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CHANGES IN EXTRACELLULAR MATRIX COMPONENTS METABOLISM IN PATIENTS WITH NONALCOHOLIC STEATOHEPATITIS ON THE BACKGROUND OF OBESITY AND COMORBIDITY WITH CHRONIC KIDNEY DISEASE

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The purpose of the study was to find out the features of the state of carbohydrate-protein components of the connective tissue of the liver and kidneys extracellular matrix in non-alcoholic steatohepatitis in patients with obesity of the 1st degree and chronic kidney disease of the I–II stages.

We examined 140 patients with non-alcoholic steatohepatitis with comorbid obesity of the 1st degree and chronic kidney disease of the I–II stages. Patients were divided into 2 groups that were randomized according to age, sex, degree of obesity, and stage of chronic kidney disease (chronic uncomplicated pyelonephritis with latent course in the phase of retinal exacerbation). The control group consisted of 30 practically healthy persons of the corresponding age and sex.

As a result of studies, it was found out that a significant increase in the synthesis of collagen and glycosaminoglycans was observed in patients with non -alcoholic steatohepatitis that arose on the background of obesity. It was accompanied by ineffective resorption of newly formed collagen due to inhibition of the collagenolytic activity of blood plasma, due to significant activation of proteinase inhibitors, significant imbalance in the system metabolism of connective tissue.

Under the conditions of comorbidity of non-alcoholic steatohepatitis and chronic kidney disease, the synthesis and resorption of collagen are activated. In spite of compensatory activation of collagenolysis, the anabolism processes predominated with a significant hyperproduction of actinic-phase proteins, fibronectin, glycosaminoglycans, fibroblast growth factor, and led to progressive fibrosis of the liver and disturbance of its functions.

Keywords: nonalcoholic steatohepatitis, chronic kidney disease, obesity, extracellular matrix.

Relationship of work with scientific programs, plans, themes. This work is a fragment of the research work «Pathogenetic mechanisms of mutual burden and clinical features of the course of non-alcoholic fatty liver disease and chronic kidney disease, justification of differentiated treatment», state registration number is 0111U006303.

Introduction. The comorbidity of non-alcoholic steatohepatitis (NASH) and chronic kidney disease (CKD) on the background of obesity has recently drawn the attention of both practitioners and researchers [1, 2]. An important role in the pathogenesis of progression of liver and kidney diseases is played by the system of components of connective tissue (CT) of the extracellular matrix (PCM) [3, 4, 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. Scientists frequently attempted to find new probable biochemical markers of fibrosis formation intensity [8, 9, 11], to increase the diagnostic value, sensitivity and specificity of existing methods, and to develop methods of influence to inhibit these processes.

The purpose of the study was to find out the features of the state of carbohydrate-protein components of the connective tissue of the liver and kidneys extracellular matrix in non-alcoholic steatohepatitis in patients with obesity of the 1st degree and chronic kidney disease of the I–II stages.

Material and methods of research. We examined 140 patients with non-alcoholic steatohepatitis (NASH) with comorbid obesity of 1st degree and chronic kidney disease (CKD) of I–II stages. Patients were divided into 2 groups that were randomized according to age, sex, degree of obesity, and stage of chronic kidney disease (chronic uncomplicated pyelonephritis with latent course in the phase of retinal exacerbation). The first group comprised 58 patients with NASH on the background of obesity (without accompanying CKD), the second group had 52 patients

with NASH on the background of obesity with a comorbid CKD I–II stages. The control group consisted of 30 practically healthy persons of the corresponding age and sex.

Changes in the metabolism of the components of the extracellular matrix were determined by the free oxyproline content in blood (FOP) by S. S. Tetyanets (1985) and protein-bound oxyproline (PBOP) by M. S. Osadchuk (1979), hexosamines (HA) by O. G. Archipova (1988), seromucoid (SM), sialic acid (SA), fucose-free protein (FFP), using Danush Ltd (Lviv), ceruloplasmin (CP) by the Revina method (1976), the level of collagenolytic activity of blood plasma (CLA): according to the intensity of azocel lysis; the content of the fibroblast growth factor (FGF) in the blood, and also on the parameters of the total fibrotest (T. Pounard) by the enzyme-linked immunosorbent assay (ELISA).

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 dated 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 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) ± standard deviation (S). Discrete values are presented in the form of absolute and relative frequencies (percentage of observations to the total number of surveyed). We used parametric tests to estimate the Student's t-criterion, Fisher's F-criterion and to compare the data with a normal distribution pattern. 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. According to the obtained results (**Table**), the intensity of the fibrous reactions in patients with NASH, depending on the presence of a comorbid CKD, indicates a probable increase in PBOP blood serum in patients of group 1 by 1.6 times compared with PHP ($p < 0.05$), patients of group 2 by 2.0 times ($p < 0.05$), indicating high activity of collagen anabolism in this contingent of patients. At the same time, the index of FOP in blood (**Table**), which is the biochemical marker of collagen catabolism, in patients with NASH in group 1 was 1.2 times lower than that in PHP ($p < 0.05$). That is, in patients with NASH an intensification of collagen formation processes was observed with the background of newly formed collagen resorption processes reduction. At the same time, in patients of group 2, the FOP content in the blood exceeded the data in the PHP by 1.4 times ($p < 0.05$), indicating an increase in collagen degradation on the background of its high synthesis. The interdependence of the abovementioned changes confirms the presence of a correlation between the content of FOP and α2-MG in blood ($r = 0.51$, $p < 0.05$), the content of PBOP and CLA ($r = 0.43$, $p < 0.05$); the content of FOP and CLA ($r = 0.53$, $p < 0.05$) in group 2.

The analysis of other elements of the extracellular matrix components of protein origin changes in blood, in particular, ceruloplasmin, indicates its probable increase in patients with steatohepatitis of all groups of observation ($p < 0,05$) with a probable prevalence in patients with NASH in group 2 (1.9 times against 1.4 times in group 1, $p < 0.05$). We established a strong direct correlation between the values of ceruloplasmin in the blood and the content of bile acids ($r = 0.67$, $p < 0.05$), with ceruloplasmin and Alkaline phosphatase activity ($r = 0.63$, $p < 0.05$). The increase in the content of osmotic phase proteins that support the quality of inflammation and are activated under conditions of cholestasis, in particular bile acids, is one of the important factors in the progression of fibrosis in the liver.

The analysis of changes in another important component of the protein-derived PCM (**Table**) – fibronectin belonging to cellular adhesion molecules indicates a probable increase in its content in the blood of patients with NASH with CKD ((1.6 times, $p < 0.05$), while in patients with NASH its growth was 1.4 times ($p < 0.05$) compared with the indicator in the PHP.

The established disturbances in the balance of collagen catabolism and anabolism analysis were accompanied by a significant increase in the factors of their regulation of those inductions, in particular, the content of fibroblasts growth factor in the blood (FGF) were more noticed in patients with NASH and CKD (an increase 3.1 times against 2.1 times in Group 1 $p < 0.05$). These phenomena explains induction phenomenon «Sinusoidal capillary» in patients with NASH with perisinusoidal star cells Ito activation, turning

them into myofibroblast-like cells with hyperproduction of collagen in Diss space, the development of pericellular, perissinusoidal, centrolobular and other types of fibrosis on the background of aseptic inflammation around dystrophic (steatosis) of hepatocytes, narrowing of sinusoids and formation of progressive disorders of portal circulation. As the obtained data showed, for the comorbidity of NASH with obesity and CKD, these phenomena were more pronounced and increase faster in comparison with the course of NASH only against 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. It 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 (α2- MG), a significant imbalance in the metabolism of CT, which leads to progressive liver fibrosis and violation of its functions. Under conditions of the comorbidity of NASH from the CKD of the I–III stages the collagen synthesis and resorption are activated, but the processes of anabolism predominate, in spite of compensatory activation of collagenolysis, with a significant hyperproduction of actinic-phase proteins, fibronectin, glycosaminoglycans, fibroblast growth factor and increased degradation of extracellular matrix fucoglycoproteins and lead to progressive fibrosis of the liver and disruption of its functions.

Table – Indicators of the connective tissue components status in patients with non-alcoholic stethogepatitis, obesity and comorbidity with chronic kidney disease

Indicators, measurement units	Groups of examined patients		
	PHP	Group I NASH with Obesity	Group II NASH with CKD and Obesity
PBOP, μmol/l	41.48 ± 3.72	64.72 ± 2.38*	83.50 ± 3.73*/**
FOP, μmol/l	12.39 ± 0.34	10.31 ± 0.50*	17.38 ± 0.54*/**
HA, mmol/l	5.54 ± 0.02	6.77 ± 0.12*	8.52 ± 0.27*/**
SC, mmol/l	1.92 ± 0.02	2.42 ± 0.03*	2.85 ± 0.02*/**
FFP, μmol/l	37.42 ± 5.79	64.22 ± 5.31*	92.56 ± 3.12*/**
CLA, c.u.	0.84 ± 0.01	0.73 ± 0.01*	0.93 ± 0.01*/**
Ceruloplasmin, mmol/l	12.63 ± 0.12	17.86 ± 0.52*	23.83 ± 1.13*/**
fibronectin, μg/ml	334.94 ± 12.04	424.21 ± 13.35*	525.30 ± 22.19*/**
α_2-MG, mmol/l	2.35 ± 0.12	4.93 ± 0.13*	6.34 ± 0.14*/**
FGF, nmol/l	17.92 ± 1.07	36.13 ± 2.52*	53.23 ± 2.29*/**

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 ($P < 0.05$).

Conclusions 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 plasma, due to significant activation of proteinase inhibitors, a significant imbalance in the system of connective tissue metabolism . Under conditions of the comorbidity of NASH with the CKD of the I–II stages the collagen synthesis and resorption are activated, but the processes of anabolism predominate, in spite of compensatory activation of collagenolysis, with a significant hyperproduction of actinic-phase proteins, fibronectin, glycosaminoglycans, fibroblast growth factor and increased degradation of extracellular matrix fucoglycoproteins and lead to progressive fibrosis of the liver and disruption of its functions.

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 I–II stages.

References

1. Babak OYa, Kolesnikova EV, Syitnik KA. Profilakticheskie meropriyatiya pri nealkogolnoy zhirovoy bolezni pecheni: suschestvuet li sposob snizit risk razvitiya zabolevaniya? [Preventive measures for non-alcoholic fatty liver disease: is there a way to reduce the risk of the disease?]. *Suchasna gastroenterol*. 2013; 3 (71): 103-9. [Russian]
2. Bueverov AO, Bogomolov PO. Nealkogol'naya zhirovaya bolezn' pecheni: obosnovanie patogeneticheskoy terapii [Non-alcoholic fatty liver disease: rationale for pathogenetic therapy]. *Klinicheskie perspektivy v gastroenterologii, gepatologii*. 2009; 1: 3-9. [Russian]
3. Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol*. 2013; 10: 330-44. PMID: 23507799. DOI: 10.1038/nrgastro.2013.41
4. Baumgarten M, Gehr T. Chronic kidney disease: detection and evaluation. *American Family Physician*. 2011; 84(10): 1138-48.
5. Brunt EM, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA, et al. NASH Clinical Research Network (CRN)ю Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. *Hepatology*. 2011; 53(3): 810-20. PMID: 21319198. PMCID: PMC3079483. DOI: 10.1002/hep.24127

6. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. Diagnosis and Management of Non-alcoholic Fatty Liver Disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Am J Gastroenterol.* 2012; 107: 811–26. PMID: 22488764. DOI: 10.1002/hep.25762
7. Castera L, Vilgrain V, Angulo P. Noninvasive evaluation of NAFLD. *Nat Rev Gastroenterol Hepatol*. 2013; 10 (11): 666–75. PMID: 24061203. DOI: 10.1038/nrgastro.2013.175
8. Cohen E, Nardi Y, Krause I, Goldberg E, Milo G, Garty M, Krause I. A longitudinal assessment of the natural rate of decline in renal function with age. *J Nephrol*. 2014; 27(6): 635-41. PMID: 24643437. DOI: 10.1007/s40620-014-0077-9
9. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med.* 2011; 43: 617-49. PMID: 21039302, DOI: 10.3109/07853890.2010.518623
10. Nascimbeni F, Pais R, Bellentani S, Day CP, Ratziu V, Loria P, Lonardo A. From NAFLD in clinical practice to answers from guidelines. *J Hepatol.* 2013; 59(4): 859-71. PMID: 23751754. DOI: 10.1016/j.jhep.2013.05.044
11. Webb M, Yeshua H, Zelber-Sagi S, Santo E, Brazowski E, Halpern Z, Oren R. Diagnostic value of a computerized hepatorenal index for sonographic quanification of liver steatosis. *Am J Roentgenol.* 2009; 192(4): 909-14. PMID: 19304694. DOI: 10.2214/AJR.07.4016

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ЗМІНИ МЕТАБОЛІЗМУ КОМПОНЕНТІВ ПОЗАКЛІТИННОГО МАТРИКСУ У ХВОРИХ НА НЕАЛКОГОЛЬНИЙ СТЕАТОГЕПАТИТ НА ТЛІ ОЖИРІННЯ ЗА КОМОРБІДНОСТІ З ХРОНІЧНОЮ ХВОРОБОЮ НИРОК

Антонів А. А.

Резюме. *Мета дослідження* – з'ясувати особливості стану вуглеводно-білкових компонентів сполучної тканини позаклітинного матриксу печінки та нирок при неалкогольному стеатогепатиті у хворих на ожиріння I ступеня та хронічну хворобу нирок I–II стадії.

Матеріали і методи. Обстежено 140 хворих на неалкогольний стеатогепатит із коморбідними ожирінням I ступеня та хронічною хворобою нирок I–II ст. Пацієнти були поділенні на 2 групи, які були рандомізовані за віком, статтю, ступенем ожиріння та стадією хронічної хвороби нирок (хронічний неускладнений пієлонефрит із латентним перебігом у фазі стихаючого загострення). Контрольну групу склали 30 практично здорових осіб відповідного віку та статі.

Результати дослідження. У результаті досліджень було встановлено, що у хворих на неалкогольний стеатогепатит, що виник на тлі ожиріння, встановлено істотне підвищення синтезу колагену та глікозаміногліканів, яке супроводжується неефективною резорбцією новоутвореного колагену внаслідок гальмування колагенолітичної активності плазми крові, внаслідок істотної активації інгібіторів протеїназ, істотного дисбалансу в системі метаболізму сполучної тканини.

Висновки. За умов коморбідності неалкогольного стеатогепатиту та хронічної хвороби нирок активуються і синтез і резорбція колагену, але процеси анаболізму переважають, незважаючи на компенсаторну активацію колагенолізу, із істотнішою гіперпродукцією гострофазових білків, фібронектину, глікозаміногліканів, фактора росту фібробластів і призводять до прогресуючого фіброзування печінки та порушення її функцій.

Ключові слова: неалкогольний стеатогепатит, хронічна хвороба нирок, ожиріння, позаклітинний матрикс.

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ИЗМЕНЕНИЯ МЕТАБОЛИЗМА КОМПОНЕНТОВ ВНЕКЛЕТОЧНОГО МАТРИКСА У БОЛЬНЫХ НА НЕАЛКОГОЛЬНИЙ СТЕАТОГЕПАТИТ НА ФОНЕ ОЖИРЕНИЕ С КОМОРБИДНОЙ ХРОНИЧЕСКОЙ БОЛЕЗНЬЮ ПОЧЕК

Антонив А. А.

Резюме. *Цель исследования* – выяснить особенности состояния углеводно-белковых компонентов соединительной ткани внеклеточного матрикса печени и почек при неалкогольного стеатогепатите у больных ожирением I степени и хронической болезнью почек I–II стадии.

Материалы и методы. Обследовано 140 больных на неалкогольный стеатогепатит с коморбидными ожирением I степени и хронической болезнью почек I–II ст. Пациенты были разделения на 2 группы, которые были рандомизированы по возрасту, полу, степени ожирения и стадией хронической болезни почек (хронический неосложненный пиелонефрит с латентным течением в фазе затихая обострения). Контрольную группу составили 30 практически здоровых лиц соответствующего возраста и пола.

Результаты исследования. В результате исследований было установлено, что у больных на неалкогольный стеатогепатит, возникший на фоне ожирения, установлено существенное повышение синтеза коллагена и гликозаминогликанов, которое сопровождается неэффективной резорбцией нового коллагена вследствие торможения колагенолитичной активности плазмы крови, вследствие существенного активации ингибиторов протеиназ, существенного дисбаланса в системе метаболизма соединительной ткани.

Выводы. В условиях коморбидности неалкогольного стеатогепатита и хронической болезни почек активируются и синтез и резорбция коллагена, но анаболические преобладают, несмотря на компенсаторную активацию колагенолиза, с существенной гиперпродукцией острофазовых белков, фибронектина, гликозаминогликанов, фактора роста фибробластов и приводят к прогрессирующему фиброзированию печени и нарушению ее функций.

Ключевые слова: неалкогольный стеатогепатит, хроническая болезнь почек, ожирение, внеклеточный матрикс.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of coauthors of the article.

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