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Осипов И.А.	
О ПРОБЛЕМЕ ВЛИЯНИЯ МОРОЗОСТОЙКОСТИ ЩЕБНЯ НА МОРОЗОСТОЙКОСТЬ БЕТОНА.....	38
Osipov I.A.	
THE PROBLEM OF THE INFLUENCE OF FROST RESISTANCE OF BREAKSTONE ON THE FROST RESISTANCE OF CONCRETE	38

Сидоренко А.Д., Скрипин А.П., Калачев П.В., Усков А.Е., Бондаренко А.А.	
ПРИМЕНЕНИЕ ВОЗОБНОВЛЯЕМОЙ ЭНЕРГЕТИКИ В ГОРОДСКИХ УСЛОВИЯХ КАК МЕТОД ПОВЫШЕНИЯ НАДЕЖНОСТИ ЭЛЕКТРОСНАБЖЕНИЯ.....	41
Sidorenko A.D., Skripin A.P., Kalachev P.V., Uskov A.E., Bondarenko A.A.	
THE USE OF RENEWABLE ENERGY IN URBAN CONDITIONS AS A METHOD OF INCREASING RELIABILITY POWER SUPPLY	41

Сидоренко А.Д., Скрипин А.П., Калачев П.В., Усков А.Е., Бондаренко А.А.	
ПЕРСПЕКТИВЫ СОЛНЕЧНЫХ ФОТОЭЛЕКТРИЧЕСКИХ СТАНЦИЙ.....	43
Sidorenko A.D., Skripin A.P., Kalachev P.V., Uskov A.E., Bondarenko A.A.	
PROSPECTS OF SOLAR PHOTOVOLTAIC PLANTS.....	43

MEDICAL SCIENCES

Антонів А.А., Коцюбійчук З.Я., Шумакова В.А., Урсакий Д.Д.	
РОЛЬ ЕНДОТЕЛІАЛЬНОЇ ДИСФУНКЦІЇ В ПАТОГЕНЕЗІ НЕАЛКОГОЛЬНОГО СТЕАТОГЕПАТИТУ ЗА КОМОРБІДНОГО ПЕРЕБІГУ З ХРОНІЧНОЮ ХВОРОБОЮ НИРОК.....	48
Antoniv A.A., Kotsyubiychuk Z.Y., Shumakova V.A., Ursakii D.D.	
THE ROLE OF ENDOTHELIAL DYSFUNCTION IN THE PATHOGENESIS OF NON-ALCOHOLIC STEATOGEPATITIS IN THE COMORBID COURSE WITH CHRONIC KIDNEY DISEASE	48

Антонів А.А., Каньовська Л.В., Юрнюк С.В., Вівсьяник В.В., Коржовська С.Ю.	
ОСОБЛИВОСТІ ЛІКУВАННЯ НЕАЛКОГОЛЬНОГО СТЕАТОГЕПАТИТУ ЗА КОМОРБІДНОГО ПЕРЕБІГУ З ХРОНІЧНОЮ ХВОРОБОЮ НИРОК (ХРОНІЧНИЙ ПІЄЛОНЕФРИТ).....	51
Antoniv A.A., Kanyovska L.V., Yurnyuk S.V., Vivsyannuk V.V., Korzhovska S.Y.	
PECULIARITIES OF TREATMENT OF NON-ALCOHOLIC STEATOGEPATITIS IN COMORBID COURSE WITH CHRONIC KIDNEY DISEASE (CHRONIC PYELONEPHRITIS).....	51

Антонів А.А., Коцюбійчук З.Я., Юрнюк С.В., Вівсьяник В.В., Руда І.І.	
АКТИВНІСТЬ ЗАПАЛЕННЯ У ПАЦІЄНТІВ З ХРОНІЧНОЮ ХВОРОБОЮ НИРОК ТА НЕАЛКОГОЛЬНИМ СТЕАТОГЕПАТИТОМ НА ФОНІ ОЖИРІННЯ, ЇХ ЗВ'ЯЗОК З ФУНКЦІОНАЛЬНИМ СТАНОМ ЕНДОТЕЛІУ.....	55
Antoniv A.A., Kotsyubiychuk Z.Y., Yurnyuk S.V., Vivsyannuk V.V., Ruda I.I.	
INFLAMMATION ACTIVITY IN PATIENTS WITH CHRONIC KIDNEY DISEASE AND NONALCOHOLIC STEATONEPATITIS ON THE BACKGROUND OF OBESITY, THEIR RELATIONSHIP WITH THE FUNCTIONAL STATE OF THE ENDOTHELIUM	55

Тарасенко І.Й., Макаренко О.А., Карман А. А.	
БІОХІМІЧНІ ПОКАЗНИКИ РОТОВОЇ РІДИНИ ДІТЕЙ ТА МАТЕРІВ ПРИ ПРОВЕДЕННІ СТОМАТОЛОГІЧНОГО ЛІКУВАННЯ В АНТЕНАТАЛЬНОМУ ПЕРІОДІ.....	59
Tarasenko I.Y., Makarenko O.A., Karman A. A.	
BIOCHEMICAL PARAMETERS OF ORAL FLUID OF CHILDREN AND MOTHERS DURING DENTAL TREATMENT IN THE ANTENATAL PERIOD.....	59

Анисимов М. В.	
ОЦЕНКА КЛИНИЧЕСКОЙ ЭФФЕКТИВНОСТИ МАНДИБУЛЯРНОЙ АНЕСТЕЗИИ В ТЕХНИКЕ BACK LOW BLOCK	63
Anisimov M.V.,	
EVALUATION OF THE CLINICAL EFFECTIVENESS OF MANDIBULAR ANESTHESIA IN THE BACK LOW BLOCK TECHNIQUE	63

MEDICAL SCIENCES

Антонів А.А.,
Коцюбійчук З.Я.,
Шумакова В.А.,
Урсакий Д.Д.

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РОЛЬ ЕНДОТЕЛІАЛЬНОЇ ДИСФУНКЦІЇ В ПАТОГЕНЕЗІ НЕАЛКОГОЛЬНОГО
СТЕАТОГЕПАТИТУ ЗА КОМОРБІДНОГО ПЕРЕБІГУ З ХРОНІЧНОЮ ХВОРОБОЮ НИРОК

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THE ROLE OF ENDOTHELIAL DYSFUNCTION IN THE PATHOGENESIS OF NON-ALCOHOLIC
STEATOHEPATITIS IN THE COMORBID COURSE WITH CHRONIC KIDNEY DISEASE

Резюме.

В даній статті узагальнено результати дослідження ролі ендотеліальної дисфункції за коморбідного перебігу неалкогольного стеатогепатиту та хронічною хворобою нирок I-II стадії. За результатами нашого дослідження було встановлено, що у даній категорії пацієнтів коморбідний перебіг супроводжується глибшим ліпідний дисбаланс, гіперхолестеролемія, у складі ліпопротеїдів низької густини, зниження вмісту ліпопротеїдів високої густини, зростання індексу атерогенності, а також гіперпродукція NO ендотелієм та лімфоцитами із прогресуючим ушкодженням ендотелію.

Resume.

This article summarizes the results of the study of the role of endothelial dysfunction in the comorbid course of non-alcoholic steatohepatitis and chronic kidney disease stage I-II. Our study found that in this category of patients comorbid course is accompanied by a deeper lipid imbalance, hypercholesterolemia, low-density lipoprotein, decreased high-density lipoprotein, increased atherogenic index, and hyperproduction of endothelial ligaments and endothelial hyperglycemia.

Ключові слова: неалкогольний стеатогепатит, хронічна хвороба нирок, ендотеліальна дисфункція.

Keywords: non-alcoholic steatohepatitis, chronic kidney disease, endothelial dysfunction.

Introduction. The comorbidity of non-alcoholic steatohepatitis (NASH) and chronic kidney disease (CKD) on the background of obesity is often recently drawn to the attention of both practitioners and researchers [1, 2]. Schematically, the development of NASH can be presented in several stages: fatty infiltration of the liver, oxidative stress, mitochondrial dysfunction, TNF / endotoxin-mediated injury, aseptic inflammation, diffused liver fibrosis, development of liver-cellular insufficiency (LCI) [1,2,3]. The first place among the causes of the development of NASH is insulin resistance syndrome. NASH most often occurs in obesity (20-81%). The prevalence of NASH in the world is 10% (600 million people) [2,4,5]. In the last 5 years in Ukraine, the incidence of steatohepatitis has increased by 76.6%. In the 12-40% of patients with liver steatosis during 8-13 years, NASH develops with early liver fibrosis (LF).

Chronic kidney disease (CKD) is an important problem in Ukraine and the world today, and the incidence rate has increased by 17% in recent years.

The frequency of occurrence of NASH in patients with CKD is unknown. The mechanisms of their joint development are described in isolated works, which

were conducted mainly in the experiments. Despite the fact that among various pathological processes in the internal organs that occur in the background of a metabolic syndrome - NASH is an extremely common disease, and quite often it occurs in patients with CKD, so far, this comorbidity remains a significant problem of the present and needs to be sufficiently studied.

The aim of the study – to find out the features of the lipid blood spectrum and the development of endothelial dysfunction in non-alcoholic steatohepatitis in patients with obesity of the 1st degree and chronic kidney disease of the 1st and 2nd stage.

Material and methods of research. 135 patients with non-alcoholic steatohepatitis (NASH) with comorbid obesity I degree and chronic kidney disease (CKD) of the 1st and 2nd stage, were examined. Patients were divided into 2 groups: of which 52 patients with non-alcoholic steatohepatitis with obesity I degree (group 1), 53 patients with non-alcoholic steatohepatitis with comorbid obesity of the 1st degree and chronic kidney disease of the I-II stage (chronic uncomplicated pyelonephritis with latent phase in subsiding exacerbation phase) (group 2). The control group consisted of 30 practically healthy persons of the corresponding age

and sex. The average age of patients was (45.8 ± 3.81) years, men were 48, and women 57 persons.

The functional state of the endothelium was studied by the content of stable metabolites of nitrogen monoxide (NO) (nitrites, nitrates) In the blood by L.C.Green et al. The number of desquamated endothelial cells in the blood was determined by the method of J.Hladovec in the modification of N.N. Petrishev et al. The statistical analysis was performed using parametric and non-parametric criteria (Student, Pearson) on RS AMD Athlon 64 using Statistica 5.1 software (StatSoft, Inc., USA) and SPSS 10.0.5. Standard Version.

The diagnosis of NASH was established in accordance with the unified clinical protocol, approved by the order of the Ministry of Health of Ukraine No. 826 from 06.11.2014, in the presence of criteria for the exclusion of chronic diffuse liver disease of the viral, hereditary, autoimmune or medicinal genesis as causes of cholestatic or cytolytic syndromes, as well as the results of the USG survey. Diagnosis and treatment of CKD were performed according to the recommendations of the clinical guidelines of the State Institute "Institute of Nephrology, NAMS of Ukraine" (2012). The analysis of clinical manifestations of NASH and CKD of the 1st and 2nd stage, biochemical, laboratory parameters of the functional state of the liver, kidneys, endothelium, ultrasonographic data was studied in dynamics after 30, 90 days of treatment, as well as 3 months after treatment.

The statistical analysis of the results was carried out in accordance with the type of research carried out

and the types of numerical data that were obtained. Distribution normality was verified using Liliefors, Shapiro-Uilka tests and the direct visual evaluation of eigenvalues distribution histograms. Quantitative indices having a normal distribution are represented as mean (M) \pm standard deviation (S). Discrete values are presented in the form of absolute and relative frequencies (percentage of observations to the total number of surveyed). For comparisons of data that had a normal distribution pattern, parametric tests were used to estimate the Student's t-criterion, Fisher's F-criterion. In the case of abnormal distribution, the median test, Mann-Whitney Rank U-Score, and Wilcox's T-criterion (in the case of dependent groups) were used for multiple comparison. Statistica for Windows version 8.0 (Stat Soft inc., USA), Microsoft Excel 2007 (Microsoft, USA) software packages were used for statistical and graphical analysis of the obtained results.

Results of the research and their discussion.

Analysis of the lipid profile of the blood in patients with NASH and obesity showed a number of changes that differed depending on the presence of CKD (table 1). Indicators of concentration in blood of total lipids in patients of 1st and 2nd groups exceeded the norm by 26.4% and 34.2%, respectively, with a statistically significant difference between the groups ($p < 0.05$). The content of total cholesterol in blood indicated that it increased by 37.4 and 46.7 ($p < 0.05$) compared with PHPs in patients of 1st and 2nd groups. Changes in the concentration of TG in the form of a significant increase (respectively, 2.2 and 2.0 times ($p < 0.05$)) were recorded in the 1st and 2nd groups of patients.

Table 1

Indicators of lipid spectrum of blood, glycemia and endothelial dysfunction in patients with non-alcoholic stethohepatitis, obesity of the I-II degree and with comorbidity with chronic kidney disease of the I-II stage (M \pm m)

Indicators, measurement units	Groups of patients surveyed		
	PHP	Group 1 NASH+Obesity	Group 2 NASH with CKD + Obesity
Total Cholesterol, mmol / l	4,72 \pm 0,11	6,89 \pm 0,38*	6,93 \pm 0,39*/**
LDL, mmol / l	2,54 \pm 0,02	4,05 \pm 0,022 *	4,58 \pm 0,04*/**
HDL, mmol / l	1,28 \pm 0,05	0,72 \pm 0,02 *	0,76 \pm 0,04 */**
TG, mmol / l	1,47 \pm 0,03	2,42 \pm 0,03*	3,19 \pm 0,07 */**
NO IN BLOOD, mmol / l	15,32 \pm 1,225	30,49 \pm 1,318 *	40,51 \pm 1,173 */**
ET-1, pmol / l	6,17 \pm 0,854	11,25 \pm 0,457 *	18,83 \pm 0,559 */**
DEC \times 104/L	3,03 \pm 0,204	3,87 \pm 0,123 *	5,80 \pm 0,127 */**

Notes: * - changes are probable in comparison with the index in PHP ($P < 0.05$);
** - changes are probable when comparing the indices in patients with NASH ($P < 0.05$);

That is, the content in TG in the blood in the comorbid flow of NASH with CKD and obesity were significantly lower than in patients with NASH and obesity.

The study of blood concentrations of proatherogenic lipoprotein fractions indicated a number of changes: the concentration of LDL in the patients of the 1st group was 1.5 times higher than the control group ($p < 0.05$), and in patients of the 2nd group LDL increased in 1.7 times ($p < 0.05$). It is also necessary to point out that with the increase in the activity of cytotoxicity, the content of the cholesterol and LDL in the blood in NASH with comorbidity with CKD and obesity - increased, which may be an important prognostic factor

in the progression of atherosclerosis in these patients. Concentration in blood of antiatherogenic lipoproteins - HDL in patients of both groups was significantly lower in comparison with control: in patients of the 1st group - in 1.5 times ($p < 0.05$), in 2nd group - 1.7 times ($p < 0.05$). As can be seen from the results of the study, the maximum suppression of HDL synthesis (Table 1) was observed in patients of the 2nd group, indicating a minimum level of protection of endothelial vessels from free radical aggression and atherogenic fractions of blood lipids. The result of these changes was a significant increase in the index of atherogenicity in patients of both groups of observation: the 1st group - 2.2 times, the 2nd group - 2.0 times with the maximum

changes in the index in patients with NASH, CKD and obesity, which testifies on the one hand, the presence of significant risk factors for the progression of atherosclerosis in these patients on the background of obesity, and on the other - on the favorable pathogenetic situation with regard to the progress of NASH. Thus, the development of NASH in patients with CKD and obesity is accompanied by a significant disorder of dis-hyperlipidemia with the highest among groups comparing with the increase in the content of cholesterol and low-density proatherogenic lipoprotein, a possible decrease in high-density anti-atherogenic lipoprotein and an increase in the atherogenicity index.

In patients of 1st and 2nd groups, a slight increase in cardiac glycemia was observed at 9.3% and 14.8%, respectively ($p < 0.05$) compared with the control group. Analysis of indicators of postprandial glycemia, obtained during Gamma glutamyltransferase (GGT), in patients of 1st and 2nd groups also showed an increase in glucose content in 120 min after loading - respectively by 16.5% and 31.2% ($p < 0.05$) compared with indicators in the PHP group. Investigation of insulin content in blood on an empty stomach revealed hyperinsulinemia, which in patients of the 1st group exceeded the index in the PHP group by 1.9 times; in patients of the 2nd group insulin content exceeded the norm by 2.2 ($p < 0.05$) times.

The results of the study showed that in patients with NASH, a significant increase in the content of NO in the blood was detected in comparison with the index in PHP ($p < 0.05$) (Table 1) in group 1 - in 2,1 times, in the 2nd group - in 2,6 times ($p < 0.05$). The role of nitrosative stress in the pathogenesis of NASH was proved, the confirmation of which is the increase in the concentration of nitrosothiols, peroxy-nitrite and other metabolites NO in the blood [2, 10]. Increased peroxy-nitrite formation due to the generation of NO by leukocytes is an important aspect of the damaging effect and inflammation process in NASH [3]. Pathological hyperproduction of NO by endothelial cells and leukocytes from inflammatory infiltrates in the liver contributes to the development of nitrosative stress in NASH. The established hypernitrate in blood may also be considered compensatory in response to hyperproduction of ET-1 in all observational groups. Thus, the content of ET-1 exceeded the index in PHP, respectively, in patients in the 1st group in 1.7 times, in the 2nd group - in 3.0 times ($p_{1-2} < 0.05$). Confirmation of the presence of endothelial dysfunction (ED) in patients with NASH with CKD resulted in a probable growth of the number of desquamated endothelial cells (DEC) in the 2nd group of patients in 1.9 times ($p_2 < 0.05$). Generation by neutrophils during the exacerbation of NASH of a significant number of active forms of oxygen and nitrogen and hyperproduction of endothelial cells and endometrial lymphocytes with progressive damage to the endothelium (growth of DEC) leads to significant ED, accompanied by mosaic angiospasm of the arteries due to hyperproduction of ET-1 and parietic vasodilatation of the veins of the portal vein system because of the hyperproduction of NO.

Conclusions Thus, in patients with non-alcoholic steatohepatitis on the background of obesity lipid distress syndrome with an increase in total cholesterol in blood, low density proatherogenic lipoproteins, and a deficiency in anti-atherogenic high-density lipoproteins is characterized. In a comorbid flow of non-alcoholic steatohepatitis and chronic kidney disease stage I and II on the background of obesity a deeper lipid imbalance (hypertriacylglycerolemia (2.1 times, $p < 0.05$), hypercholesterolemia (1.5 times, $p < 0.05$), including in the low density lipoprotein (1.8 times, $p < 0.05$), decrease in the content of high density lipoprotein (1.8 times, $p < 0.05$), increase in the atherogenic index (at 2, 7 times, $p < 0.05$), as well as hyperproduction of the endothelium of the NO and lymphocytes with progressive damage to the endothelium (growth of DEC)) was confirmed.

The prospect of further scientific research in this direction is the development of a method for the early prevention of non-alcoholic steatohepatitis on the background of obesity and the accompanying CKD of the 1st and 2nd stage.

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ОСОБЛИВОСТІ ЛІКУВАННЯ НЕАЛКОГОЛЬНОГО СТЕАТОГЕПАТИТУ ЗА КОМОРБІДНОГО ПЕРЕБІГУ З ХРОНІЧНОЮ ХВОРОБОЮ НИРОК (ХРОНІЧНИЙ ПІСЛОНЕФРИТ)

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PECULIARITIES OF TREATMENT OF NON-ALCOHOLIC STEATOHEPATITIS IN COMORBID COURSE WITH CHRONIC KIDNEY DISEASE (CHRONIC PYELONEPHRITIS)

Анотація.

У статті представлено теоретичне узагальнення результатів клінічної ефективності S-аденозилметіоніну у хворих на неалкогольний стеатогепатит (НАСГ) при супутніх захворюваннях з ожирінням і хронічною хворобою нирок (ХНП) 1-2 стадії, що продукує потужний мембрано-стабілізуючий вплив на уражені гепатоцити, що надовго усуває клінічні прояви захворювання, інтенсивність цитолізу, холестази, мезенхімально-запального синдрому, гальмує прогресування гепатоцелюлярної та ниркової дисфункції (підвищує альбумін-синтезувальну та гломерну функцію печінки). швидкість фільтрації) шляхом оптимізації контролю фіброзу печінки та нирок.

Abstract.

The article presents a theoretical generalization of the results of the clinical efficacy of S-adenosylmethionine in patients with non-alcoholic steatohepatitis (NASH) in comorbidity with obesity and chronic kidney disease (CKD) of the 1st-2nd stages, which produces powerful membrane-stabilizing effects on the affected hepatocytes, which permanently eliminates clinical manifestations of the disease, the intensity of cytolysis, cholestasis, mesenchymal-inflammatory syndrome, inhibits the progression of the hepatocellular and renal dysfunction (increases the albumin-synthesizing function of the liver and the glomerular filtration rate) by optimizing the control of liver and kidneys fibrosis.

Ключові слова: *неалкогольний стеатогепатит, хронічна хвороба нирок, S-аденозилметіонін.*

Keywords: *non-alcoholic steatohepatitis, chronic kidney disease, S-adenosylmethionine.*

Introduction. The comorbid flow of non-alcoholic steatohepatitis (NASH) and chronic kidney disease (CKD) has often recently attracted the attention of both practitioners and researchers [4, 9]. Without correction of clinical and biochemical syndromes of liver and kidney damage by interrupting the cascade of interactions, the cessation of the progression of their inflammation, the fibrosing of both organs and the restoration of their functional state can not be corrected [1, 7, 8, 9]. An important place in the pathogenesis of both diseases is the disturbance of carbohydrate and lipid homeostasis, oxidative and nitrosatitistic stress, endogenous intoxication that helps to accelerate apoptosis of hepatocytes, endothelium, and further their cytolysis on the background of autoimmune cytokine mechanisms

activation of inflammation progression and fibrosing reactions, which leads to progressive functional lack of organs [4, 9].

Therefore, among modern methods of treating patients with NASH and CKD, the use of correctors of several parts of the pathogenesis of most components with comorbidity with the probable normalization of the maximum number of parameters of homeostasis is important. [1, 4].

One of these drugs is S-adenosylmethionine (SAM), which, according to the literature, has detoxification, antioxidant, membrane-stabilizing properties (promoting the synthesis of glutathione), the ability to eliminate intrahepatic cholestasis (by activating enzymes that provide transport of bile micelles on the