RENAL FUNCTIONAL STATUS AND INFLAMMATION ACTIVITY IN PATIENTS WITH CHRONIC KIDNEY DISEASE AND NONALCOHOLIC STEATOHEPATITIS ON THE BACKGROUND OF OBESITY

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**Keywords:** chronic kidney disease, nonalcoholic steatohepatitis, glomerular filtration rate, oxidative stress, endotoxicosis, lipid distress syndrome, functional state of the endothelium.

The aim of the study was to find out the probable effect of the comorbid flow of nonalcoholic steatohepatitis on the functional state of the kidneys and the activity of inflammation of the kidneys in patients with chronic kidney disease (pyelonephritis) of the I–III stages, to determine the pathogenetic role of endothelial dysfunction, lipid distress syndrome, endotoxicosis and oxidative stress in the mechanisms of their mutual burden.

**Material and methods.** 240 patients with chronic kidney disease (chronic bilateral pyelonephritis) of the I–III stages were examined, 145 of which had comorbid nonalcoholic steatohepatitis and obesity of the 1st degree (group 1), 95 patients were diagnosed with stages I–III of chronic kidney disease stages without comorbid pathology. Depending on the stage of the chronic kidney disease, both groups were divided as follows: the 1st group — into 3 subgroups: 51 patients with stage 1 of chronic kidney disease, 53 patients with stage 2 of chronic kidney disease, 41 patients with stage 3 of chronic kidney disease. The 2nd group was divided into 3 subgroups: 32 patients with stage 1 of chronic kidney disease, 35 patients with stage 2 of chronic kidney disease, 28 patients with stage 3 of chronic kidney disease. The control group consisted of 30 practically healthy persons.

**Results.** As a result of the research it was established that nonalcoholic steatohepatitis affects the functional state of the kidneys in patients with stages I–III of chronic kidney disease I-III stages with a possible reduction of nitrogen function, velocity of glomerular filtration, increase in the intensity of hypoalbuminemia, proteinuria, leukocyturia, erythrocyturia, cylinduria, bacteriuria than in isolated course of chronic kidney disease.

**Conclusions.** For the comorbidity of the chronic kidney disease with nonalcoholic steatohepatitis and a decrease in glomerular filtration rate, an increase in the intensity of oxidative stress, endotoxicosis, lipid distress syndrome, degree of violation of the functional state of the endothelium: increased activity of iNOS, nitrite/nitrate content, endothelin-1, homocysteine, cytokeratin-18, decrease in the activity of arginase, H2S content, which correlate with the intermediate and high power interactions with the index of glomerular filtration rate.
Цель исследования — выяснить возможное воздействие коморбидности хронической болезни почек на ФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ И АКТИВНОСТЬ ВОСПОЛЕНИЯ ПОЧЕК У БОЛЬНЫХ ХРОНИЧЕСКОЙ БОЛЕЗНИЮ ПОЧЕК И НЕАЛКОГОЛЬНЫМ СТЕАТОГЕПАТИТОМ НА ФОНЕ ОЖИРЕНИЯ.


ФКНКЦИОНАЛЬНОЕ СОСТОЯНИЕ И АКТИВНОСТЬ ВОСПОЛЕНИЯ ПОЧЕК У БОЛЬНЫХ ХРОНИЧЕСКОЙ БОЛЕЗНЮ ПОЧЕК И НЕАЛКОГОЛЬНЫМ СТЕАТОГЕПАТИТОМ НА ФОНЕ ОЖИРЕНИЯ

А.А. Антонов

Ключевые слова: хроническая болезнь почек, неалкогольный стеатогепатит, скорость клубочковой фильтрации, оксидативный стресс, эндотоксикоз, липидный дистресс-синдром, функциональное состояние эндотелия.
Relevance of the problem. Comorbidity of chronic kidney disease (CKD) with nonalcoholic steatohepatitis (NASH) in obesity patients has a significant increase in the frequency of this type of comorbidity (15–30%) [1, 2, 3, 4, 5]. CKD affects up to 8% of the adult population of the world, and its prevalence increases significantly in the category of the elderly (up to 38%) that suffering from such diseases as obesity, metabolic syndrome, diabetes, arterial hypertension, and smoking [1, 3, 4, 6, 7, 8, 9]. In our previous studies, it was found that the clinical course of NASH significantly impairs the comorbidity of CKD, which, in progress, is accompanied by an increasing degree of endogenous intoxication, oxidative and nitrosativistis stress against the suppression of the antioxidant defense system and the natural system of detoxification, lipid distress syndrome, functional state of the endothelium, disorders of microcirculation, peripheral and organ blood circulation, growing fatty degeneration of hepatocytes (steatosis), cytolytic and cholestatic syndromes, activation of mesenchymal inflammation with the activation of biosynthesis of protein, carbohydrate-protein components of connective tissue extracellular matrix of the liver, kidneys and myocardium with development of their diffuse fibrosis [9, 10]. The above mechanisms are important links in the pathogenesis of CKD and NASH mutual burden, especially if they occur on the background of obesity, which confirm the results of our studies and the results obtained by other researchers [2, 5, 11, 12, 13]. At the same time, the degree of these disorders and features of the functional state of the kidneys for the comorbidity of the CKD with NASH have not been established yet.

The aim of the study. To establish the probable effect of the comorbid flow of nonalcoholic steatohepatitis on the functional state of the kidneys and the activity of kidney inflammation in patients with chronic kidney disease (pyelonephritis) of the I–III stages and to determine the pathogenetic role of endothelial dysfunction, lipid distress syndrome, endotoxicosis and oxidative stress in mechanisms of their mutual burden.

Material and methods of research. 240 patients with CKD (chronic bilateral peylonephritis) of the I–III stages were examined, 145 of which had comorbid NASH and obesity of the 1st degree (group 1), 95 patients were diagnosed with CKD I-III stages without comorbid pathology. Depending on the stage of the CKD, both groups were divided as follows: 1st group — into 3 subgroups: 51 patients with 1st stage CKD, 53 patients with 2nd stage CKD, 41 patients with 3rd stage CKD. The 2nd group was divided into 3 subgroups: 32 patients with 1st stage CKD, 35 patients with 2nd stage CKD, 28 patients with 3rd stage CKD. The control group consisted of 30 practically healthy persons (PHPs). The average age of patients was (49.8 ± 5.8) years. 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, autoimmunne or medicinal genesis as causes of cholestatic or cytolytic syndromes, as well as the results of the USG survey. Diagnosis of obesity was established on the basis of calculating the body mass index (BMI) by the formula of Kettle: BMI = body weight (kg)/height2 (m). On the basis of an increase in BMI of 30–34.9 kg/m2, 1st degree it was established, with BMI 35–39.9 kg/m2–2nd degree, BMI above 40 kg/m2–3rd degree obesity. The diagnosis of CKD was carried out in accordance with the recommendations of the clinical guidelines of the State Institute "Institute of Nephrology, NAMS of Ukraine" (2012) [2]. The study included patients with CKD I-III stages without a nephrotic syndrome with chronic uncomplicated pyelonephritis in the phase of exacerbation. The glomerular filtration rate (GFR) was investigated by creatinine clearance, calculated using the Cockcroft-Gault formula, as well as by the universal automatic calculator CKD-EPI [2]. In addition to standard methods of research (blood creatinine, urea, proteinuria, ionograms, urinalysis, urine analysis by the methods of Nechyporenko, Zimnytsky, urine culture with
the definition of the pathogen, its amount and sensitivity to antibiotics, etc.) we studied the intensity of oxidative stress — by malondialdehyde (MA) content in the blood, intensity of oxidative modification of proteins (OMP) — by the content of aldehyde- and ketone dinitrophenyl-hydrazones neutral (AKDNPH N) and basic (AKDNPH B). The degree of endogenous intoxication was studied based on the content of the medium molecular peptides (MMP) in the blood and the activity of arginase. The lipid spectrum of blood was studied by the contents of common lipids in blood; total cholesterol (TC), low and high density cholesterol, lipoproteins and triacylglycerol (TG) using a set of reagents of the company Danish LTD (Lviv).

The functional state of the endothelium and its regulation were studied in terms of the content of nitrogen monoxide (stable NO metabolites: nitrite/nitrate), hydrogen sulfide (H2S), endothelin-1, homocysteine, cytoketatin-18, induction and endothelial NO synthase activity (iNOS, eNOS) using enzyme-linked immunosorbent assay (ELISA) by using the sets of reagents of the firm “VSM Ukraine” (Ivano-Frankivsk city). The statistical analysis of the results was carried out in accordance with the type of research and the types of numerical data that were obtained. Distribution normality was verified using Lilliefors, Shapiro-Ulka tests and the direct visual evaluation of eigenvalues distribution histograms. Quantitative indices having a normal distribution are represented as mean (M) ± standard deviation (s). 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. To measure the relationship between variables, Pearson's correlation analysis using parametric distribution and the Spirman rank correlation coefficient was applied in the case of a distribution of indicators that significantly differed from the normal one. 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.

**Research results.** Analysis of indicators of the functional state of the kidneys showed that the creatinine content in the blood of the 1st and the 2nd group of patients of CKD I st. statistically significantly different. Thus, in patients of group 1, the indicator exceeded the data in the PHPs by 1.5 times (p <0,05), in group 2 — in 1.3 times (p <0,05) (table 1). In patients with CKD II st. In group 1, the creatinine content exceeded the index in PHPs by 1.7 times against 1.5 times in group 2 (p <0,05). Accordingly, in patients with CKD of the III st. the content of creatinine in patients with group 1 exceeded the data in PHPs by 2.3 times (p <0,05), in group 2 — by 1.9 times (p <0,05), in all cases with the probable difference between groups (p <0,05) (table 1). Thus, comorbidity with NASH significantly affects the functional parameters of the state of the kidneys, in particular, their nitrogen-containing function. This position is confirmed by the obtained data on the content of urea in the comparative aspect between the groups (table 1). Thus, the urea content in blood in patients with CKD I st. exceeded the indicators in PHPs, respectively, in 1st and 2nd group — in 2,4 and 2,2 times (p <0,05). In patients with CKD II st. in group 1 the urea content exceeded the index in PHPs by 2.5 times compared with 2.4 times in group 2 (p <0,05). Accordingly, in patients with CKD of the III st. the content of urea in patients with group 1 exceeded the data in the PHPs by 2.9 times (p <0,05), in group 2 — by 2.5 times (p <0,05), with the presence of a probable difference between the groups (p <0,05). An analysis of albumin content in blood also points to a significant difference between the comparison groups. In particular, the index of blood albumin content in patients with CKD I st. was lower than that in PHPs, respectively, in groups 1 and 2 — in 1,3 and 1,2 times (p <0,05), however, the probable difference between the groups was not established (p >0,05). In patients with CKD II st. In group 1, the albumin content was 1.4 times lower than that of PHPs and 1.3 times in group 2 (p <0,05). Accordingly, in patients with CKD of the III st. albumin content in patients with group 1 was lower than the standard one in 1,5 times (p <0,05), in group 2 — in 1,4 times (p <0,05), with the probable difference between the groups of patients with comorbidity with NASH and CKD II st. and CKD III st. and for CKD without comorbid diseases (p <0,05). As a result of the established changes, there was a significant decrease in the GFR for creatinine clearance by the Cockroft-Gault formula and calculated by the CKD-EPI (table 1). Thus, the indicator of clearance of creatinine by the Cockroft-Gaulta formula in patients with CKD I st. was lower than that in PHPs only in group 1 patients (11.8%) (p <0,05); in patients of the group 2, changes were unlikely and no significant difference was found between the groups (p > 0.05). In patients with CKD II st. in group 1, the creatinine clearance score was lower than the PHPs by 39.2% versus a decrease of 25.5% in group 2 (p <0,05) with a confirmation of statistically significant difference between the groups (p <0,05). At the same time, in patients with CKD III st. the rate of creatinine clearance in patients in group 1 was lower than the normative at 55.9% (p <0,05), in group 2 — by 44.1% (p <0,05), with the presence of a probable difference between patients with a combined course NASH and CKD in comparison with patients with CKD without comorbid diseases (p <0,05). Calculation of GFR using CKD-EPI points to a higher accuracy of GFR evaluation, since the index significantly differed from the comparison groups, indicating the probability of our working hypothesis. So, the index of GFR in patients with CKD I st. was lower than that in PHPs in patients of group 1 in 1,5 times (p <0,05), in patients of group 2 — in 1,3 times (p <0,05) with confirmation of statistically significant difference between groups (p <0,05). In patients with CKD II st. in group...
1 GFR was 1.9 times lower than the PHPs, compared with a decrease of 1.6 times in group 2 (p < 0.05), with a statistically significant difference between the groups (p < 0.05). At the same time, patients with CKH III st. the rate of GFR in patients in group 1 was lower than the standard in 2.7 times (p < 0.05), in group 2 — in 2.2 times (p < 0.05), with the presence of a probable difference between patients with a comorbid flow of NASH and CKD II st. and CKD III st. in comparison with patients with isolated CKD of the corresponding stage (p < 0.05).

Thus, the functional state of the kidneys in patients with CKD and comorbidity with NASH regarding the rates of excretion of nitrogenous slags, albumin loss and integral index — GFR is significantly lowered compared to those in patients with CKD without comorbidity.

In the study of indicators of inflammatory process activity in patients with CKD and comorbidity with NASH in comparison with the isolated course of CKD, the following data were obtained (table 2). When comparing the number of leukocytes in urine analysis by Nechyporenko method, a significant difference in the indicators was established. So, in patients with CKD I st. in group 1 indicators exceeded the data in the PHPs by 6.9 times (p < 0.05), and in 2 groups — by 5.7 times (p < 0.05) (table 2). In patients with CKD II st. in group 1, the number of leukocytes in 1 ml of urine exceeded the normative by 7.9 times against the increase in 6.8 times in group 2 (p < 0.05). In patients with CKD III st. the content of leukocytes in the urine in patients of group 1 exceeded the normal values by 11.1 times (p < 0.05), in group 2 — by 8.2 times (p < 0.05), in all cases with the probable difference between the groups (p < 0.05). When comparing the number of erythrocytes in the analysis of urine by Nechyporenko method we found that in patients with CKD I st. in group 1 exceeded the data in the PHPs by 5.7 times (p < 0.05), and in group 2 — by 4.6 times (p < 0.05) (table 2). In patients with CKD II st. in group 1, the number of erythrocytes in 1 ml of urine exceeded the normative by 6.9 times against the increase in 5.6 times in group 2 (p < 0.05). In patients with CKD of the III st. the content of red blood cells in patients in group 1 exceeded the normal values by 7.4 times (p < 0.05), in group 2 — by 6.0 times (p < 0.05), in all cases with a probable difference between the groups (p < 0.05).

Analysis of the daily proteinuria showed a significant difference between the comparison groups (table 2). At patients with CKD I st. in group 1 exceeded the data in PHPs by 7.5 times (p < 0.05), and in group 2 —

<table>
<thead>
<tr>
<th>Indicators, units measurement</th>
<th>PHPs (n=30)</th>
<th>Group 1 (NASH, CKD) (n=145)</th>
<th>Group 2 (CKD) (n=95)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CKD I st. (n=51)</td>
<td>CKD II st. (n=53)</td>
</tr>
<tr>
<td>Creatinine, μmol/l</td>
<td>75.0±2.0</td>
<td>113.2±2.2*</td>
<td>125.2±1.4*</td>
</tr>
<tr>
<td>Urea, mmol/l</td>
<td>3.8±0.1</td>
<td>9.0±0.3*</td>
<td>9.5±0.1*</td>
</tr>
<tr>
<td>Albumin, g/l</td>
<td>40.2±1.3</td>
<td>32.2±0.8*</td>
<td>27.2±0.5*</td>
</tr>
<tr>
<td>Creatinine Clearance ml/min</td>
<td>102.2±2.6</td>
<td>90.0±1.2*</td>
<td>62.0±1.1*</td>
</tr>
<tr>
<td>GFR CKD-EPI, ml/min/1.72m2</td>
<td>101.2±1.6</td>
<td>68.0±1.3*</td>
<td>54.0±1.0*</td>
</tr>
</tbody>
</table>

Notes: 1. * - changes are probably compared to the index in the PHPs (p < 0.05); ** ** - changes are probably in comparison with the indicator in the group of patients of the corresponding stage of CKD with a comorbid flow of NASH and obesity (p < 0.05).
by 7.0 times (p <0.05). In patients with CKD II st. in group 1 of proteinuria exceeded the index in the PHPs by 8.5 times against the increase in 8.0 times in group 2 (p <0.05). In patients with CKD of the III st. urine protein loss in group 1 exceeded the norm by 9.5 times (p <0.05), in group 2 — by 8.5 times (p <0.05), in all cases with a probable difference between the groups (p <0.05).

The probable results were obtained by us in relation to cylinduria in the comparison of data in patients in group 1 and 2 with CKD I st.: growth of 4.9 and 3.6 times (p <0.05), in CKD II st. — in 6.3 and 3.6 times (p <0.05), and in CKD III st. — growth in 7.8 and 6.9 times (p <0.05), with the presence in all cases of the probable difference between the groups (p <0.05). The analysis of indicators of bacteriuria also showed the presence of a probable intergroup difference in the analysis of indices in patients with CKD with NASH and without comorbidity pathology (p <0.05) (table 2). The correlation analysis shows that there is an average strength and a strong correlation between the GFR indices and the intensity of lipoperoxidation (increase MA content in blood) and the oxidative modification of the proteins (increase in the AKDNPH B content in blood) (table 3), the degree of endotoxiosis (increase of MMP in the blood, decrease in the activity of arginase), growth of fractions of proatherogenic fractions: LDL, cholesterol, TG and lowering of blood HDL — antiatherogenic LP in blood, due to their dysregulation by adipocytokines: hyperleptinemia, hypoadiponectinemia, hypercytocreatinemia (p <0.05), indicating the participation of these factors in the reduction of GFR for comorbidity with NASH and the progression of CKD.

It should be noted a significant impact on GFR indicators that contribute to endothelium dysfunction, and its direct biochemical markers. In particular, the significant influence of hydrogen sulfide deficiency, hyperhomocysteinemia, hyperproduction of endothelin-1 and over-expression of INOS on GFR was established, resulting in hyperproduction and violation of the excretion of metabolites of nitrogen monoxide with activation of nitrosatistic stress and redistributive impaired renal vascular tone [9], which also affected the decrease in GFR in

### Table 2

**Characteristics of the intensity of the inflammatory process in patients with chronic kidney disease and non-alcoholic stethohepatitis obesity and chronic kidney disease without comorbid pathology depending on the stage of the chronic kidney disease(M±m)**

<table>
<thead>
<tr>
<th>Indicators, units measurement</th>
<th>PHPs (n=30)</th>
<th>Groups of patients surveyed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group 1 (NASH, CKD) (n=145)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 1 st. (n=51)</td>
</tr>
<tr>
<td>Number of leukocytes / 1 ml</td>
<td>753.0±23.5</td>
<td>5239.0±101.4*</td>
</tr>
<tr>
<td>Number of erythrocytes / 1 ml</td>
<td>214.3±12.1</td>
<td>1223.1±25.1*</td>
</tr>
<tr>
<td>Amount of protein (g / day)</td>
<td>0.02±0.001</td>
<td>1.5±0.02*</td>
</tr>
<tr>
<td>Number of cylinders</td>
<td>2.5±0.2</td>
<td>12.2±0.4*</td>
</tr>
<tr>
<td>Number of bacteria / ml</td>
<td>0.56x102±0.1</td>
<td>4.8x105±0.2*</td>
</tr>
</tbody>
</table>

**Notes:** 1. * - changes are probably compared to the index in the PHPs (p <0.05); ** ** - changes are probably in comparison with the indicator in the group of patients of the corresponding stage of CKD with a comorbid flow of NASH and obesity (p <0.05).
patients with CKD and NASH (p <0,05). The obtained data substantially complement the concept of the pathogenesis of the mutual burden of CKD and NASH with obesity [10], contribute to the search for new, previously unknown mechanisms for their progression.

**Conclusions**

1. Nonalcoholic steatohepatitis affects the functional state of the kidneys in patients with chronic kidney disease of I-III stages with a possible reduction of nitrogen function, the velocity of glomerular filtration, increase in the intensity of hypoaalbuminemia, proteinuria, leukocyturia, erythrocyturia, cylindria, bacteriuria than in isolated course chronic kidney disease.

2. For the comorbidity of the chronic kidney disease with nonalcoholic steatohepatitis and a decrease in glomerular filtration rate, an increase in the intensity of oxidative stress, endotoxicosis, lipid distress syndrome, degree of violation of the functional state of the endothelium: increased activity of iNOS, nitrite/nitrate content, endothelin-1, homocysteine, cytokeratin-18, decrease in the activity of arginase, H2S content, which correlate with the intermediate and high power interactions with the index of glomerular filtration rate.

The prospect of further research in this direction is to study the factors of regulation of renal functions, the functional state of the endothelium and the development of methods for their correction in patients with a comorbid flow of nonalcoholic steatohepatitis and chronic kidney disease: chronic pyelonephritis.

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