old. All sera were examined for iCp, eCp and serum Cu. The following methods were used: iCp – immunoturbidimetric method (SigmaAldrich, Pentra ABX); eCp – enzymatic method with chromogenic substrate o-dianisidine (Sigma-Aldrich, Pentra ABX); serum Cu – flame atomic absorption (Perkin Elmer, AAnalyst 400 Plus).

Results: Results are presented as mean ± SD and range: iCp – 36,9 ± 5,4 (27,9 to 50,99) mg/dL; eCp – 106,7 ± 20,4 (34,32 to 156,13) IU/L and serum Cu – 15,4 ± 1,8 (12,4 to 20,16) μmol/L. Slight significant difference between male and female eCp (p = 0,0458) was found, with higher values in females (111.9 ± 18,2 IU/L) vs. males (98,6 ± 20,5 IU/L). The correlation between iCp and eCp was high – r = 0,838. Mild correlation was established between Cu and iCp (r = 0,520), and also between Cu and eCp (r = 0,43).

Conclusion: This is the first study on eCp in healthy Bulgarians. We found mean values similar to those reported in the literature for other populations. In 5% (n = 2) of individuals eCp was out of the reference range for the method (60-140 IU/L). Good correlation was established between iCp and eCp, and also for Cp (concentration and enzymatic activity) and Cu. Recently, increased clinical implication of eCp is observed. Copper status seems to be important not only for rare disorders but in social significant diseases also. Deeper knowledge on eCp (reference ranges, factors of variations, clinical significance) could be necessary in disordered copper balance.

LEVELS OF COPPER AND ZINC IN SALIVA

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Saliva is composed of salivary glands secrets, cellular debris, upper respiratory tract fluid and microorganisms in the oral cavity [1]. Saliva testing for monitoring and diagnosis of both oral and systemic conditions is a challenge in accordance with the contemporary development of "omics" sciences. Research applications are directed to dental diseases, hormonal, neurological and emotional status, and human behaviour [1, 2]. Essential micronutrients copper (Cu) and zinc (Zn), simultaneously with cortisol and other laboratory salivary findings as adrenocorticotropic hormone, catecholamines and various cytokines, could be useful stress biomarkers [3]. Zn with neurotransmission and receptor functions is involved in mental health [2]. Cu and Zn are suggestive to participate in sleep duration as antagonists of N-methyl-D-aspartate ionotropic receptor of the sleep mediator glutamate [4]. Systemic zinc supplementation may alter salivary stress hormone levels, particularly these of cortisol [2]. The aim of the present study is to assess salivary Cu and Zn levels in adults during different time of the day.

The study comprises 40 volunteers at shift work (men:women = 31:9), average age 37 ± 9 years (men:women = 36:46). Cu and Zn are measured in two salivary samples: 1st sample released at 9-11am and 2nd released at 15-16 pm in the same day. Non-stimulated salivary samples are collected in Salivette tubes, Sarstedt. After centrifugation Cu and Zn levels are analysed by atomic absorption spectrophotometry (AAnalyst 400) with a deuterium background corrector.

The following results are established (mean ± SD, μmol/L): 1st saliva – Cu 0.61 ± 0.6; Zn 1.73 ± 1.1 and 2nd saliva – Cu 0.52 ± 0.8; Zn 1.49 ± 0.7. Lower concentrations of Cu and Zn are observed in the afternoon samples without statistically difference: Cu $p = 0.508$ and Zn $p = 0.953$.

The tendency for decreasing afternoon Cu and Zn values could be explained either by possible diurnal biological variation or accumulated during the working day stress. Present data are compatible with previously described effects of stress social factors [5]. Complex deeper knowledge in this aspect could be beneficial for oral health and disease prevention.

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БИОМАРКЕРИ В ЛИКВОР И СЕРУМ ПРИ РАННА ДИАГНОЗА НА БОЛЕСТ НА АЛЦХАЙМЕР

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Резюме към изследователски проект Договор 59/2013
Сборник резюмета на изследователски проекти 2012-2013, МУ – София, стр. 119

Резултати: Проучването включи 29 индивиди, от които 19 контроли и 10 с болест на Алцхаймер (БА), които бяха изследвани за: обща и „свободна“ мед в серум, ликвор/серум отношение на албумина (CSF/S Alb), както и мед, β-амилоид-42 (Aβ42) и тау-протеин в ликвор. В контролната група (11 мъже и 8 жени) се получиха следните резултати: обща серумна мед – 16.5 ± 3.1 μmol/l; несвързана с церулоплазмина мед (NCC) – 3.3 ± 0.24 μmol/l; CSF/S Alb – 5.75 ± 1.9, ликворна мед – 0.49 ± 0.1 μmol/l; Aβ42 – 754.4 ± 198.9 pg/ml; тау-протеин – 254.22 ± 112.2 pg/ml; резултатите при пациентите с БА (5 жени и 5 мъже) са респективно: 14.33 ± 0.8 μmol/l; 1.42 ± 1.6 μmol/l; 6.99 ± 1.2 μmol/l 0.50 ± 0.75 μmol/l; 192.42 ± 104.88 pg/ml; 397.8 ± 269.4 pg/ml. Наблюдава се тенденция за по-висока серумна мед при контролите, без статистическа значимост ($p = 0.070$). Ликворните нива на медта са почти еднакви и в двете групи ($p = 0.8$), както и в двете групи функцията на кръвно-ликворната бариера, оценена чрез отношението CSF/S Alb, е запазена (< 10.2). Статистически значима разлика ($p < 0.001$) с по-ниски нива в групата с БА се установи за Aβ42, докато тау-протеинът е с несигнификантно по-високи нива при БА ($p = 0.19$). По непубликувани наши данни средната стойност за серумна мед в българската популация е 16.04 ± 3.32 μmol/l, а за NCC – 4.22 ± 0.86 μmol/l ($n = 379$), т.е. изследваните болни с БА са с тенденция за по-ниски нива на обща серумна мед (14.3 μmol/l) и NCC (1.42 μmol/l).

СРАВНИТЕЛЕН АНАЛИЗ НА МЕД И ЦИНК В СЕРУМ И УРИНА ПРИ БЪЛГАРСКИ ПАЦИЕНТИ С ЧЕРНОДРОБНА ИЗЯВА НА БОЛЕСТТА НА УИЛСЪН И С ДРУГИ ХРОНИЧНИ ЧЕРНОДРОБНИ ЗАБОЛЯВАНИЯ

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Въведение: Черният дроб има ключова роля в метаболизма на медта и цинка както в норма, така и в патология. Промяната в медната обмяна е най-изразена при болестта на Уилсън (БУ), но редица отклонения се наблюдават и при други остри и хронични чернодробни заболявания. Намалени серумни нива на цинка се описват предимно при чернодробна цироза. Въпреки това липсват сравнителни проучвания на промените в серумните нива и уринната екскреция на медта и цинка при БУ и други хронични чернодробни заболявания.

Материал и методи: Изследвани са 169 лица, от които 50 здрави контроли, 39 с БУ, 25 с първична билиарна цироза (ПБЦ), 49 с хроничен вирусен хепатит (ХВХ; 35 HCV и 14 HBV) и 6 с неалкохолен стеатозен хепатит (НАСХ). Измерени са: серумни нива на мед ($Cu_{(S)}$) и цинк ($Zn_{(S)}$), концентрация на церулоплазмин (iCp), 24-часова купри- ($Cu_{(U)}$24 h) и цинкуреза ($Zn_{(U)}$24 h).

Резултати: Значимо най-ниска $Cu_{(S)}$ се установи при пациенти с БУ – 8.7 ± 5.8 μmol/L ($p < 0.01$), а значимо най-висока – при ПБЦ – 21.4 ± 4.2 μmol/L ($p < 0.05$). Най-ниски нива на $Zn_{(S)}$ са установени при лицата с ХВХ, като разликата е значима спрямо здравите контроли ($p < 0.002$). Групата с ХВХ показа значимо най-високи нива на iCp ($p < 0.03$), а тази с БУ – значимо най-ниски ($p < 0.001$) спрямо контролите. Повишена куприуреза се установи при БУ и ПБЦ, а при ХВХ и НАСХ – в референтен интервал. Значимо най-висока цинкуреза установихме при БУ, като при 53,8% от болните стойностите са над референтен обхват. При 32% от изследваните с ХВХ, на фона на $Zn_{(U)}$24 h в референтна област, е налице дефицит на $Zn_{(S)}$ (срещу 8% при здравите).

Дискусия: Познаването на статуса на медта и цинка, и на протеини, свързани с тяхната обмяна, разкрива фини аспекти в патологичния процес и дава възможности за по-добро диференциране на БУ от другите хронични чернодробни заболявания и индивидуализиран терапевтичен подход по отношение на извличането на излишната мед от организма и корекция на цинковия дефицит.

ОКСИДАЗНА АКТИВНОСТ НА ЦЕРУЛОПЛАЗМИН – СРАВНИТЕЛЕН АНАЛИЗ МЕЖДУ ЗДРАВИ И ПАЦИЕНТИ С БОЛЕСТ НА УИЛСЪН

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Според съвременни проучвания ензимната активност на церулоплазмина (eCp) е потенциален неинвазивен маркер за диагноза на болест на Уилсън (БУ). Целта на настоящото проучване е да се сравнят концентрациите на Cp (iCp, измерени чрез имунотурбидиметрия) и стойностите за eCp между здрави контроли и пациенти с БУ (от българската популация) на дългогодишна терапия с пенициламинов препарат.

Материал и методи: В изследването са включени 41 здрави доброволци (м:ж = 16:25; ср. възраст 43 ± 13 год.) и 28 пациенти с БУ (м:ж = 14:14; ср. възраст 38 ± 12 год.), които са на дългогодишно лечение с пеницилamин със средна доза 1000 mg/24 h. На всички индивиди са определени: серумна мед $Cu_{(S)}$ μmol/L с пламъкова атомно-абсорбционна спектрофотометрия, iCp g/L – турбидиметрично, eCp чрез ензимен метод с хромоген субстрат o-dianisidine и е изчислено съотношението eCp/iCp IU/g x 10^{-1}.

Резултати: Получени са следните резултати (mean ± SD) – за здрави: $Cu_{(S)}$ 15.4 ± 1.9; iCp 0.28 ± 0.03; eCp 103 ± 9; eCp/iCp 4.1 и в групата с БУ: $Cu_{(S)}$ 4.4 ± 5.2; iCp 0.08 ± 0.09; eCp 48 ± 31 и eCp/iCp 6. Двете сравнявани групи не се различават значимо според възрастта (p = 0.13). Установена е значима разлика в стойностите за $Cu_{(S)}$, iCp, eCp (p < 0.001) и eCp/iCp; (p < 0.003). Установена е висока корелация между нивата на iCp и eCp както при здрави (R = 0.83), така и при пациентите с БУ (R = 0.96), които са на дългогодишна терапия с пенициламинов препарат.

Дискусия: По данни от литературата като диагностичен критерий при пациенти с новооткрита БУ eCp има по-висока диагностична специфичност в сравнение с iCp (100% vs. 78.8%). При новодиагностицирани пациенти корелационната зависимост между iCp и eCp е по-ниска в сравнение със здрави (R 0.70 vs. 0.94). Получените от нас резултати показват обратното, което дава основание да се заключи, че определянето на eCp към рутинните маркери за охарактеризирането на медния статус би могло да бъде обещаващ показател и при проследяване хода на терапията.

25-OH VITAMIN D3/D2 INSUFFICIENCY IN HEALTHY SUBJECTS AND PATIENTS WITH AUTOIMMUNE DISORDERS

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Introduction: Vitamin D compounds D2 and D3 are obtained from dietary sources, including supplements or produced (as D3) in the skin upon ultraviolet exposure. These two forms are subsequently converted in the liver into 25-OH vitamin D3/D2 (25OHD) which concentration in blood is a relevant indicator of total supply in human body. Vitamin D deficiency vitamin D is worldwide spread and is associated with many pathological conditions: cardiovascular, neurological, metabolic, cancer, musculoskeletal, autoimmunity disorders. The aim of the study was to define the sufficiency in vitamin D levels in healthy individuals and to compare with patients with autoimmune diseases.

Materials and methods: The study comprises 90 adults in total, 26 men and 64 women: healthy controls (HC) – 42 (men:women – 16:26) and patients with suspected autoimmune disorders (systemic lupus, rheumatoid arthritis, vasculitis) – 48 (men:women – 10:38). All participants are tested for 25OHD serum levels using automatic competitive enzyme linked immunosorbent assay (ELISA) method on Alegria® device. The blood is taken after fasting over night, during the period of November to March.

Results: Levels measured (ng/ml) in the healthy controls (HC) are 17.9 ± 6 (men/women = 17 ± 4/18.4 ± 7) without significant differences depending on gender (p = 0.442). The observed levels for the patient group are 14.6 ± 5 (men/women = 14.5 ± 3.5/13.8 ± 5) with significant difference between genders (p = 0.0184). 25OHD concentrations of the patients with immunological disorders are significantly lower in comparison to healthy controls (p = 0.006). In both groups, levels above 30 ng/ml are not established. More frequent vitamin D deficiency (< 20 ng/ml) is found for patients in comparison to healthy individuals: 23% (n = 11) vs. 16.6% (n = 7). There are no samples with measured levels ≥ 50 ng/ml 25OHD as a marker of sufficiency.

Conclusion: The data of the current study is in agreement with already established prevalence of vitamin D deficiency for Bulgarian population, although active ultraviolet radiation over prolonged time period (over 8 months) is typical for the country. The results imply that immunomodulatory effect of vitamin D could be useful therapeutic tool in the treatment of various autoimmune conditions.

СТАТУС НА ВИТАМИН D ПРИ ЗДРАВИ БРЕМЕННИ ЖЕНИ

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Нарушеният баланс на витамин D е често срещан при жени в репродуктивна възраст от различни популации. Етиологията вероятно е мултифакторна, но отчасти се дължи на намален хранителен прием и ограничено излагане на слънчева светлина. При бременност тежкият дефицит се свързва с нарушена костна хомеостаза, вродени рахити и фрактури при новороденото. Досега не са напълно изяснени ефектите на недостатъчност за майката и плода. Липсват специфични за бременността препоръки за прием поради съществуващите неадекватни данни, които не диференцират нуждите при бременни жени от тези при небременни.

Цел: да се проучат при здрави бременни и небременни жени серумните нива на 25-хидроксивитамин D3 (25-OH-D3) като най-добър индикатор за насищане.

Модел на проучването: Изследвани са общо 53 жени (20-40 г.), разделени в две групи: здрави небременни (n = 29) и здрави бременни жени (24 ± 7 г.с.); (n = 24). Проучването е извършено в периода февруари-май. Анализите са извършени чрез високоспециализиран дефинитивен принцип: течна хроматография с тандем масспектрометрична детекция и изотопно разреждане (ID LC-MSMS). Данните са изразени като средна стойност ± SD.

Резултати: Серумната концентрация на депо-формата 25-OH-D3 при здрави небременни жени е 41 ± 13 nmol/L, характерна за ниво на недостатъчност (недостатъчност 25-80 nmol/L). 14% (n = 4) от всички жени в групата са с дефицит (< 25 nmol/L). Няма жени с резултат, показващ достатъчност (80-200 nmol/L). 86% от групата са с концентрации, показващи общо недостатъчност (n = 25): 68% от тази подгрупа са с изразена недостатъчност и 32% с лека недостатъчност.

Групата здрави бременни жени се характеризира с недостатъчност (25-OH-D3 60 ± 22 nmol/L): 33% (n = 8) са суплементирани (25-OH-D3 96 ± 29 nmol/L), останалите 67% са без суплементиране (25-OH-D3 60 ± 29 nmol/L). Само една бременна жена (4%) е с дефицит. Сравнителният анализ между двете групи

жени (здрави бременни и небременни) показва статистически силно повишение на серумните нива на 25-OH-D3 при нормална бременност с ($p > 0.001$; Student's t-test, ниво на значимост $p < 0.05$).

Заключение: Данните са в потвърждение на известни вече факти за широкото разпространение на нарушен статус на витамин D при жени в репродуктивна възраст и по време на бременност. Интригуващо, нивата на 25-OH-D3 при бременните жени са подчертано по-високи от тези при небременните. Суплементирането при нормална бременност в периодите с ограничена слънчева светлина би позволило да се излезе от зоната на недостатъчност и да се стигне до стойности, типични за достатъчност. Изглежда вероятно бременността да се свързва със специфични физиологични механизми, насочени не само към преодоляване на неонаталния дефицит, но и към осигуряване общо на по-високи нива на насищане у майчиния организъм.