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ROLE OF ENDOTHELIAL DYSFUNCTION IN DEVELOPMENT OF NONALCOHOLIC STEATOHEPATITIS

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РОЛЬ ЭНДОТЕЛИАЛЬНОЙ ДИСФУНКЦИИ В ФОРМИРОВАНИИ НЕАЛКОГОЛЬНОГО СТЕАТОГЕПАТИТА

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The aim of the study was to determine the clinical significance of the blood levels of endothelin-1 (E-1) and nitric oxide (NO) as indicators of endothelial function, as well as serum endotoxin (ET) in patients with non-alcoholic fatty liver disease (NAFLD). The body of patients included 142 persons with NAFLD – 90 patients with hepatic steatosis (Group I), 52 patients with non-alcoholic steatohepatitis (NASH) (Group II). All the patients had their plasma content of E-1 determined by ELISA, as well as the level of NO (by colorimetric method). The level of ET in blood serum was determined employing the chromogenic method Hbt LAL. The values for endothelial dysfunction (ED) in the group of the patients with steatosis revealed no difference from the control values. The content of E-1 and NO in the blood of the patients with NASH exceeded those in healthy individuals and in Group I. The ET levels in the blood of the patients in Group II were higher compared to the healthy persons as well the patients with hepatic steatosis. There has been positive correlation identified between the E-1 and HOMA-index, E-1 and ET in case of NASH, yet not in Group I. The dynamics of 1 month into the therapy, which included dietary measures, Metformin and hepatoprotector, revealed a decrease in the blood levels of E-1 and no change in NO. The outcomes demonstrate a role of ED and endotoxinemia in the progress from steatosis to steatohepatitis. It has been shown that, in case of NASH, comprehensive therapy may exert positive effect on endothelial function.

Key words: non-alcoholic fatty liver disease, steatosis, nonalcoholic steatohepatitis, endothelial dysfunction, endotoxinemia

В исследовании определялось клиническое значение содержания в крови эндотелина-1 (Э-1) и оксида азота (NO) как показателей функции эндотелия, а также количества сывороточного эндотоксина (ЭТ) у больных неалкогольной жировой болезнью печени (НАЖБП). Обследовано 142 больных НАЖБП: 90 больных со стеатозом печени – группа I, 52 пациента – с неалкогольным стеатогепатитом (НАСГ) – группа II. У всех пациентов определяли плазменное содержание Э-1 методом ИФА и уровень NO колориметрическим методом. Количество ЭТ в сыворотке крови определялось хромогенным методом Hbt LAL. Показатели эндотелиальной дисфункции (ЭД) в группе больных со стеатозом не отличались от контроля. Содержание Э-1 и NO в крови больных НАСГ превышало показатели у здоровых и в группе I. Уровень ЭТ в крови больных группы II был выше, чем у здоровых и больных стеатозом печени. Выявлена положительная корреляция между Э-1 и HOMA-индексом, Э-1 и ЭТ при НАСГ, но не в группе I. В динамике 1 месяца терапии, включающей диетические меропрятия, метформин и гепатопро-

тектор, отмечено снижение содержания в крови Э-1 и отсутствие изменений NO. Полученные данные демонстрируют роль ЭД и эндотоксинемии в эволюции стеатоза в стеатогепатит. Показана возможность позитивного влияния комплексной терапии при НАСГ на функциональное состояние эндотелия.

Ключевые слова: неалкогольная жировая болезнь печени, стеатоз, неалкогольный статогепатит, эндотелиальная дисфункция, эндотоксинемия

Non-alcoholic fatty liver disease (NAFLD) is currently regarded as one of the diseases related to metabolic syndrome or one of the manifestations of metabolic syndrome associated with obesity and insulin resistance (IR) [5]. The clinical significance of the disease stems from the possibility of transformation of steatosis into nonalcoholic steatohepatitis (NASH) and development of hepatic cirrhosis. Addition of inflammatory component and evolution of steatosis into steatohepatitis are among the most difficult issues in the pathogenesis of NASH which have not been resolved yet. There is the hypothesis of «two strikes» or «two steps» proposed by C.P. Day in 1998 [3]. Enhanced liver delivery of free fatty acids under visceral obesity and IR develop hepatic steatosis. Further on, there is gradually (or simultaneously) developing oxidative stress, which comes as a result of dissociation of oxidation and phosphorylation under the influence of free fatty acids, proinflammatory cytokines (TNF-α, IL-8, etc.), and some other substances. Oxidative stress being a result of mitochondrial fatty acid oxidation and expression of proinflammatory cytokines is considered a secondary causative factor of liver injury, fibrosis and inflammation [11, 15, 18]. The basis of the «second strike» mechanism in the development of steatohepatitis may be the additional effects of portal endotoxinemia [3, 10]. In this case, liver can be considered not only as an immediate source of proinflammatory cytokines yet also as a target organ for a systemic inflammatory response. The cascade of inflammatory reactions ultimately leads to endothelial dysfunction (ED) and damage to the hepatocytes themselves thus creating a vicious circle.

IR is well known to play a key role in the pathogenesis of NAFLD. H.O. Steinberg et al. [16] demonstrated a link between IR and endothelial dysfunction, which leads to improper (increased or decreased) formation of various biological substances in the endothelium. Some argue that endothelin-1 (E-1) is able to limit the action of insulin in people with excessive body weight and IR [9]. Under physiological conditions, insulin causes vasodilation and redistribution of blood flow to ensure metabolism in the skeletal muscles, while under IR this effect is disturbed. Thus, an increase in endogenous endothelin induced by intraarterial antagonists of its receptors, enforced IR in skeletal muscles, and facilitated ED under obesity and diabetes [9].

The clinical data on ED at NAFLD remain scarce. Endothelium-dependent and endothelium-independent vasodilation, when studied ultrasonically, was found to be reduced in patients with steatohepatitis compared to cases of simple steatosis, although there were no differences detected between the group with steatosis and healthy people. The conclusion drawn then was that the treatment strategy for NASH should include drugs affecting ED, which is aiming at the cardiovascular diseases risk reduction in patients with NAFLD [14]. Other data suggest that in case of NASH, if compared with simple steatosis, only endothelium-dependent dilatation changed significantly [19].

Endotoxin (ET) (LPS) is the major component of the Gram-negative bacterial cell membrane. Changes in intestinal microbiota, as well as bacterial translocation can elicit immune response, stimulate the synthesis of proinflammatory cytokines, and contribute to the progression of fibrosis [13]. Once in the liver, ET interacts with sinusoidal endothelial cells, leading to the emergence of ED. Understanding the impact that endotoxin has on the development of ED in patients with NAFLD appears a promising area within the point under discussion, which might help resolve certain still pending issues of pathogenesis, as well as indicate the path for some novel therapeutic strategies.

Aim: to detect the clinical significance of endothelin-1, nitric oxide (NO) and the role of serum endotoxin in patients with non-alcoholic fatty liver disease.

Material and Methods. 142 patients with NAFLD (77 males and 65 females) were examined. The mean age of the patients was 49±0.9. The patients had an increased body weight, with an average BMI of 33.54±0.78. 90 patients were diagnosed with steatosis (Group I); in 52 cases the diagnosis was nonalcoholic steatohepatitis (Group II). The groups were matched by age and gender. ALT activity in the group with NASH (72.78±4.83 U/l) was above that in the patients with hepatic steatosis (26.07±1.18 U/l). The insulin resistance was assessed by the HOMA-index. IR was typical of all patients yet in Group II HOMA index (4.22±0.28) proved higher than that in the patients with steatosis (3.81±0.35 (p=0.01)). In patients with NASH blood levels of cholesterol were higher, too, if compared to Group I patients (6.03±0.13 and 5.79±0.16 mmol/l; p=0.04) while the contents of other lipid indicators turned comparable.

The control group included 20 healthy volunteers.

All the patients had their plasma levels of E-1 (ELISA; Biomedica-Gruppe) as well as NO levels by colorimetry (R&D Systems) determined in them. The level of ET in blood serum was measured through the chromogenic method Hbt LAL (Hycult Biotech).

The results were statistically processed using the Microsoft Excel 2007 software with the add-in Attestat for Excel 12.0.5. Since the distribution of the data was abnormal, nonparametric Mann-Whitney test was used to compare the groups, and during the correlation analysis Spearman test was applied.

Results and Discussion. In all the patients with NAFLD, the blood levels of E-1, a vasoconstrictor polypeptide produced by the endothelium and playing a crucial role in vascular homeostasis, exceeded the values in healthy individuals (Table). At the same time, the mean values of E-1 in the patients with hepatic steatosis were not significantly different from control, while the patients with NASH revealed its highest performance going beyond the corresponding values both in the control group and in the patients with steatosis. Serum levels of E-1 in patients with steatohepatitis exceeding the similar indicator in the steatosis, were reported by other researchers as well, who also noted a correlation between the activity of ALT, severity of fibrosis and the level of E-1 [4]. The importance of E-1 in progression of liver pathology was demonstrated in experimental cirrhosis in rats induced by a diet rich in fats and low in methionine and choline. The resulting hyperleptinemia which is characteristic of steatohepatitis, increased vasoconstrictor response to E-1 impairing the microcirculation, increasing the levels of TGF-β, TNF-α, and exacerbating portal hypertension and fibrosis [20].

Table

Indicators of endothelial dysfunction in patients with non-alcoholic fatty liver disease ($\bar{X} \pm s_{\bar{x}}$)

Groups	Content of endothelin-1 (fmol / ml)	Content of nitric oxide (μmol / ml)
Healthy (n=20)	0.48±0.05	21.67±2.69
Patients with NAFLD (n=142)	0.66±0.05*	40.23±5.16
Patients with hepatic steatosis (n=90)	0.61±0.04	29.07±3.48
Patients with steatohepatitis (n=52)	0.78±0.12*(**)	47.93±7.31*(**)

Note: * – p <0.05 compared to healthy controls; ** – p <0.05 compared to patients with hepatic steatosis.

IR values are known to be significantly higher in patients with NASH compared to those with the steatosis [1, 14]. As noted before, in our study IR values in Group II patients were higher than in those of Group I. Correlation analysis revealed a direct link between the blood levels of E-1 and the values of HOMA index in the patients with NASH (r_s =0.373; p=0.006), whereas no such link was to be detected in the patients with simple steatosis. This confirms the role of IR in the deterioration of endothelial function in patients with NAFLD, which can contribute to the transformation of liver pathology from steatosis to steatohepatitis.

An opinion holds that in the progression of NAFLD an important role belongs to the deficit of NO, an endothelial relaxing factor known to be a trigger of activation of endothelium-produced biologically active substances [12]; however, there is also data denying increased levels of NO in the blood of patients with NASH [8].

In our study, the average blood level of NO in the patients with NAFLD did not exceed the norm, while the average values of NO in the patients with NASH were higher than those in healthy individuals and patients with steatosis (Table). The obtained data on the NO increase in steatohepatitis were confirmed with experimental results. For instance, in rats with NASH, the amounts of NO and NO-metabolites were increased in blood (especially from the portal vein), in the liver tissue, and in the visceral fat, where high activity of NO synthase was found. According to one of the ideas proposed, the induction of NO-synthase and increased levels of NO in visceral fat cause directed diffusion of NO and NO-metabolites into the liver tissue, thus leading to inflammation, apoptosis and fibrosis, which are the main components of NASH [6].

There is also an opposite view of the importance of elevated levels of NO, holding that there is a positive impact of increased NO, which is able to prevent or hinder the occurrence of atherogenic cardiovascular diseases associated with cases of insulin resistance [22]. Given that, high total mortality in patients with NAFLD was observed in the cases with NASH (but not steatosis) with high levels of mortality associated with liver damage [2, 21].

We found no significant difference in the levels of endotoxemia in patients with NAFLD compared to the healthy controls (p=0.069) (Fig. 1). The serum levels of ET in Group I also did not differ from the average control data. However, the patients with NASH (Group II) showed increased content of ET, including when it came to comparing them to the group of patients with hepatic steatosis.

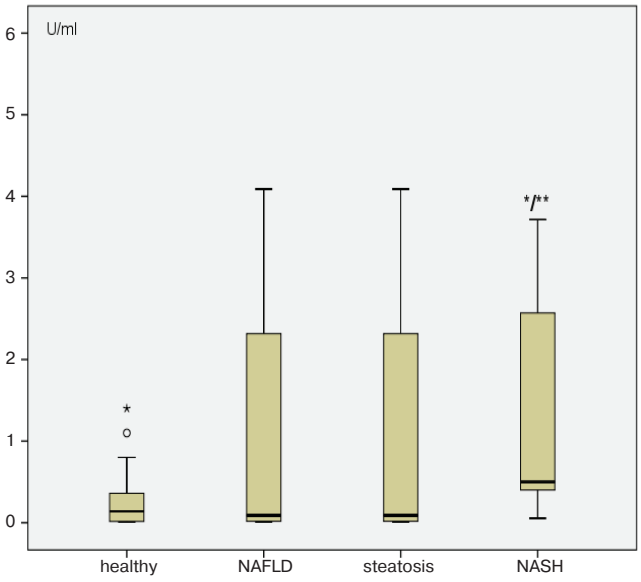

Fig. 1. Endotoxin content in the blood of patients with non-alcoholic fatty liver disease. * – p=0.0001 compared to healthy controls; ** – p=0.001 compared to patients with hepatic steatosis

A significant increase in serum ET in patients with NAFLD allowed proposing it as an early non-in-

vasive indicator of liver damage with no significant differences in the levels of endotoxemia under the steatosis and steatohepatitis [7]. During that, the role of ET in the evolution of steatosis under NASH was convincing enough in experimentally induced NAFLD in rats, where by Week 8 against the developed hepatic steatosis the rise of serum ET was seen, followed by evolution (by Week 12) of simple steatosis into steatohepatitis [17].

Besides, we also found weak but significant correlation between the indicators of ET and E-1 (r_s =0.294; p=0.033) in the patients of Group II, which was evidence to potential involvement of endotoxin in the development of endothelial dysfunction. There was no such dependency identified in Group I (no signs of steatohepatitis). The data yielded show presence of ED in patients with NASH.

To assess the reversibility of these changes in 20 patients with NASH, the dynamics of the ED indices was studied – before and 1 month after the comprehensive therapy, including dietary restriction and, given the current IR, Metformin. Phosphogliv (containing essential phospholipids and sodium glycyrrhizinate) was administered as a hepatoprotector.

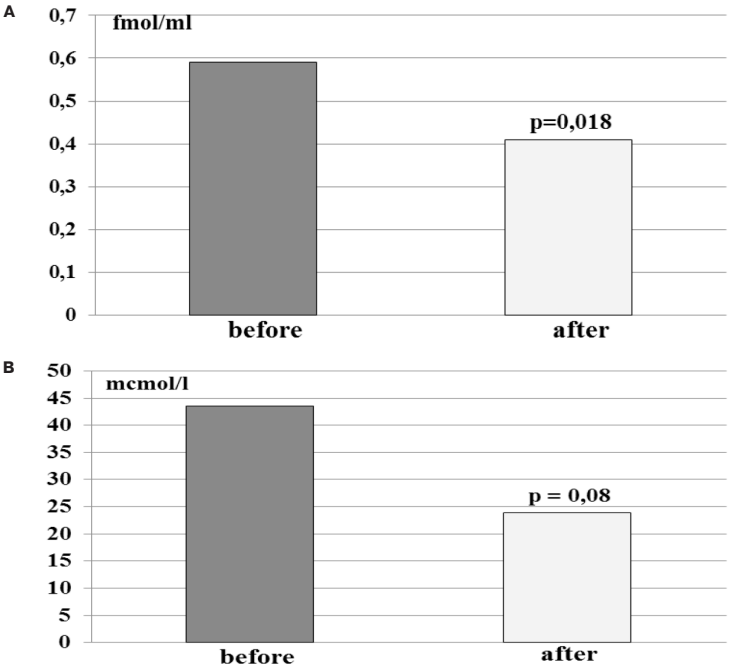

Fig. 2. Content of E-1 (A) and NO (B) in the blood of patients with NASH prior to comprehensive therapy and afterwards

After the treatment, there could be seen a drop in the body weight of 3.1±1.3 kg and a decrease in ALT activity: 69.89±4.75 to 48.81±5.32 U/l; p=0.005. Parallel to this, there was a decrease in E-1 levels (p=0.018) while the NO content in blood (p=0.08) remained high (Fig. 2). The resulting favorable dynamics of ED suggests possible therapeutic effect on endothelial function. Obtaining more significant positive changes, may, obviously, take longer courses of therapy, which would include dietary measures and insulin-sensitizing agents as well as hepatoprotective drugs.

Conclusions. The data above demonstrate the presence of endothelial dysfunction in patients with non-alcoholic steatohepatitis. Increased E-1 and NO can serve an additional criterion witnessing inflammation present in the liver under steatosis to steatohepatitis evolution. Increased E-1 at NASH is associated with a rise in insulin resistance. Evolution of steatosis in patients with NAFLD comes accompanied by an increase in the level of endotoxemia and emerging direct dependence between ET and E-1 indicators, whose levels in the liver steatosis remain within norm thus demonstrating the absence of ED.

Therefore, the clinical significance of endotoxemia has been shown in the development of the inflammatory process in the liver under NAFLD. There is also a demonstration of possible influence that comprehensive therapeutic measures can work on the state of endothelial dysfunction in patients with non-alcoholic steatohepatitis.

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PATHOGENETIC ASPECTS OF DENTAL PULP PATHOLOGY

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ПАТОГЕНЕТИЧЕСКИЕ АСПЕКТЫ ПАТОЛОГИИ ПУЛЬПЫ ЗУБОВ

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The item offers a view on the effects of morphological, functional, and structural changes in overpulpal dentine and pulp while using multicomponent paste for treatment of deep caries and acute focal pulpitis. The material for the experiment included 13 teeth: 5 canines, eight premolars and molars (6 clinically outbred dogs aged 2–5 years). As found out, the use of the paste accelerated the dynamics of reparative dentinogenesis. The data from electronic microscopy scanning suggest optimization in inflammatory re-