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Abstract **УДК 615.244+615.272]:616.36-003.826+616.61-036.12]-056.527**

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ROLE OF FIBRINOLYTIC ACTIVITY OF BLOOD IN PATHOGENESIS OF NON-ALCOHOLIC FATTY LIVER DISEASE AND CHRONIC KIDNEY DISEASE (ORIGINAL RESEARCH)

The aim of the research − to find out of changes fibrinolytic activity of blood in patients with non-alcoholic fatty liver on the background of obesity, depending on the presence of comorbid chronic kidney disease.

Material and methods of research: 444 patients were examined: 84 of them were with NAFLD and class I obesity (group 1), which contained 2 subgroups: 32 patients with non-alcoholic steatosis (NAS) and 52 patients with non-alcoholic steatohepatitis (NASH); 270 patients with NAFLD with comorbid class I obesity and CKD I–III stage (group 2), including 110 patients with NAS and 160 patients with NASH. The control group consisted of 90 patients with CKD of I–III stage with normal body weight (group 3). To determine the dependence of the NAFLD course on the form and stage of the CKD, the group of patients was randomized according to age, sex, degree of obesity, and activity of NASH.

Research results. The study of fibrinolytic activity of blood showed that total fibrinolytic activity (TFA) of blood plasma in patients of all groups was significantly lower than the control indexes: in patients with NAS – by 7.1%, patients with NAS with CKD – by 14.9%, patients with NASH – by 17.2%, patients with NASH with CKD – by 18.9%, patients with CKD – by 10.6% ($p < 0.05$) with the presence of a probable intergroup difference between groups with comorbidity and isolated course of CKD ($p < 0.05$). The suppression of TFA occurred through the decrease of EF: in patients with NAS the index is significantly lower than that in the controls by 1.2 times, in patients with NAS with CKD – by 1.4 times, in patients with NASH – by 1.7 times, in the group of patients with NASH and CKD – by 1.9 times, while in the group of patients with CKD, the suppression of EF was registered – 1.3 times ($p < 0.05$). At the same time, the NEF in patients of all groups increased in comparison with the AHP group: in patients with NAS – by 1.2 times, in patients with NAS with CKD – by 1.3 times, in patients with NASH – by 1.4 times, in the group of patients with NASH with CKD – 1.5 times, while in the group of patients with CKD the activation of NEF was registered 1.2 times ($p < 0.05$), with the presence of a probable difference between the groups with comorbidity and isolated course of CKD ($p < 0.05$).

Conclusion. Analysis of hemostasis and fibrinolysis indices in

examined patients with NASH, depending on the stage of CKD showed that with the growth of the CKD stage, the activity of the cohort increases, with the exception of the fibrinogen content (most likely due to coagulopathy consumption), the activity of the anti-coagulants decreases, the total and enzymatic activity of fibrinolysis is reduced, and non-enzymatic compensator increases. Thus, metabolic intoxication, oxidative stress, which accompany the flow of NAFLD with obesity and CKD, promote the activation of the kallikrein-kinin system, the formation of plasma and thrombin, with subsequent disturbance of equilibrium between them, the development of stasis, slag phenomenon, the formation of platelet and erythrocyte aggregates in blood circulation system. The consequence of significant activation of hemocoagulation against the suppression of total fibrinolytic activity (TFA) is the local clotting of blood in the arteries.

Key words: nonalcoholic fatty liver disease, chronic kidney disease, fibrinolytic activity.

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Резюме

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РОЛЬ ФІБРИНОЛІТИЧНОЇ АКТИВНОСТІ ПЛАЗМИ КРОВІ У ПАТОГЕНЕЗІ ПРОГРЕСУВАННЯ НЕАЛКОГОЛЬНОЇ ЖИРОВОЇ ХВОРОБИ ПЕЧІНКИ ТА ХРОНІЧНОЇ ХВОРОБИ НИРОК

Мета роботи − з'ясувати зміни фібринолітичної активності крові у пацієнтів на неалкогольну жирову хворобу печінки на тлі ожиріння залежно від наявності коморбідного хронічного захворювання нирок.

Матеріал та методи дослідження: Обстежено 444 хворих: з яких 84 хворих на НАЖХП із ожирінням I ступеня (1 група), яка містила 2 підгрупи: 32 хворих на неалкогольний стеатоз (НАСП) та 52 хворих на неалкогольний стеатогепатит (НАСГ); 270 хворих на НАЖХП із коморбідним ожирінням I ступеня та ХХН I–III стадії (2 група), у тому числі 110 хворих на НАСП та 160 хворих на НАСГ. Групу контролю склали 90 хворих на ХХН I–III стадії із нормальною масою тіла (3 група). Для визначення залежності перебігу НАЖХП від форми та стадії ХНН групи хворих були рандомізовані за віком, статтю, ступенем ожиріння, активністю НАСГ.

Результати дослідження. Дослідження фібринолітичної активності крові показало, що сумарна ферментативна активність (СФА) плазми крові у хворих усіх груп була вірогідно нижча від контрольних показників: у хворих на НАСП – на 7,1 %, хворих на НАСП із ХХН – на 14,9 %, хворих на НАСГ – на 17,2 %, хворих на НАСГ із ХХН – на 18,9 %, хворих на ХХН – на 10,6 % ($p < 0,05$) із наявністю вірогідної міжгрупової різниці між групами з коморбідністю та ізольованим перебігом ХХН ($p < 0,05$). Гальмування СФА відбувались за рахунок зниження ФФА: у хворих на НАСП показник вірогідно нижчим за контрольні у 1,2 рази, у хворих на НАСП із ХХН – у 1,4 рази, у хворих на НАСГ – у 1,7 рази, у групі хворих на НАСГ із ХХН – у 1,9 рази, у той час як у групі хворих на ХХН було зареєстровано пригнічення ферментативної фібринолітичної активності (ФФА) – у 1,3 рази ($p < 0,05$). Водночас, неферментативна фібринолітична активність (НФА) у хворих усіх груп зростала у порівнянні з групою практично здорових осіб (ПЗО): відповідно у хворих на НАСП – у 1,2

рази, у хворих на НАСП із ХХН – у 1,3 рази, у хворих на НАСГ – у 1,4 рази, у групі хворих на НАСГ із ХХН – у 1,5 рази, у той час як у групі хворих на ХХН було зареєстрована активація НФА – у 1,2 рази ($p < 0,05$), із наявністю вірогідної різниці між групами з коморбідністю та ізольованим перебігом ХХН ($p < 0,05$).

Висновок. Аналіз показників гемостазу та фібринолізу у обстежених хворих на НАСГ залежно від стадії ХХН показав, що із зростанням стадії ХХН активність зсідання зростає, за виключенням вмісту фібриногену (найбільш ймовірно внаслідок коагулопатії споживання), активність чинників протизсідаючої системи зменшується, сумарна та ферментативна активність фібринолізу знижуються, а неферментативна компенсаторно зростає. Таким чином, метаболічна інтоксикація, оксидативний стрес, які супроводжують перебіг НАЖХП за умов ожиріння та ХХН, сприяють активації калікреїн-кінінової системи, утворенню плазміну та тромбіну з подальшим порушенням рівноваги між ними, розвитку стазу, сладж-феномену, утворенням тромбоцитарних та еритроцитарних агрегатів у системі кровообігу.Наслідком значної активації гемокоагуляції на тлі пригнічення СФА є місцеве згортання крові в артеріях.

Ключові слова: неалкогольна жирова хвороба печінки, хронічна хвороба нирок, фібринолітична активність.

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Introduction

An important problem in internal medicine is the problem of the comorbidity of non-alcoholic fatty liver disease (NAFLD) with obesity and chronic kidney disease (CKD), which has a significant overall medical and social significance [1, 2, 3]. The incidence of non-alcoholic fatty liver disease on the background of obesity increases every year and attracts the attention of clinicians [1, 2]. Non-alcoholic fatty liver disease is the most common non-alcoholic steatohepatitis. Pathogenesis development of NASH can be represented in several stages: fatty infiltration of the liver, oxidative stress, mitochondrial dysfunction, injury mediated by TNF/endotoxin, aseptic inflammation, diffuse liver fibrosis, development of hepatic-cell disease. Insulin resistance syndrome ranks first among the causes of our development. NASH is most common in obesity (20–81%). According to the literature, the prevalence of NASH in the world is 10% (600 million people), including in Ukraine the incidence of steatohepatitis increased by 76.6% [1, 2, 3].

Chronic kidney disease (CHF) is an important problem in Ukraine and in the world today, and the incidence rate has increased by 17% in recent years [5, 6, 7]. 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.

The purpose of the study: to find out of changes fibrinolytic activity of blood in patients with non-alcoholic fatty liver on the background of obesity, depending on the presence of comorbid chronic kidney disease.

Material and methods. 444 patients were examined: of which 84 patients with class I obesity (group 1), which contained 2 subgroups: 32 patients with NAS and 52 patients with NASH; 270 patients with NAFLD with comorbid class I obesity and CKD I–III stage (group 2), including 110 patients with NAS and 160 patients with NASH. The control group consisted of 90 patients with CKD of I–III stage with normal body weight (group 3). To determine the dependence of the NAFLD course on the form and stage of the CKD, the group of patients was randomized according to age, sex, degree of obesity, and activity of NASH.

The diagnosis of NAFLD 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 and recommendations of the European Association for the Study of Liver (EASL), the European Association for the Study of Obesity (EASO). Diagnosis of obesity was established on the basis of calculating the body mass index (BMI) by the

formula of Kettle: BMI = body weight (kg)/height^2 (m). Class I obesity was established on the basis of increase in BMI of 30–34.9 kg/m^2, class II – with BMI 35–39.9 kg/m^2, class III – BMI above 40 kg/m^2. Diagnosis and treatment of CKD were performed in accordance with the recommendations of the clinical guidelines of the State Institute "Institute of Nephrology, NAMS of Ukraine" (2012).

The total coagulation potential of blood (prothrombin time (PT)), plasma fibrinolytic activity, plasminogen potential activity (PPA), fibrinogen level in blood plasma, activity of antithrombin III (AT III), activity of XIII factor were studied using the sets of reagents of the company "Simko Ltd" (Lviv) according to the methods of N. Titsa. Using the reagents of the same company, we studied the state of enzymatic (EFS) and non-enzymatic fibrinolysis (NEF) in blood plasma. The principle of the method is as follows: azofibrin is incubated with a standard amount of plasminogen in the presence of fibrinolysis activators that are contained in blood plasma, thus plasmin is formed, which activity is estimated by the degree of coloring of the solution in alkaline medium in the presence of E-amino-capronic acid (EF) or without it (NEF). The difference between them determines the state of the EFS. By the same method, but without the use of plasminogen and E-aminocaproic acid, the proteolytic activity of blood plasma was determined using azoalbumin, azocasein, azocol (Simko Ltd, Lviv), and the total activity of proteinases by M. Kunitz.

The statistical processing of the results of the study was carried out using parametric (t-criterion Student, Fisher's F-criterion) and non-parametric methods (Mann–Whitney U-criterion, Wilcoxon's T-criterion) of variation statistics. For statistic and graphical analysis of the obtained results we used software packages of Statistica for Windows version 10.0 Pro (Stat Soft inc., USA), Microsoft Excel 2013 (Microsoft, USA).

Results and discussion. Analysis of the results of the 2nd phase of the coagulation hemostasis showed that the PT was significantly lower in patients of all groups of observation (Table 1). The maximum decrease in the rate was observed in patients with NASH and CKD – 1.9 times compared with the indicator in the AHPs ($p < 0.05$) with the presence of intergroup difference; in patients with NASH without CKD, PT was 1.6 times lower than that in apparently healthy persons (AHPs) ($p < 0.05$).

Table 1 – Indicators of hemostasis and fibrinolysis in patients with non-alcoholic liver steatosis and steatohepatitis depending on comorbidity with CKD (M ± m)

Indicators, units measurement	AHP, n=30	Groups of patients				
		NAS, n=32	NAS, CKD, n=110	NASH, n=52	NASH,CKD, n=160	CKD, n=90
PT, sec.	22.12±0.46	18.41±0.32*	15.73±0.23$^{*/**}$	13.56±0.21$^{*/**}$	11.38±0.25$^{*/***}$/$^{\#}$	16.37±0.29$^{*/***}$/$^{\#\#}$
Fibrinogen, g/l	3.81±0.12	3.38±0.15*	3.15±0.11*	2.69±0.17$^{*/**}$	1.87±0.10$^{*/***}$/$^{\#}$	4.35±0.09$^{*/***}$/$^{\#\#}$
TT, sec	16.95±0.87	15.75±0.36	12.31±0.27$^{*/**}$	11.84±0.23$^{*/**}$	10.25±0.15$^{*/***}$/$^{\#}$	13.27±0.20$^{*/***}$/$^{\#\#}$
AT III, %	95.48±2.01	82.81±3.18*	78.33±3.21*	73.38±2.86*	67.27±2.24$^{*/***}$	80.27±3.28*/$^{\#\#}$
Total fibrinolytic activity (TFA), E440/ml/hour	1.69±0.02	1.58±0.02*	1.47±0.01*	1.40±0.01$^{*/**}$	1.37±0.004$^{*/***}$/$^{\#}$	1.52±0.01$^{*/***}$/$^{\#\#}$
Non-enzymatic fibrinolytic activity (NFA), E440/ml/hour	0.49±0.02	0.60±0.01*	0.63±0.003*	0.69±0.004$^{*/**}$	0.75±0.01$^{*/***}$/$^{\#}$	0.57±0.002$^{*/***}$/$^{\#\#}$
Enzymatic fibrinolytic activity (EFA), E440/ml/hour	1.20±0.01	0.98±0.01*	0.84±0.01$^{*/**}$	0.71±0.004$^{*/**}$	0.62±0.01$^{*/***}$/$^{\#}$	0.95±0.01$^{*/***}$/$^{\#\#}$
Hageman-dependent fibrinolysis, min.	19.45±0.19	22.52±1.33*	30.21±1.18$^{*/**}$	34.53±1.15$^{*/**}$	37.31±1.28$^{*/***}$	29.39±1.07*/$^{\#\#}$
XIII Factor, %	99.91±2.45	97.32±2.41	82.43±1.12*	70.82±1.13$^{*/**}$	68.18±1.29$^{*/***}$	80.25±2.34*/$^{\#\#}$
Potential plasminogen-activating activity, min.	15.23±0.27	18.31±0.21*	22.20±0.18$^{*/**}$	26.38±0.13$^{*/**}$	30.15±0.12$^{*/***}$/$^{\#}$	24.01±0.11$^{*/***}$/$^{\#\#}$

Notes:
* - the difference is probable compared to the indicator in the AHP ($p < 0.05$);
** - the difference is probable in comparison with the indicator in patients with NAS ($p < 0.05$);
*** - the difference is probable compared with the index in patients with NASH ($p < 0.05$);
$^{\#}$ - the difference is probable in comparison with the index in patients with NAS with CKD ($p < 0.05$);
$^{\#\#}$ - the difference is probable compared with the index in patients with NASH with CKD ($p < 0.05$).

In patients with NAS, less intensive changes were observed: PT in the group without comorbidity was 1.2 times lower (P <0.05), in patients with NAS with CKD – 1.4 times lower (p <0.05). In patients with isolated CKD, the decrease in PT was 1.4 times (p <0.05) (Table 1). The study of the 3rd phase of coagulation hemostasis suggested that in patients the content of blood fibrinogen was reduced: in patients with NASH and NASH with CKD – respectively, by 1.4 and 2.0 times (p <0.05) against growth by 1.2 times in patients with isolated CKD (p <0.05); in patients with NAS – the decrease was 12.7% and 17.1% (p <0.05), the indicator was significantly different in comparison with the intergroup aspect (p <0.05). Reducing the fibrinogen content in the blood of patients with NAFLD with CKD and obesity suggests a lack of synthesis of Factor I of coagulation in the liver and/or activation of the hemostasis system in response to inflammation, the development of hypercoagulation, the formation of microthrombus and the addition of a certain amount of fibrinogen in this process. Registration of low content of fibrinogen in patients with obesity is indicative of the development of coagulopathy of consumption, that is, the use of fibrinogen in the processes of intravascular blood coagulation with the simultaneous exhaustion of the circulating pool of this factor. At the same time, the increase in the fibrinogen content in patients with CKD without comorbid pathology indicates activation of blood clotting due to chronic inflammation.

Changes in the activity of AT III (Table 1) indicate an insufficiency of the anticoagulation potential of the blood. In particular, the inhibition of AT III activity in all groups of comparison with the maximum inhibition of patients with NASH with CKD was determined 1.4 times (p <0.05) versus a decrease of 1.3 times in patients with NASH (Table 1). In the groups of patients with NAS and NAS with CKD, a moderate difference was not established. It should also be noted that in patients with CKD without comorbid conditions, the activity of AT III was significantly reduced by 1.2 times (p <0.05).

The study of fibrinolytic activity of blood showed that TFA of blood plasma in patients of all groups was significantly lower than the control indexes: in patients with NAS – by 7.1%, patients with NAS with CKD – by 14.9%, patients with NASH – by 17.2%, patients with NASH with CKD – by 18.9%, patients with CKD – by 10.6% (p <0.05) with the presence of a probable intergroup difference between groups with comorbidity and isolated course of CKD (p <0.05). The suppression of TFA occurred through the decrease of EFA: in patients with NAS the index was significantly lower than that in the controls by 1.2 times, in patients with NAS with CKD – by 1.4 times, in patients with NASH – by 1.7 times, in the group of patients with NASH and CKD – by 1.9 times, while in the group of patients with CKD, the suppression of EFA was registered – 1.3 times (p <0.05). At the same time, the NFA in patients of all groups increased in comparison with the AHP group: in patients with NAS, by 1.2 times, in patients with NAS with CKD – by 1.3 times, in patients with NASH – by 1.4 times, in the group of patients with NASH with CKD – 1.5 times, while in the group of patients with CKD the activation of NFA was registered 1.2 times (p <0.05), with the presence of a probable difference between the groups with comorbidity and isolated course of CKD (p <0.05). That is, in patients with NASH with CKD NFA acquired compensatory maximum intensity (p <0.05). At the same time, there was a probable decrease in the activity of Hageman-dependent fibrinolysis: respectively, in patients with NAS – 1.2 times, in patients with NAS and CKD – 1.6 times, in patients with NASH – 1.8 times, in the group patients with NASH with CKD – 1.9 times, while in the group of patients with CKD decrease in Hageman-dependent fibrinolysis activity was 1.5 times (p <0.05) with the probable difference between groups with comorbidity and isolated flow of CKD (p <0.05). The activity of the fibrin stabilizing factor in patients with NASH and NASH with CKD decreased respectively by 1.4 and 1.5 times (p <0.05), indicating a violation of the postcoagulation phase of blood coagulation. In groups of patients with NAS changes were unlikely, and in patients with NAS with CKD and isolated CKD – reduction was 1.2 times (p <0.05) (Table 1).

Patients with CKD had a probable reduction in PPA: in patients with NAS – 1.2 times, patients with NAS with CKD – 1.5 times, patients with NASH – 1.7 times, patients with NASH with CKD – by 2.0 times, in the group with CKD without comorbidity – the decrease was 1.6 times (p <0.05) with the presence of a probable difference between the groups with comorbidity and the isolated course of CKD (p <0.05) (Table 1).

Analysis of hemostasis and fibrinolysis indices in examined patients with NASH depending on the stage of CKD showed that with the growth of the CKD stage, the activity of the cohort increases, with the exception of the fibrinogen content (most likely due to consumption coagulopathy), the activity of the anti-coagulation factors decreases, the total and enzymatic activity of fibrinolysis is reduced, and non-enzymatic compensator increases. Thus, metabolic intoxication, oxidative stress, which accompany the flow of NAFLD with obesity and CKD, promote the activation of the kallikrein-kinin system, the formation of plasma and thrombin, with subsequent disturbance of equilibrium between them, the development of stasis, slag phenomenon, the formation of platelet and erythrocytic aggregates in blood circulation system. The consequence of significant activation of hemocoagulation against the suppression of TFA is the local clotting of blood in the arteries. The function of Hageman-dependent fibrinolysis is the regular deprivation of the circulatory system from fibrin clots formed under conditions of inflammation. The results of our study indicate a decrease in the rate of enzymatic, Hageman-dependent fibrinolysis, which causes the compensatory activation of NEF. Slowdown of blood circulation in the liver and kidneys due to the formation of microthrombi in the microcirculatory system promotes progression of hypoxia, formation of reactive oxygen species (ROS) and free radicals with subsequent damage to cellular membranes of hepatocytes, cytolysis, reduction of glomerular filtration rate (GFR) and closure of the "vicious" circle of the progression pathogenesis of NAFLD and CKD.

Conclusions

The role of chronic inflammation in CKD in the formation of hemostasis disorders and in the pathogenesis of progression of NASH on the background of obesity, which in general can be characterized as hypercoagulation syndrome due to significant inhibition of anti-coagulation factors and fibrinolytic systems and activation of plasma coagulation factors (fibrinogen) due to chronic inflammation.

Prospects for future research

The prospect of further scientific research in this direction is the development of a method for correction of hemostasis and fibrinolysis indices in patients with NAFLD depending on the stage of CKD.

Conflict of interest

The authors declare no conflict of interest.

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