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Część 1

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ALT, μmol / hour×1	0,38± 0,014	1	1,4±0,02 *	1,2±0,08 *	0,8±0,03 */**	0,7±0,05 */**
		2	1,4±0,02 *	0,6±0,02 */**/#	0,5±0,02 */**/#	0,4±0,02 **/#
		3	1,4±0,01 *	0,5±0,02 */**/#/****	0,4±0,01 **/#/****	0,4±0,01 **/#
Total protein, g/l	76,13± 2,12	1	60,30±2,11*	65,26± 2,25 *	66,5±2,39 *	70,3±2,53 **
		2	60,31±1,92*	75,8±2,31**	78,2±2,04 **/#	81,2±2,31 **/#
		3	60,28±1,84*	80,2±2,37 **/#	82,3±2,13 **/#	82,6±2,12 **/#
Albumin, %	59,37±2,23	1	43,63±2,33*	45,32± 1,97 *	50,42±1,79 *	51,0±1,92 *
		2	43,62±2,34 *	54,83± 1,35**/#	59,27±1,25 **/#	59,8±1,18 **/#
		3	43,63±2,35 *	57,15± 1,42 **/#	60,42±1,34 **/#	60,1±1,24 **/#
Bile acid, mmol/l	1,27± 0,01	1	2,83±0,06 *	2,74±0,35 *	2,72±0,53 *	2,60±0,17 *
		2	2,81±0,08 *	2,12±0,03 */**/#	1,57±0,05 **/#	1,39±0,04 **/#
		3	2,82±0,07 *	1,94±0,05 */**/#/****	1,36±0,02 **/#/****	1,20±0,05 **/#/****
Thymol test, conditional units	2,82± 0,13	1	4,30±0,15*	4,24±0,21*	4,13±0,13*	4,01±0,21 *
		2	4,33±0,13*	3,53±0,17 */**/#	3,21±0,07 */**/#	3,09±0,08 **/#
		3	4,32±0,12*	3,41±0,10 */**/#	2,90±0,06 */**/#/****	2,76±0,07 **/#/****
Glomerular fil- tration rate, ml/min	117,0±3,37	1	78,5± 3,26*	80,2± 3,75*	82,7± 3,14*	87,3± 3,79*
		2	78,3± 3,25*	96,5± 2,43 */**/#	100,2± 2,64 **/#	105,8± 2,28 **/#
		3	78,6± 3,28*	106,8± 2,27 */**/#/****	112,5± 2,51 **/#/****	116,1± 2,39 **/#/****

Notes: 1. \* the difference is probable compared to the indicator for practically healthy persons (p <0,05); 2. \*\* the difference is probable compared with the indicator before treatment (p <0,05); 3. # - the difference is probable compared to the indicator after treatment in patients in group 1 (p <0,05); 4. \*\*\*\* - the difference is probable compared to the indicator after treatment in patients in group 2 (p <0,05).

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**АКТИВНІСТЬ ЗАПАЛЕННЯ У ПАЦІЄНТІВ З ХРОНІЧНОЮ ХВОРОБОЮ НИРОК ТА  
НЕАЛКОГОЛЬНИМ СТЕАТОГЕПАТИТОМ НА ФОНІ ОЖИРІННЯ, ЇХ ЗВ'ЯЗОК З  
ФУНКЦІОНАЛЬНИМ СТАНОМ ЕНДОТЕЛІО**

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**INFLAMMATION ACTIVITY IN PATIENTS WITH CHRONIC KIDNEY DISEASE AND  
NONALCOHOLIC STEATOHEPATITIS ON THE BACKGROUND OF OBESITY, THEIR  
RELATIONSHIP WITH THE FUNCTIONAL STATE OF THE ENDOTHELIUM**

**Abstract.**

*The article summarizes the clinical study, which showed that in non-alcoholic steatohepatitis that develops on the background of obesity and chronic kidney disease I-III stage, the presence of fibrotic changes in the liver tissue was found, which according to the biochemical index of fibrosis, exceeds those in patients with non-alcoholic steatohepatitis without comorbidity with kidney pathology. In patients with non-alcoholic steatohepatitis, which was accompanied by obesity, a significant increase in the synthesis of collagen and glycosaminoglycans*

*which was accompanied with an ineffective resorption of newly formed collagen due to inhibition of the collagenolytic activity of blood plasma, due to significant activation of proteinase inhibitors ( $\alpha$ 2-MG) was observed with a significant imbalance in the system of connective tissue metabolism.*

**Keywords:** *non-alcoholic steatohepatitis, chronic kidney disease, liver fibrosis.*

**Резюме.** У статті узагальнено клінічне дослідження, яке показало, що при неалкогольному стеатогепатиті, що розвивається на тлі ожиріння та хронічної хвороби нирок I-III стадії, виявлено наявність фіброзних змін у тканині печінки, які за біохімічним показником фіброз, перевищує показники у хворих на неалкогольний стеатогепатит без супутньої патології нирок. У хворих на неалкогольний стеатогепатит, який супроводжувався ожирінням, відзначається значне збільшення синтезу колагену та глікозаміногліканів, що супроводжується неефективною резорбцією новоутвореного колагену через пригнічення колагенолітичної активності плазми крові. інгібіторів протеїнази ( $\alpha$ 2-MG) спостерігався значний дисбаланс у системі метаболізму сполучної тканини. **Ключові слова:** неалкогольний стеатогепатит, хронічна хвороба нирок, фіброз печінки.

**Introduction.** An important role in the pathogenesis of the progression of liver and kidney diseases is played by the components of the connective tissue system of the extracellular matrix [3, 7]. According to the literature, non-alcoholic fatty liver disease (NAFLD) in progress leads to the development of both liver cirrhosis and hepatocellular carcinoma, the incidence of which on the background of NAFLD substantially exceeds the indicators in the population. There are numerous attempts by scientists to find new probable biochemical markers of the intensity of fibrosis formation [9, 10], increasing the diagnostic value, sensitivity and specificity of existing methods, and developing methods of influence to inhibit these processes.

The "golden standard" for the diagnosis of fibrosis is considered to be a liver biopsy with morphological study of biopsy and evaluation of fibrosis stages on one of the proposed scales (R.Knodell, Ishak, V.J.Desmet, METAVIR, E. Brunt). Widespread implementation of non-invasive research methods: liver fibro elastography, ultrasonographic elastography of the liver after the wave shift of the HSG signal, as well as biochemical fibrotest, patented by T. Pounard. Despite the high level of study of the pathomorphological picture and the patterns of liver fibrosis progression in non-alcoholic steatohepatitis (NASH) on the background of obesity [9], the features of fibrosis in the liver tissue and its metabolic prerequisites in NASH with comorbidity with chronic kidney disease (CKD), depending on the stage, have not been studied sufficiently.

**The objective of the article:** to find out the features of biochemical markers of liver fibrosis with non-alcoholic steatohepatitis in patients with I-II degree obesity and chronic kidney disease I-III stage

**Material and methods of research:** 98 patients with non-alcoholic steatohepatitis on the background of I-II degree obesity were examined: 52 patients with non-alcoholic steatohepatitis (1st group) (without accompanying chronic kidney disease), 46 patients with

non-alcoholic steatohepatitis with a comorbid chronic kidney disease I-III stage (2nd group). The control group consisted of 20 practically healthy persons (PHPs) with the corresponding age and sex. Biopsy of the liver was performed on 32 patients with non-alcoholic steatohepatitis with the accompanying of chronic kidney disease I-III stage, 28 patients with non-alcoholic steatohepatitis without chronic kidney disease. Patients on both groups of non-alcoholic steatohepatitis received heparhizine treatment (glycyrrhizin 40 mg, glycine 400 mg, L-cysteine hydrochloride 20 mg) by intravenous administration of 20 ml of the drug for 10 days followed by enteral administration of 2 tablets of heparhizine (1 tablet: glycyrrhizin 25 mg, glycine - 25 mg, methionine - 25 mg) 3 times a day for 80 days. Patients with non-alcoholic steatohepatitis with a comorbid flow of non-alcoholic steatohepatitis, obesity and chronic kidney disease of the I-III stage, except heparisin, they received baseline therapy of chronic kidney disease I-III stage: chronic pyelonephritis (course of antibacterial drugs, uroseptics, cainfron). The examinations were carried out prior to treatment and on the 90th day of treatment.

Changes in the metabolism of the components of the extracellular matrix were determined by the content of free oxyproline in the blood by S.S. Tetanets (1985) and protein-bound oxyproline according to M.S Osadchuk (1979), hexosamines according to O.G. Arhipova (1988), seromucoid, sialic acids, non-protein-bound fucose, with the help of Danush Ltd company (Lviv), ceruloplasmin by the method of Revin (1976), the level of collagenolytic activity of blood plasma : by the intensity of azokol lysis; the content of the fibroblast growth factor in the blood, and also on the parameters of the total fibrotest (T.Pounard) by the method of immunoassay analysis. The statistical analysis was performed using parametric and non-parametric criteria (Student, Pearson) on PC AMD Athlon 64 using Statistica 5.1 software (StatSoft, Inc., USA) and SPSS 10.0.5. Standart Version.

**Results of the research:** Based on the obtained results, among the examined patients with NASH in 1st group, the zero stage of fibrosis (F0) occurred in 28.6% of patients, while 42.8% of patients registered probable fibrotic changes (F1) in the liver tissue. In patients with NASH 1st group F2 stage was registered in 17.9% of patients, F3 - in 10.7%. Thus, fibrotic changes in the F1 stage were most often recorded.

In the group of patients with NASH 2nd group, F0 stage of fibrosis was observed in 9.4% of patients. F1 stage was recorded in the ratio of 28.1%, F2 - 37.5%, F3 stage was registered in 25.0% of patients in 2nd group. F4 stages in this contingent were not detected. The obtained results indicate the involvement of chronic kidney disease in the induction of liver tissue fibrosis with the background of NASH and obesity.

In order to identify possible risk factors in the progression of liver fibrosis and additional biochemical markers of the intensity of fibrous reactions, we carried out a correlation analysis between the biochemical index of fibrosis and markers of basic biochemical syndromes of NASH, which established the existence of a potential direct correlation between the biochemical index of fibrosis and ALT activity ( $r = 0.67$ ,  $p < 0.05$ ), alkaline phosphatase activity ( $r = 0.53$ ,  $p < 0.05$ ), blood bile acid content ( $r = 0.51$ ,  $p < 0.05$ ). The given data indicate that the intensity of the fibrous reactions in patients with NASH, developed on the background of obesity, depends on the activity of the cytolytic syndrome and cholestasis. With the progression of the stage of fibrosis, the detoxification function of the liver decreases (with the activity of arginase ( $r = -0.62$ ,  $p < 0.05$ )).

The analysis of the intensity of the fibrous reactions in patients with NASH, depending on the presence of a comorbid chronic kidney disease, indicates a probable increase in the content of protein-bound oxyproline in the blood of patients in the 1st group - 1.6 times compared with practically healthy person ( $p < 0.05$ ), patients in 2nd group - 2.0 times ( $p < 0.05$ ), which indicates the high activity of collagen anabolism in this contingent of patients. At the same time, the index of free oxyproline in blood (Table 1), which is the

biochemical marker of collagen catabolism, in patients with NASH in the 1st group was 1.2 times lower than that in a practically healthy person ( $p < 0.05$ ). That is, in patients with NASH an intensification of collagen formation processes is observed with the background of resorption processes reduction of newly formed collagen. At the same time, in patients in the 2nd group, the free oxyproline content in the blood exceeded the content in a practically healthy person by 1.4 times ( $p < 0.05$ ), indicating an increase in collagen degradation in the background of its high synthesis. The interdependence of the above-mentioned changes confirms the presence of a correlation between the content of free oxyproline and  $\alpha_2$ -MG ( $r = 0.51$ ,  $p < 0.05$ ), the content of protein-bound oxyproline and collagen anabolism ( $r = 0.43$ ,  $p < 0.05$ ); the content of free oxyproline and collagen anabolism ( $r = 0.53$ ,  $p < 0.05$ ) in the 2nd group.

We established a strong direct correlation between the values of ceruloplasmin in the blood and the content of bile acids ( $r = 0.67$ ,  $p < 0.05$ ), ceruloplasmin and alkaline phosphatase activity ( $r = 0.63$ ,  $p < 0.05$ ). The increase in the content of acute phase proteins that support the quality of inflammation and are activated under the conditions of cholestasis, in particular bile acids, is one of the important factors in the progression of fibrosis in the liver.

Table 1

**Biochemical parameters of the connective tissue components condition in patients with non-alcoholic steatohepatitis, obesity I-II degree and with the comorbidity of chronic kidney disease I-III stages in the heparisin treatment dynamics ( $M \pm m$ )**

Indicators, measurement units.	Groups of surveyed patients				
	practically healthy person	NASH prior to treatment.	NASH after treatment.	NASH with Chronic kidney disease prior to treatment.	NASH with Chronic kidney disease after treatment.
FibroTest, C.U	0,18±0,01	0,29±0,02*	0,20±0,01#	0,46±0,01 */**	0,23±0,02 #
Protein-bound oxyproline, $\mu\text{mol/l}$	41,48±3,72	64,72±2,38*	43,25±3,23#	83,50±3,73 */**	58,25±3,15 */#
Free oxyproline, $\mu\text{mol/l}$	12,39±0,34	10,31±0,50 *	12,76±0,38#	17,38±0,54 */**	13,25±0,98 #
hexosamines, $\text{mmol/l}$	5,54±0,02	6,77±0,12*	5,68±0,15 #	8,52±0,27 */**	6,13±0,23 */#
sialic acids, $\text{mmol/l}$	1,92±0,02	2,42±0,03*	2,03±0,01#	2,85±0,02 */**	2,38±0,02 */#
Fucose not linked to the protein, $\mu\text{mol/l}$	37,42±5,79	64,22±5,31*	41,70±3,52#	92,56±3,12 */**	67,15±4,27 */#
Collagenolytic activity, C.U	0,84±0,01	0,73±0,01 *	0,86±0,01#	0,93±0,01 */**	1,25±0,01 */#
Ceruloplasmin, $\text{mmol/l}$	12,63±0,12	17,86±0,52*	12,75±0,61#	23,83±1,13 */**	16,71±0,71 */#
Fibronectin, $\mu\text{g/ml}$	334,94±12,04	424,21±13,35*	345,28±10,72 */#	525,30±22,19 */**	417,37±12,38 */#
$\alpha_2$ -MG, $\text{mmol/l}$	2,35±0,12	4,93±0,13*	3,21±0,11 */#	6,34±0,14 */**	4,83±0,16 */#
Fibroblasts growth factor, $\text{nmol/l}$	17,92±1,07	36,13±2,52 *	21,25±1,37 */#	53,23±2,29 */**	31,63±2,13 */#

Notes: \* - changes are probable in comparison with the index in practically healthy person ( $P < 0.05$ );  
\*\* - changes are probable when comparing the indices in patients with NASH ( $P < 0.05$ );  
# - changes are possible when comparing the indicators before treatment ( $P < 0.05$ ).

The established disturbances in the balance of collagen anabolism and catabolism analysis were accompanied by a significant increase in the factors of their regulation and induction, in particular, the content of the fibroblasts growth factor in the blood - more noticed in patients with NASH and CKD (an increase 3.1 times against 2.1 times in 1st group  $p < 0.05$ ). This induction explains the phenomenon of "Capillarization of Hepatic Sinusoid" in patients with NASH with the activation of perisinusoidal star cells of Ito, their transformation into myofibroblast-like cells with hyperproduction of collagen in the Disse space, the development of pericellular, perisinusoidal, centrolobular and other types of fibrosis against the background of aseptic inflammation around the dystrophically altered (steatosis) hepatocytes, narrowing of sinusoids and formation of progressive disorders of portal circulation.

As the data show, in the comorbidity of non-alcoholic steatohepatitis, obesity with chronic kidney disease, phenomena are more expressed and increase faster in comparison with non-alcoholic steatohepatitis on the background of obesity.

The obtained data testify that in patients with NASH, which arose on the background of obesity, a significant increase in the synthesis of collagen and glycosaminoglycans was observed, which was accompanied by an ineffective resorption of newly formed collagen due to inhibition of collagenolytic activity of blood plasma at NASH, which arose as a result of activation of proteinase inhibitors ( $\alpha_2$ -MG), a significant imbalance in the metabolism of connective tissue, which leads to progressive liver fibrosis and violation of its functions. Under conditions of the comorbidity of NASH with CKH of the I-III stages, synthesis and resorption of collagen are activated, but the processes of anabolism prevail, despite the compensatory activation of collagenolysis, with a significant hyperproduction of acute-phase proteins, fibronectin, fibroblasts growth factor and increased degradation of fucoglycoproteins.

The use of the drug heparizin showed the presence of its effect on the substantial correction of the revealed disturbances of homeostasis components extracellular matrix. Thus, the average index of fibro test in patients with NASH in 1st group after treatment was decreased by 1.5 times ( $p < 0.05$ ), in 2nd group - 2.0 times ( $p < 0.05$ ) (Table 1). Blood content of protein-bound oxyproline in patients of the 1st group decreased by 1.5 times ( $p < 0.05$ ), and in patients in the 2nd groups - 1.4 times ( $p < 0.05$ ), indicating inhibition of collagen anabolism processes under the influence of the drug. At the same time, the content of  $\alpha_2$ -MG blood (Table 1) after treatment decreased by 1.5 and 1.3 times, respectively ( $p < 0.05$ ). The content of ceruloplasmin in the blood after treatment decreased 1.4 times in both groups ( $p < 0.05$ ), and the content of fibronectin decreased - 1.2 and 1.3 times respectively ( $p < 0.05$ ). We found a significant effect of heparizin on the content of fibroblasts growth factor in the blood-reduction in both groups after treatment in 1.7 times ( $p < 0.05$ ). Thus, we have established a significant corrective effect of heparizin on the metabolic rate of the extracellular matrix connective tissue system of the liver, both in terms of comor-

bidity with and without chronic kidney disease. In particular, heparizin therapy contributed to the achievement of the collagen catabolism balance by activating collagen lysis, inhibiting the activity of proteolytic inhibitors and collagen lysis, inhibition of the secretion of fibroblast growth factor, acute phase inflammation indicators, degradation of fucoglycoproteins of the liver, and in general, reducing the activity of reparative processes in the connective tissue, which proved to be a decrease in the index of liver fibrosis according to the fibro test within 1.5-2.0 times.

#### Conclusion.

1. In non-alcoholic steatohepatitis that develops on the background of obesity and chronic kidney disease I-III stage, the presence of fibrotic changes in the liver tissue, which according to the biochemical index of fibrosis exceeds those in patients with NASH without comorbidity with kidney pathology, has been established.

2. A significant increase in the synthesis of collagen and glycosaminoglycans was observed in patients with NASH, which was accompanied by an ineffective resorption of newly formed collagen due to inhibition of the collagenolytic activity of blood plasma, due to significant activation of proteinase inhibitors ( $\alpha_2$ -MG), a significant imbalance in the system of connective tissue metabolism. Under conditions of the comorbidity of NASH with CKH I-III stages, synthesis and resorption of collagen is activated, but the processes of anabolism prevail in spite of compensatory activation of collagenolysis, with a significant hyperproduction of acute-phase proteins, fibronectin, glycosaminoglycans, fibroblast growth factor and elevated degradation of extracellular matrix fucoglycoproteins and lead to progressive fibrosis in the liver and disturbance of its functions.

3. Heparizin therapy for 3 months contributed to the achievement of a collagen ana- and catabolism balance by activating collagen lysis, inhibiting the activity of proteolytic inhibitors and collagenolysis, inhibition of fibroblast growth factor secretion, acute phase inflammation indicators, degradation of liver extracellular matrix fucoglycoproteins, and in general, reducing the activation of connective tissue components, by evidence of a decrease in the index of liver fibrosis according to the fibro test in the range of 1.5-2.0 times.

**The prospect of further research** in this direction is the development of early diagnosis method of liver fibrosis by biochemical markers of fibrosis formation in non-alcoholic steatohepatitis on the background of obesity and the accompanying of chronic kidney disease I-III stage.

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## **БІОХІМІЧНІ ПОКАЗНИКИ РОТОВОЇ РІДИНИ ДІТЕЙ ТА МАТЕРІВ ПРИ ПРОВЕДЕННІ СТОМАТОЛОГІЧНОГО ЛІКУВАННЯ В АНТЕНАТАЛЬНОМУ ПЕРІОДІ**

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## **BIOCHEMICAL PARAMETERS OF ORAL FLUID OF CHILDREN AND MOTHERS DURING DENTAL TREATMENT IN THE ANTENATAL PERIOD**

### **Анотація**

Розроблений лікувально-профілактичний комплекс супроводу стоматологічного лікування вагітних жінок, до складу якого входили препарати «Кальцикер», «Алфавіт для вагітних», «Вітафтор» та зубні пастки «R.O.C.S.», «R.O.C.S. Medical mineral», «R.O.C.S. Bionica» і «Lacalut fluor», мав позитивний вплив на біохімічні показники ротової рідини як у жінок під час вагітності, так і у їхніх народжених дітей. Так, у дітей, які отримували антенатальну профілактику, в порівнянні з дітьми, які не отримали терапію під час внутрішньоутробного розвитку, була встановлена краща мінералізуюча здатність ротової рідини. У дітей основної групи в ротовій рідині також наблизилися до норми показники активності лізоциму, уреазу та ступеня дисбіозу, що свідчить про високий рівень неспецифічного антимікробного захисту. У вагітних жінок запропонована терапія за 4 місяці застосування призвела до збільшення в ротовій рідині активності лізоциму та каталази, а також до зменшення активності уреазу, еластази та вмісту малондіальдегіду.

### **Abstract**

A medical and preventive complex for supporting dental treatment of pregnant women was developed, which included preparations "Calciker", "Alphabet for pregnant women", "Vitafor" and toothpastes "R.O.C.S.", «R.O.C.S. Medical mineral», «R.O.C.S. Bionica "and" Lacalut fluor", had a positive effect on the biochemical parameters of oral fluid both in women during pregnancy and in their children born. Thus, children who received antenatal prophylaxis, in comparison with children who did not receive therapy during intrauterine development, had a better mineralizing ability of oral fluid. In children of the main group, the indicators of lysozyme activity, urease and the degree of dysbiosis in the oral fluid also approached normal, which indicates a high level of non-specific antimicrobial protection. In pregnant women, the proposed therapy for 4 months of use led to an increase in the activity of lysozyme and catalase in the oral fluid, as well as to a decrease in the activity of urease, elastase and malondialdehyde content.