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FEATURES OF IRON HOMEOSTASIS IN PATIENTS WITH STEATOHEPATITIS OF ALCOHOLIC AND NON-ALCOHOLIC ETIOLOGY AND ITS CORRELATION WITH THE INTENSITY OF OXIDATIVE STRESS AND APOPTOSIS

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Abstract

Topicality. Comorbid course of alcoholic steatohepatitis (ASH) and non alcoholic steatohepatitis (NASH) with dysmetabolic iron overload syndrome (DIOS) is caused by the cascade of interload reactions, which may contribute to the progression of the disease. DIOS is a pathological condition characterized by a quantitative increase in the content of elemental iron in the body, which leads to the damage of organs and tissues due to the toxic effects of excess iron. DIOS formation is associated with congenital or acquired insufficiency of mechanisms of the regulation of iron excretion, accumulation in the population of gene mutations of proteins-regulators of iron metabolism; inefficient erythropoiesis and insufficient utilization of iron in the bone marrow, with the changes in the quality of nutrition (predominant consumption of meat products); increasing life expectancy, living in geozones

with high iron content in water bodies, soils, associated both with natural features and industrial production. Other risk factors leading to the development of DIOS include multiple blood transfusions, unreasonable, uncontrolled prescription of iron-containing drugs, alcohol abuse, tobacco smoking, obesity, diabetes mellitus (DM), hormone replacement therapy with steroid hormones and estrogens, and more.

The aim of the work is to establish the features of iron homeostasis in patients with steatohepatitis of alcoholic and nonalcoholic etiology, to identify the dependence of steatohepatitis, oxidative-antioxidant homeostasis and the intensity of hepatocyte apoptosis on the presence of iron overload syndrome.

Materials and methods. 125 patients were examined, including 60 patients with NASH and 65 patients with ASH, 25 practically healthy persons of the corresponding age and sex. Among the examined patients with NASH, there were 15 male patients (25.0%) and 45 female patients (75.0%). The mean age of the examined patients was (46.3 ± 5.2) years. Among the examined patients with ASH there were 56 male patients (86.2%) and 9 female patients (13.8%). The mean age of patients with ASH was (47.4 ± 5.1) years. The control group consisted of 25 healthy individuals (PHIs), namely male - 11 (44.0%) and female - 14 (56%). The mean age of PHIs was (41.3 ± 2.1) years.

The diagnosis of NASH and ASH was established according to the unified clinical protocol approved by the order of the Ministry of Health of Ukraine № 826 from 06.11.2014, in the presence of the criteria for excluding the possibility of chronic diffuse liver disease of viral, hereditary, autoimmune or drug genesis being a cause of cytolytic, cholendoma-cholestatic-melestatic , as well as the results of ultrasonography of the liver with shear wave elastography, Steato-test, ASH-test, NASH-test (BioRedictive, France).

Results. The frequency of clinical syndromes in NASH varied depending on the presence of DIOS as follows: under DIOS conditions the frequency of cholestatic syndrome prevailed (3.1 times, $p < 0.05$), abdominal discomfort in the right hypochondrium (3.5 times, $p < 0,05$), the incidence of splenomegaly (7.0 times, $p < 0,05$). The analysis of biochemical syndromes in patients with ASH depending on the presence of DIOS indicates a significantly higher incidence of cholestasis (2.7 times, $p < 0.05$), hepatocellular insufficiency (4.3 times, $p < 0.05$), as well as impaired glucose tolerance (2.8 times, $p < 0.05$) in patients with DIOS compared with the incidence of these syndromes in ASH without DIOS. The analysis of the biochemical parameters of ferrokinetics indicates a probable increase in serum iron in patients with NASH with DIOS - 1.7 times ($p < 0.05$) compared with PHIs, and in the absence of DIOS- iron content corresponded to the reference values ($p > 0.05$). The course of ASH is

accompanied by a manifest DIOS in 61.5% of patients, also accompanied by hypersideremia, hyperferritinemia, hypertransferinemia and a significant increase in the percentage of TS (in the range of 1.7-4.3 times), which causes increased activity of cytolytic syndrome compared to ASH without DIOS. A strong correlation was found between the content of iron in the blood and the activity of AST ($r = 0.61$, $p < 0.05$), the content of transferrin in the blood and AST ($r = 0.67$, $p < 0.05$), the content of blood ferritin and AST ($r = 0.75$, $p < 0.05$) in patients in this group. At the same time, patients with ASH without DIOS are also characterized by, but less intense (within 1.3-1.6 times), an increase in blood iron, ferritin and transferrin, the percentage of TS, which confirms the opinion of scientists that the indicators of ferrokinetics should be used as a significant diagnostic criterion for ASH. Among the patients with NASH on the background of obesity, the manifestation of DIOS was registered in 30.0% of patients with hypersideremia, hyperferritinemia, hypertransferinemia, the increase in the percentage of TS (within 1.5-2.7 times). The course of NASH without DIOS was characterized by hyperferritinemia ($p < 0.05$), which can be regarded as a marker of active mesenchymal inflammation.

Conclusions. 1. The course of ASH is accompanied by a manifest iron overload syndrome in 61.5% of patients, which is accompanied by hypersideremia, hyperferritinemia, hypertransferrinemia and a significant increase in the percentage of iron transferrin saturation, which causes increased activity of cytolytic syndrome without comparison. 2. In patients with NASH on the background of obesity, the manifestation of DIOS was registered in 30.0% of cases, in which hypersideremia, hyperferritinemia, hypertransferrinemia, increase in the percentage of TS, which can be regarded as a marker of inflammatory activity. 3. The course of alcoholic and non-alcoholic steatohepatitis is accompanied by significant oxidative stress, which increases with the accession of DIOS. 4. The main signs of disintegration of the parameters of the antiradical defense system in patients are compensatory, in response to the activation of oxidative stress and endotoxicosis, increased glutathione peroxidase activity and progressive decrease in erythrocytes of reduced glutathione, the depot of which is depleted in proportion to intensity. 5. The presence of DIOS significantly enhances the intensity of hepatocyte apoptosis.

Key words: steatohepatitis; dysmetabolic iron overload; obesity; hepatocytes; free radical oxidation; apoptosis.

The relevance of the study of the comorbid course of alcoholic steatohepatitis (ASH) and non-alcoholic steatohepatitis (NASH) with dysmetabolic iron overload syndrome (DIOS)

is due to the cascade of interload reactions, which may contribute to the progression of the disease [2, 3]. DIOS is a pathological condition characterized by a quantitative increase in the content of elemental iron in the body, which leads to damage to organs and tissues due to the toxic effects of excess iron [4]. In contrast to the disease of hemochromatosis - an inherited disorder of iron metabolism [1, 9, 12], the formation of DIOS is associated with congenital or acquired insufficiency of mechanisms of regulation of iron excretion, accumulation in the population of gene mutations that regulate iron metabolism; inefficient erythropoiesis and insufficient utilization of iron in the bone marrow, with the changes in the quality of nutrition (predominant consumption of meat products); increasing life expectancy, living in geozones with high iron content in water bodies, soils associated both with natural features and industrial production [7, 8, 10, 13, 16, 20]. Other risk factors leading to the development of DIOS are multiple blood transfusions, unreasonable, uncontrolled prescription of iron-containing drugs, alcohol abuse, tobacco smoking, obesity, diabetes mellitus (DM), hormone replacement therapy steroid hormone and estrogen therapy, etc. [3, 4, 7, 10, 13, 20].

A significant medical and social problem in the field of internal medicine is a significant increase in the incidence of alcoholic fatty liver disease. In terms of prevalence and social significance, it ranks second after liver diseases of viral etiology [18]. The systematic consumption of toxic doses of alcohol (40 ml per day for men, 20 ml per day for women for more than 6 months) leads primarily to the development of alcoholic steatosis of the liver, then to alcoholic steatohepatitis (ASH) (fatty dystrophy with centrolobular necrosis of hepatocytes, mesenchymal-inflammatory reaction), and with further progression - alcoholic cirrhosis of the liver (LC) (irreversible process of septal liver fibrosis F4 stage with nodes of hepatocyte regeneration and impaired hepatic architectonics) on the background of systemic manifestations of alcoholism, pancreatitis, etc. [6, 18]. In the United States, up to 10% of the population suffers from chronic alcoholism, which requires medical supervision, 15% of them develop LC within 10-20 years [18]. Hypersideremia, which occurs in 70-90% of patients with alcoholism, is one of the probable unfavorable diagnostic criteria for ASH, causally related to the progression of ASH in LC [2, 3, 4, 7, 23].

The problem of diagnosis and treatment of nonalcoholic fatty liver disease (NAFLD) is the interdisciplinary problem of internal medicine, as recent studies show an increase in the number of obese, type 2 diabetes, which are background diseases for NAFLD, increasing its complications and possible transformation into LC [5, 6, 14]. Today in developed countries NAFLD is observed in 20-30% of the adult population. LC on the background of NASH is the cause of 5-8% of liver transplants performed in the United States and in the European

Union [17, 21]. Given the frequent comorbidity of NASH with obesity and type 2 diabetes, which are risk factors of the development of DIOS, this syntropy is a serious concern of scientists. Secondary DIOS, which occurs on the background of NAFLD, is observed in every third or fourth patient (in 20-30% of cases) [4, 20]. According to the data obtained, 36.7% of patients with NAFLD find mutant genes in the heterozygous state (C282 / N, H63D / N), which themselves do not lead to the development of NASH, but in the presence of liver pathology contribute to the manifestation of DIOS [17, 20, 23]. Therefore, early diagnosis and treatment of DIOS in patients with ASH and NASH can prevent progression to LC and significantly reduce the risk of hepatocellular carcinoma (HCC) [2, 3, 4, 23].

The term "iron overload" defines a wide range of pathological conditions, from the pronounced accumulation of iron in the tissues to metabolic and organ dysfunctions caused by disorders of iron metabolism [3]. Oversaturation of the body with non-heme iron (ie iron that is not part of heme) creates the conditions for increasing non-protein (free) fraction of iron, which is a catalyst for the formation of highly toxic hydroxyl radicals in the reactions of Fenton and Harber-Weiss [2]. R. Morand and co-authors (1997) described dysmetabolic iron overload syndrome (DIOS) [4].

Excess iron is no less pathogenic than its deficiency, because iron is a modulator of auto-oxidative processes, and the body does not have effective ways to remove its excess [2, 23]. Hypersideremia has been shown to induce hemosiderin synthesis by hepatocytes [3, 4]. In DIOS, excess iron is deposited in the liver, spleen, bone marrow and, increasing the formation of free radicals, leads to local tissue damage [8, 10]. It is believed that low molecular weight iron complexes can contribute to tissue damage due to catalysis of free radical lipid oxidation (FRLO) and oxidative modification of proteins (OMP) and, thus, contribute to the development of such diseases as steatohepatitis with progressive 2 fibrosis, liver cancer, pathology of the cardiovascular system [3, 8, 16]. The "toxicity" of iron is targeted at the enzymes of the mitochondrial electron transport chain [3, 9, 20], which occurs due to the formation of dinitrosyl complexes of iron with iron and sulfur-containing proteins [20]. Recent data suggests that the concentration of labile iron is one of the important factors in regulating the relationship between cell proliferation and apoptosis [16, 22]. Thus, iron-induced oxidative stress (OS) is the leading pathogenetic mechanism of DIOS, which is also an important part of the pathogenesis of NASH and ASH [2, 5, 6, 11, 15]. It is known that an excess of elemental iron acts as a catalyst for FROL, stimulates apoptosis, cytolysis, collagen formation and tissue fibrosis at the sites of micronutrient deposition [3, 12, 22]. Direct

damage to cell DNA induces the initiation of carcinogenesis, in particular, increases the risk of hepatocellular carcinoma [16].

Thus, determining the concentration of iron in the form of low molecular weight complexes can be important and useful for the diagnosis and monitoring of pathological conditions associated with DIOS. Increased concentrations of deposited iron in DIOS are also a risk factor for tissue damage due to increased radiation and chemical processes in an environment filled with highly dispersed material with high density, which is biological tissue enriched with iron in the inorganic "core" of proteins that transport it or depot. ferritin / hemosiderin) [3, 4, 16, 23]. Studies using electron spectroscopy and magnetic properties have also revealed the presence of iron in the form of "biogenic magnetite" in various tissues [8]. The presence of ferromagnetic material in human tissues creates a real basis for the realization of possible mechanisms of interaction with the surrounding magnetic fields and violation of neurohumoral regulation of homeostasis in general [10, 20, 23]. Therefore, for the diagnosis and monitoring of DIOS it is important to study the range of indicators of iron metabolism, in particular, the pool of its transport forms: transferrin, ferritin / hemosiderin, as well as its low molecular weight complexes in conjunction with the manifestation of leading clinical syndromes. -antioxidant homeostasis and the intensity of cell apoptosis.

The aim of our study was to establish the features of iron homeostasis in patients with steatohepatitis of alcoholic and nonalcoholic etiology, to identify the dependence of steatohepatitis, oxidative-antioxidant homeostasis and the intensity of apoptosis of hepatocytes from the presence of dysmetabolic iron overload syndrome.

Material and methods of research

125 patients were examined, including 60 patients with NASH and 65 patients with ASH, 25 practically healthy persons of the corresponding age and sex. The examinations were performed in the gastroenterological and therapeutic departments of the Emergency Hospital in Chernivtsi in 2015-2020. Among the examined patients with NASH there were 15 male patients (25.0%) and 45 female patients (75.0%). The mean age of the examined patients was (46.3 ± 5.2) years. Among the examined patients with ASH, there were 56 male patients (86.2%) and 9 female patients (13.8%). The mean age of patients with ASH was (47.4 ± 5.1) years. The control group consisted of 25 practically healthy individuals (PHIs): male - 11 (44.0%) and female - 14 (56%). The mean age of PHIs was (41.3 ± 2.1) years.

The diagnosis of NASH and ASH was established according to the unified clinical protocol approved by the order of the Ministry of Health of Ukraine № 826 from 06.11.2014, in the presence of criteria for exclusion of chronic diffuse liver disease of viral, hereditary,

autoimmune or drug genesis as a cause of cytolytic, cholendoma-cholestatic-melestatic , as well as the results of ultrasonography of the liver with shear wave elastography, Steato-test, ASH-test, NASH-test (BioRedictive, France). Additionally, in the diagnosis of steatohepatitis of alcoholic origin, anamnestic data on daily consumption of toxic doses of alcohol, consultation with a narcologist, availability of records in a narcological dispensary, changes in the de Ritis coefficient were taken into account.

The diagnosis of obesity was established according to the classification of the WHO International Working Group on Obesity (1997). Patients were measured for height and body weight, calculated body mass index (BMI) according to the Kettle formula (1):

BMI = weight (kg) / height2 (m) (1)

The diagnosis of obesity was established when the BMI value is more than 30 kg/m2.

The presence of DIOS was determined, under conditions of alcoholic and non-alcoholic steatohepatitis, by three of the following laboratory markers: increase in blood ferritin content of more than 300 μg / l in men and menopausal women and more than 200 μg / l in women of childbearing age; increase in serum iron content above reference values, but not higher than 50 μmol / l (hemochromatosis); decrease in the total iron-binding capacity of blood serum; increase in iron saturation of transferrin by more than 45% [4].

Upon admission to the hospital, the functional state of the liver was determined according to the approved list of enzyme activity, markers of pigment metabolism, mesenchymal inflammation, proteinogram, lipidogram, ionogram, calculation of the de Ritis coefficient according to conventional methods.

Iron homeostasis was studied by the content of iron, transferrin, ferritin in the blood, the calculation of the percentage of transferrin saturation (TS) (1).

TS = serum iron content / transferrin content × 3,9) [4].

The content in the blood of the molecular product of lipid peroxidation (LPO) - malonic aldehyde (MA) in blood plasma was studied by Yu.A. Vladimirov, AI Archakov. The content of reduced glutathione (RG) in the blood was determined by the titration method according to OV Travina in the modification of IF Meshchishena, I.V. Petrova. The activity of the enzyme of the antioxidant defense system (ADS) glutathione peroxidase (GP) was studied by IF We moved. The intensity of endotoxicosis was studied by the content in the blood of medium molecular weight peptides (MMP) by the method of NI Gabrielyan at a wavelength of 254 and 280 nm. The blood content of M3 fraction of cytokeratin-18 (C-18) as a marker of the intensity of apoptosis of hepatocytes was determined by enzyme-linked immunosorbent assay using ELISA reagents.

Statistical analysis of the results was performed according to the type of study and the types of numerical data that were obtained. The normality of the distribution was checked using Liliefors, Shapiro-Wilk tests and the method of direct visual evaluation of histograms of the distribution of eigenvalues. Quantitative values that had a normal distribution are presented 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 subjects). For comparisons of the data that had a normal distribution, we used parametric tests to assess Student's t-test, Fisher's F-test.

In the case of abnormal distribution, used: median test, calculation of the Mann-Whitney rank U-test, for multiple comparison - Wilcoxon T-test (in the case of the study of dependent groups). To assess the significance of the frequency of clinical manifestations of NASH and ASH depending on the DIOS, we used the method of calculating the odds ratio - Odds Radio (OR) and determining its 95% confidence interval. To assess the degree of dependence between variables, we used Pearson correlation analysis in the parametric distribution and Spearman's rank correlation coefficient in the case of the distribution of indicators that were significantly different from normal. For statistical and graphical analysis of the obtained results we used software packages Statistica for Windows version 8.0 (Stat Soft inc., USA), Microsoft Excel 2007 (Microsoft, USA).

Results of the research. Depending on the indicators of ferrokinetics, the examined patients were divided into 4 groups. Among patients with ASH - DIOS was found in 40 patients (61.5%), in 25 patients ASH was without DIOS (38.5%) (Table 1). Among patients with NASH in 18 patients (30.0%) was diagnosed with DIOS, in 42 people (70.0%) NASH was without DIOS (see Table 1).

Table 1 - Distribution of the examined patients with steatohepatitis depending on the etiology and the presence of iron overload syndrome (n,%)

№	DIOS	PHIs, n=25	ASH, n=65	NASH, n=60
1.	Present	-	40 (61,5 %)	18 (30,0 %)
2.	Absent	25 (100%)	25 (38,5 %)	42 (70,0 %)

Analysis of the frequency of clinical ASH syndromes depending on the presence of DIOS indicates a statistically significant predominance of the frequency of dyspeptic syndrome in patients with DIOS (2.6 times, $p < 0.05$), cholestasis syndrome (2.8 times, p

<0.05), abdominal discomfort in the right hypochondrium (2.4 times, p <0.05), the incidence of splenomegaly (3.0 times, p <0.05) (Table 2).

At the same time, the frequency of clinical syndromes in NASH varied depending on the presence of DIOSs as follows: under DIOS conditions the frequency of cholestatic syndrome (3.1 times, p <0.05), abdominal discomfort in the right hypochondrium prevailed (3.5 times, p <0.05), the incidence of splenomegaly (7.0 times, p <0.05) (Table 3).

Table 2 - Frequency of manifestation of clinical and biochemical syndromes of alcoholic steatohepatitis depending on the presence of dysmetabolic iron overload syndrome, (n,%)

Syndromes	Groups of patients				OR	
	ASH with DIOS, n=40		ASH, n=25		OR	95% DI
	Abs	%	Abs	%		
Astheno-vegetative	28	70,0	17	68,0	1,02	0,47-2,25
Dyspeptic	38	95,0	9	36,0*	2,64	1,09-6,37
Cholestatic	27	67,5	6	24,0*	2,80	1,02-7,77
Abdominal pain	39	97,5	10	40,0*	2,44	1,04-5,74
Hepatomegaly	40	100,0	25	100,0	1,0	0,49-2,03
Splenomegaly	29	72,5	7	28,0*	3,02	1,10-8,30
Cytolytic	40	100,0	25	100,0	1,0	0,49-2,03
Cholestatic without cholecestitis	30	75,0	7	28,0*	2,68	1,12-7,01
Mesenchymal-inflammatory	40	100,0	22	88,0	1,14	0,55-2,34
Mesenchymal-inflammatory	34	85,0	5	20,0*	4,25	1,47-12,31
Impaired glucose tolerance	40	100,0	9	36,0*	2,78	1,15-6,69
Note: * - the difference is statistically significant compared to the indicator in the group of patients with ASH with DIOS (p <0,05)						

Analysis of biochemical syndromes in patients with ASH depending on the presence of DIOS indicates a significantly higher incidence of cholestasis (2.7 times, p <0.05), hepatocellular insufficiency (4.3 times, p <0.05), as well as impaired glucose tolerance (2.8 times, p <0.05) in patients with DIOS compared with the incidence of these syndromes in ASH without DIOS (see Table 2).

Table 3 - Frequency of manifestation of clinical and biochemical syndromes of non-alcoholic steatohepatitis depending on the presence of iron overload syndrome, (n, %)

Syndromes	Groups of patients				OR	
	NASH, Obesity with DIOS, n=18		NASH, Obesity, n=42		OR	95% DI
	Abs	%	Abs	%		
Astheno-vegetative	6	33,3	29	69,0	1,47	0,59-3,66
Dyspeptic	11	61,1	39	92,9	1,51	0,64-3,62
Cholestatic	16	88,9	12	28,6*	3,11	1,23-7,89
Abdominal pain	15	83,3	10	23,8*	3,50	1,32-9,25
Hepatomegaly	18	100,0	33	78,6	1,27	0,57-2,82
Splenomegaly	12	66,7	4	9,5*	7,0	1,99-24,66
Cytolytic	18	100,0	42	100,0	1,0	0,46-2,18
Cholestatic without cholecestitis	17	94,4	12	28,6*	3, 31	1,31-8,32
Mesenchymal-inflammatory	15	83,3	8	19,0*	4,38	1,58-12,14
Mesenchymal-inflammatory	11	61,1	6	14,3*	4,28	1,37-13,95
Impaired glucose tolerance	18	100,0	42	100,0	1,0	0,46-2,18
Note: * - the difference is statistically significant compared to the indicator in the group of patients with NASH with DIOS (p <0,05)						

Analysis of markers of biochemical syndromes in patients with NASH depending on the presence of DIOS indicates a significantly higher incidence of cholestasis (3.3 times, p <0.05), mesenchymal inflammation (4.4 times, p <0.05), hepatic -cellular insufficiency (4.3 times, p <0,05) in patients with DIOS in comparison with the frequency of these syndromes in NASH without DIOS (see table. 3).

Analysis of biochemical parameters of ferrokinetics indicates a probable increase in serum iron in patients with NASH with DIOS - 1.7 times (p <0.05) compared with PHIs, and in the absence of DIOS - iron content corresponded to the reference values (table. 4) (p> 0.05).

Table 4 - Biochemical parameters of cytolysis activity of hepatocytes, blood ferrokinetics, blood content of malonic aldehyde, reduced glutathione, medium molecular weight peptides, cytokeratin-18, glutathione peroxidase activity in patients with nonalcoholic and alcoholic steatohepatitis

Indicator, units measurement	PHIs, n=25	NASH, n=60		ASH, n=65	
DIOS		DIOS, n=18	Without DIOS, n=42	DIOS, n=40	Without DIOS, n=25
ALT, U/L	22,8 ±1,7	69,8 ± 2,8*	58,3 ± 2,4 */**	82,4 ±6,3 */#	67,8±4,8 */#
AST, U\L	25,2 ± 1,5	48,2± 1,4*	41,6 ± 2,2 */**	125,8±5,8 */#	102,0±5,5 */**/#
De Rittis ratio	1,1± 0,01	0,7± 0,01*	0,7± 0,01*	1,5± 0,01 */#	1,5±0,01 */#
Serum iron, μmol / l	17,6±1,2	32,3±1,2*	19,2±1,1**	39,1±0,9*/#	25,6±1,0 */**/#
Ferritin, μg / l	80,3± 5,8	219,8 ±7,1*	103,7±5,1 */**	341,9±4,3 */#	227,6±5,8 */**/#
Transferrin, g / l	2,4±0,01	2,9±0,01 *	2,6±0,01 **	3,3±0,01 */#	3,2±0,01 */**/#
Transferrin saturation,%	27,6±1,4	43,4±1,3*	28,8±1,4 **	46,2±1,6 *	31,2±1,5 **
Malone aldehyde, μmol / l	3,9±0,1	6,8±0,1*	6,0±0,2 */**	8,1±0,4 */#	7,2±0,2 */**/#
GR, μM / g Hb	6,1±0,3	3,8±0,1 *	4,3±0,1 */**	3,0±0,1 */#	3,6±0,1 */**/#
GP, nM GV / min г Нв	157,6±6,7	291,4 ± 9,0*	272,8± 15,1*	246,0± 10,9*/#	208,7± 14,5*/#
MMP 254, USD / l	0,21 ± 0,001	0,35 ± 0,002*	0,30± 0,001 */**	0,43 ± 0,002 */#	0,39 ± 0,001 */**/#
Cytokeratin-18 M3, U / l	51,2 ± 5,3	325,7 ± 15,2*	319,4± 12,8*	143,7 ± 21,6*/#	119,5± 18,3*/#
Note: * - changes are probable (p <0,05) in comparison with the indicator in PHIs. ** - changes are probable (p <0,05) in comparison with the indicator in patients with steatohepatitis with DIOS; # - changes are probable (p <0,05) in comparison with the indicator in patients with NASH.					

In contrast to these data, in patients with ASH with DIOS, the increase in serum iron was more intense - 2.2 times (p <0.05), however, and in the absence of DIOS in ASH, the iron content was significantly increased - in 1 , 4 times (p <0,05) in comparison with the indicator

in PHIS though did not exceed the upper limit of norm, with existence of probable difference with similar groups of patients with NASH ($p < 0,05$) (tab. 4).

The level of ferritin in the blood of patients with NASH with DIOS also exceeded the indicator in PHIs 2.7 times ($p < 0.05$), and in patients with ASH with DIOS - 4.3 times ($p < 0.05$) with the presence of a probable intergroup difference ($p < 0.05$). In patients with NASH without DIOS, the ferritin content exceeded the value in PHIs by 1.3 times ($p < 0.05$), and in patients with ASH without DIOS - the excess was 1.6 times with the presence of intergroup difference ($p < 0.05$) both in similar groups of patients with NASH and in groups of patients with DIOS ($p < 0.05$).

The results of the analysis of transferrin content in the blood of patients with NASH with DIOS showed a significant excess of data in PHIs 1.5 times ($p < 0.05$), and in patients with ASH with DIOS - 1.7 times ($p < 0, 05$) with the presence of a probable intergroup difference ($p < 0.05$). In patients with NASH without DIOS, the transferrin content had only a tendency to increase ($p > 0.05$), and in patients with ASH without DIOS - the excess was 1.3 times with the presence of an intergroup difference ($p < 0.05$) as with a similar group patients with NASH, and with a group of patients with ASH with DIOS ($p < 0,05$).

Indicators of iron transferrin saturation were increased in the observation groups of patients with NASH and ASH with DIOS: respectively in NASH - 1.6 times and ASH - 1.7 times ($p < 0.05$), which indicates the presence of iron overload syndrome. Accordingly, in the comparison groups - patients with NASH and ASH without DIOS, the TS values were within the reference values ($p > 0.05$).

Thus, the course of ASH is accompanied by a manifest DIOS in 61.5% of patients, which is accompanied by hypersideremia, hyperferritinemia, hypertransferinemia and a significant increase in the percentage of TS (in the range of 1.7-4.3 times), which causes increased activity of the cytolytic syndrome compared to the course of ASH without DIOS. A strong correlation was found between the content of iron in the blood and the activity of AST ($r = 0.61$, $p < 0.05$), the content of transferrin in the blood and AST ($r = 0.67$, $p < 0.05$), the content of blood ferritin and AST ($r = 0.75$, $p < 0.05$) in patients in this group. At the same time, patients with ASH without DIOS are also characterized by an increase in blood iron, however less intense (within 1.3-1.6 times), ferritin and transferrin, the percentage of TS, which confirms the opinion of scientists that the indicators of ferrokinetics should be used as significant diagnostic criteria for ASH.

Among the patients with NASH on the background of obesity, the manifestation of DIOS was registered in 30.0% of patients with hypersideremia, hyperferritinemia,

hypertransferinemia, increase in the percentage of TS (within 1.5-2.7 times). The course of NASH without DIOS was characterized by hyperferritinemia ($p < 0.05$), which can be regarded as a marker of active mesenchymal inflammation.

Analysis of the intensity of lipoperoxidation (according to the content of MA in the blood) indicates a higher intensity of oxidative stress (OS) in patients with ASH with DIOS with an increase in the content of MA in the blood by 2.1 times ($p < 0.05$), and in the absence of DIOS - 1.8 times ($p < 0.05$) with the presence of a probable intergroup difference ($p < 0.05$). In patients with NASH, the intensity of OS was lower: in NASH with DIOS, the content of MA exceeded the value in PHIs 1.7 times, and in the absence of DIOS - 1.5 times ($p < 0.05$). The content of MA in the blood was in a direct strong correlation with the indicator of the content of iron in the blood (at ASH $r = 0.77$, $p < 0.05$, at NASH $r = 0.64$, $p < 0.05$).

In parallel with the activation of the OS, we analyzed the changes in the integrated markers of the intensity of endogenous intoxication (EI) depending on the presence of DIOS. The results of the study indicate a maximum increase in the intensity of endotoxicosis in patients with ASH with DIOS (by increasing the content of MMP in the blood by 2.0 times ($p < 0.05$) against an increase in ASH without DIOS - by 1.9 times ($p < 0, 05$)). The content of iron in the blood was in direct interrelation with the content of MMP: with ASH $r = 0.71$ ($p < 0.05$), with NASH $r = 0.54$ ($p < 0.05$). In patients with NASH, the intensity of EI was lower: in NASH with DIOS, the content of MMP exceeded the index in PHIs 1.7 times, and in the absence of DIOS - 1.4 times ($p < 0.05$), which also indicates a significant level of endotoxicosis , however, it was lower than in ASH.

Analysis of the indicators of the state of protective anti-radical systems revealed the following changes (Table 4). First of all, a decrease in the content of GR in the blood should be elucidated - the maximum decrease was observed in patients with ASH with DIOS – 2.0 times ($p < 0,05$), whereas in NASH with DIOS the decrease was 1.6 times ($p < 0,05$) . The content of iron in the blood was inversely related to the content in the erythrocytes of GR: at ASH $r = -0.78$ ($p < 0.05$), at NASH $r = -0.65$ ($p < 0.05$). In the absence of DIOS, the content of HF in the blood also decreased by 1.7 and 1.4 times - respectively in ASH and NASH ($p < 0.05$) compared with the indicator in PHIs.

Significant changes have also been identified in the functioning of glutathione-dependent enzymes. In particular, the activity of the enzyme glutathione peroxidase, which is involved in the neutralization of hydrogen peroxide, preventing the formation of extremely reactive hydroxyl radical, in patients with NASH with DIOS was increased among the comparison groups - 1.9 times, in NASH without DIOS - 1, 7 times ($p < 0.05$). In patients with

ASH with DIOS, compensatory activation of the enzyme was also registered 1.6 times ($p < 0.05$), and in the absence of DIOS - the activity exceeded the index in PHIs 1.3 times ($p < 0.05$).

Thus, the main signs of disintegration of the parameters of the antiradical protection system studied are compensatory, in response to OS activation, increase in GP activity, and progressive decrease in erythrocyte content of reduced glutathione, the depot of which is depleted in direct proportion to the intensity of iron accumulation and intensity. EI with maximum manifestations in patients with ASH with DIOS.

Thus, the nature of dysmetabolic, inflammatory-necrotic processes in the liver and the prognosis are closely related to the functioning of antiradical defense systems and ROS formation, which allows us to consider the liver as a target organ of oxidative damage or oxidative stress [5, 6, 15]. It is known that the processes of cytolysis are preceded by the processes of apoptosis, which is enhanced by the activation of OS, IR and reduced activity of antioxidant protection factors [22]. Iron-mediated intramitochondrial and lysosomal oxidative reactions, which cause mitochondrial "death", partial rupture of lysosomal membranes and subsequent activation of apoptosis [6, 22], have been described in the literature. Analysis of the content of M3 fraction of cytokeratin-18 (CC-18), which is an active marker of the intensity of hepatocyte apoptosis in patients with NASH [22], revealed its intensive growth in patients with NASH: in the presence of DIOS - 6.4 times, for absence - 6.3 times ($p < 0.05$). At the same time, the content of CC-18 in the blood of ASH increased less intensively - 2.8 and 2.3 times, respectively, compared with PHIs ($p < 0.05$). The presence of the correlation between the content of iron in the blood and CC-18 (NASH $r = 0.51$, $p < 0.05$, ASH $r = 0.36$, $p < 0.05$).

Thus, excess iron can play an important role in the damage to cells caused by oxygen free radicals, OS, EI, ranging from a slight increase in apoptosis to significant cytolysis of hepatocytes in both ASH and NASH [2, 3, 6, 18]. Free iron can catalyze the Fenton reaction, which proves a crucial role in the pathogenesis of ASH. As a result of continuous hydrolysis of iron-containing compounds, lysosomes contain a pool of redox iron. Chronic ethanol consumption causes an obvious increase in lysosomal redox iron, accompanied by persistent oxidative damage. Iron-mediated oxygen free radicals can increase lysosomal membrane permeability, mitochondria, and subsequent cell apoptosis due to mitochondrial-lysosomal "death" [2, 3, 4, 7, 18, 22, 23]. Therefore, for adequate treatment and rehabilitation of patients with ASH, as well as NASH on the background of obesity, it is necessary to carefully monitor

ferrokinetics and in case of DIOS to take measures to eliminate excess iron from the body and strengthen the antioxidant defense system and detoxification ability of hepatocytes.

Conclusions. 1. The course of alcoholic steatohepatitis is accompanied by a manifest syndrome of iron overload in 61.5% of patients, which occurs with hypersideremia, hyperferritinemia, hypertransferrinemia and a significant increase in the percentage of transferrin iron saturation (within 1.7-4.3 times, $p < 0.05$) , which causes increased activity of the cytolytic syndrome in comparison with the course of ASH without DIOS. The correlation between the content of iron in the blood and the activity of AST ($r = 0.61$, $p < 0.05$), the content of transferrin in the blood and AST ($r = 0.67$, $p < 0.05$), the content in the blood ferritin and AST ($r = 0.75$, $p < 0.05$) An increase in blood iron, ferritin and transferrin, TS can be characteristic, but less intense (in the range of 1.3-1.6 times, $p < 0.05$) of patients with ASH without DIOS.

2. In patients with non-alcoholic steatohepatitis on the background of obesity, the manifestation of DIOS was registered in 30.0% of cases, in which hypersideremia, hyperferritinemia, hypertransferrinemia, increase in the percentage of TS (within 1.5-2.7 times, $p < 0.05$). The course of NASH without DIOS was characterized by hyperferritinemia ($p < 0.05$), which can be regarded as a marker of inflammatory activity.

3. The course of alcoholic and non-alcoholic steatohepatitis is accompanied by significant oxidative stress, which increases with the accession of DIOS (increase in MA content by 1.8 - 2.1 times in ASH, 1.5-1.7 times in NASH, $p < 0,05$), as well as the syndrome of endogenous intoxication (increase in the content of MMP by 1.9 - 2.0 times in ASH, 1.4-1.7 times in NASH, $p < 0,05$), markers of which correlate with the content of iron ($p < 0.05$).

4. The main signs of disintegration of the parameters of the antiradical protection system in patients are compensatory, in response to the activation of oxidative stress and endotoxicosis, increased glutathione peroxidase activity (maximum in NASH with DIOS 1.9 times, $p < 0,05$), and progressive decrease in erythrocytes of reduced glutathione (maximum in ASH with DIOS 2.0 times, $p < 0.05$), the depot of which is depleted in proportion to the intensity of iron accumulation in the body ($p < 0.05$).

5. The presence of DIOS significantly increases the intensity of apoptosis of hepatocytes: an increase in blood M3 fraction of cytokeratin-18 maximum in NASH with DIOS - 6.4 times ($p < 0,05$), which also depends on the content of iron in the blood 0.51, $p < 0.05$).

Prospects for further research in this area are the development of effective treatment programs for patients with ASH and NASH with comorbid iron overload syndrome.

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