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DIAGNOSTIC SIGNIFICANCE OF LABORATORY MARKERS, INFLAMMATORY AND ANTI-INFLAMMATORY CYTOKINES IN THE DEVELOPMENT OF NON-ALCOHOLIC FATTY LIVER DISEASE STEATOSIS AND STEATOHEPATITIS

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Abstract: This article studied clinical and laboratory analyzes of 98 patients with non-alcoholic fatty liver disease. With steatohepatitis, higher ALT and AST values are noted than in patients with hepatic steatosis. The main differential difference between non-alcoholic steatosis and steatohepatitis, available in clinical practice, may be the severity of the biochemical syndrome of cytolysis. The article contains clinical features and prognosis in patients with fatty hepatosis and non-alcoholic steatohepatitis (NASH). Dyslipidemia (hypertriglyceridemia, decreased HDL, increased LDL) occurs in about 65-85% of patients. The basal insulin level, according to the results of our studies, in persons with NAFLD at the stage of steatosis and steatohepatitis was significantly increased. With NAFLD compared with JS, the level of liver inflammation was rated as higher with SG activity. It has been proved that there is a correct correlation with the indices of cytolysis, according to which the functional state of the liver is assessed.
Key words: non-alcoholic fatty liver disease, nonalcoholic steatohepatitis, cytokines, inflammation

The urgency of the problem. Due to the progressive growth of non-alcoholic fatty liver disease (NAFLD), widespread (20-40%) and observation of the population while the working capacity is preserved, ineffectiveness of special treatment based on non-specific clinical signs at the initial stage, obvious clinical signs are observed in the late stages of the disease, the actuality of clinical medicine is considered one of the problems [1,2,6,8,12,16,18].

NAFLD - chronic multifactorial developing steatosis of the liver - accumulation of fat in the liver (fatty dystrophy of hepatocytes), steatohepatitis - formation of inflammatory infiltrate around the center of necrosis in liver cells, non-alcoholic fibrosis - cirrhosis is a disease complicated by a violation of the architecture of the liver and the growth of connective tissue. and foreign hepatologists are the main focus. When the disease progresses periodically, 12-40% of patients may develop non-alcoholic steatohepatitis after 8-13 years, and 15% of patients may develop liver cirrhosis and liver failure. In 7% of patients, liver cirrhosis can develop into hepatocellular carcinoma after 10 years [1,2,3,8,9,10,11,17].

The problem of the growth pattern of NAFLD is assessed by the tendency of the population to be obese. However, as the level of obesity increases, so does the severity of the disease. NAFLD occurs in 30-100% with obesity [1,2,3,8,9,10,11,17].

NAFLD is a multifactorial and multistage disease, which is mainly associated with metabolic syndrome. In the development of nonalcoholic steatosis and steatohepatitis, insulin resistance is formed based on compensatory hyperinsulinemia, which in turn increases lipogenesis and gluconeogenesis in the liver, decreases lipolysis, and accumulates fat in cells. Due to increased lipolysis, free fatty acids (FFA) are released from visceral fat. As a target organ, the liver develops steatosis as a result of the release of fat reserves in obesity. The progressive development of steatosis lays the groundwork for steatohepatitis. Additional oxidized stress, peroxyoxidized lipids disrupt the cell defense mechanism and inflammation and necrosis appear. Inflammatory cytokines tumor necrosis factor α (FNO-α) and interleukin 6 (IL-6) and anti-inflammatory cytokine interleukin 10 (IL-10) are important in the progression of NAFLD [3,7,15,17].

At the moment, a lot of attention is being paid to the consistent study of the symptoms that make up the laboratory picture in the stage of NAFLD steatosis and steatohepatitis. However, the number of studies that allow to confirm this opinion is extremely small, the information in them is not convincing.

Based on these, ***the purpose of our work*** is to evaluate the development of steatosis and steatohepatitis by determining the amount of lipids in the blood and the biochemical indicators of FNO-α, IL-6 and IL-10 in patients with NAFLD.

Materials and research methods. To solve the set tasks, 98 patients with NAFLD were examined, including 67 (68.3%) patients at the stage of hepatic steatosis (HS) and 31 (31.6%) patients with steatohepatitis (SG). Of these, 45 (46%) men and 53 (54%) women aged 20 to 75 years (average age 49.2 ± 4.2). The research results were recorded in the developed clinical information cards (questionnaire). The consent of the participants and members of the ethical committee for human rights in biomedicine at the Bukhara medical institute was obtained for the study. When selecting patients, we took into account the criteria for including and not including patients in the study. Criteria for the inclusion of patients in the study: - men and women aged 20 - 75 years; - the presence of fatty hepatosis and steatohepatitis; - the presence of a signed informed consent. The exclusion criteria were suspicion of alcohol or drug dependence, drug, viral, autoimmune liver damage, storage diseases, oncological diseases, severe diseases (uncorrected arterial hypertension (AH), type 2 diabetes in the stage of decompensation, chronic heart failure (CHF) III – IV functional class, heart attacks, strokes), pregnancy, lactation and low compliance. The criterion for not including from the survey was alcohol consumption in patients with fatty liver disease. We took into account the data of the anamnesis (absence of alcoholic beverages consumption regularly). We also used a special CAGE questionnaire [4].

We compared the results obtained in the course of the study with the indicators of the control group, formed of 24 apparently healthy individuals aged 20 to 65 years, who had no abnormalities in the hepatobiliary system. The diagnosis of non-alcoholic fatty liver disease was made on the basis of anamnesis, laboratory tests, and ultrasound examination of the liver. To detect NAFLD, ultrasound of the hepatobiliary system and liver elastography were performed. Ultrasound examination of the hepatobiliary system was performed in 500 patients with risk factors for NAFLD: obesity, dyslipidemia, impaired carbohydrate tolerance. Ultrasound of the liver revealed steatosis and steatohepatitis in 98 patients with NAFLD. The following signs of hepatic steatosis were noted: an increase in the size of the liver, an increase in its echogenicity, a relatively reduced density of the liver compared to the spleen (hepatic-splenic index less than 1), a decrease in sound conductivity, and a deterioration in visualization of the branches of the portal and hepatic veins. Ultrasound elastography was performed in 98 patients in order to exclude fibrosis in the liver parenchyma. Lipid metabolism was studied in terms of serum cholesterol (CS), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and triglycerides (TG). The LDL and VLDL values were calculated using the formula: VLDL = TG / 2, LDL = CHCR- (VLDL + HDL). Based on the results obtained, the atherogenic coefficient (CA) was calculated using the formula:

CA = CS LDL + CS VLDL / HDL. Determination of the degree of obesity was carried out according to the Quetelet index, calculating it by the formula: BMI = weight (kg) / height (m2).

FNO-α, IL-6 and IL-10 in blood serum were analyzed by IFA method in all examined group patients. The obtained data were statistically processed using the Student's t-test, and the difference of the results with $R<0.05$ was recognized as reliable.

Results and discussion. According to the results of our study, the ratio of women to men was 1,25:1. The distribution of patients with hepatic steatosis and steatohepatitis by age is shown in Table 1.

Table 1
Distribution of steatosis and steatohepatitis among patients with NAFLD depending on age, n (%)

Age sick	Women Abs (%)		Men Abs (%)	
	HS	SG	HS	SG
Up to 39 years old	1 (2,7%)	1 (6,25%)	2 (6,6%)	1 (6,6%)
40–49 years old	6 (16,2%)	2 (12,5%)	5 (16,6%)	2(13,3%)
50–59 years old	17 (45,9%)	6 (37,5%)	9 (30%)	5(33,3%)
60-74 years old	13 (35%)	7 (43,7%)	14(46,6%)	7 (46,6%)
Total	37	16	30	15

Analyzing the age criterion that HS occurs at any age, the able-bodied population is most susceptible to it (in persons from 40-59 years old - 55.2%, of them in women 34.3%, in men 20.8%; over 60 years old - in women 19%, in men over 60 years old - 20.8%), and SG occurs more often in older age (40-49 years old - 13%, in people over 60 years old - 45%). Demographic and anthropometric parameters were studied in all patients included in the study with non-alcoholic fatty liver disease (Table 2). When questioning patients, errors in nutrition were noted by 74 (76.5%) patients (irregular nutrition, abundant food, the presence of fatty and fried foods).

Table 2.
Comparison of demographic and anthropometric parameters of indicators of the main and control groups

Index	CG (n=24)	HS (n=67) 1	SG n=31 2	P_{1-2}
Age	36,4±2,30	40,2±2,2	48,2±4,2	> 0,005
Body weight, kg	63,0±1,03	72,0±3,2	82,0±4,22	0,001
Height, cm	170±4,2	165 ±4,33	167 ±3,25	>0,005
BMI, kg / m2 (25-30)	22,0±0,37	26,2±1,6	28,1±1,8	0,001
BMI, kg / m2 (25-30)	23,0±0,25	31,4±1,5	32,4±2,5	0,001
BMI, kg / m2 (25-30)	24,0±0,2	36,4±1,4	37,4±2,5	0,001
BMI, kg / m2 (25-30)	24,0±0,5	38,2±2,4	40,2±2,6	0,001

Patients in the observation group had increased body weight (Quetelet body mass index up to 30) DP in 29 (43%); SG 8 (26%) cases. Obesity I degree SP (body mass index (BMI) 30 - 34.9) was observed in 17 (25%), SG in 14 (45%) patients. Obesity II degree (BMI from 35 to 39.9) - in HS 15 (22.3%); SG 5 (16%). Obesity III degree (BMI 40 or more) - in HS 6 (9%); SG 4 (12.9%) patients.

When studying the functional state of the liver, we were interested in the state of lipid metabolism of NAFLD. The level of total cholesterol (TCS) was assessed according to the classification of the European Atherosclerotic Society [13]: up to 5.2 mmol / l - normal level; 5.3-6.5 mmol / l - mild hypercholesterolemia (HCS); 6.6-7.8 mmol / l - moderate; more than 7.8 mmol / l - high. Determined the expanded lipid profile: triglycerides (TG), cholesterol (CS) low density lipoprotein (LDL) and high density lipoprotein cholesterol (HDL). The content of very low density lipoprotein cholesterol (VLDL) was calculated. According to the Russian recommendations (V revision) "Diagnostics and correction of lipid metabolism disorders for the prevention and treatment of atherosclerosis" [13], the normal TG level did not exceed 1.7 mmol / L, the target LDL cholesterol value was less than 2.6 mmol / L, cholesterol HDL is higher than 1.15 mmol / L. Lipid metabolism indicators are presented in Table 3. Disorders of lipid metabolism in NAFLD are one of the cardinal signs of the disease [13]. According to our data, severe HCS (more than 6 mmol / L) was recorded more often.

Table 3.
Indicators of lipid metabolism in patients of the surveyed groups

Index	CG (n=24)	HS (n=67) 1	SG n=31 2	P_{1-2}
Cholesterol (mmol / L)	5,12±0,04	6,35±0,85	7,3±0,18	>0,005
Cholesterol VLDL (mmol / l)	0,37±0,06	0,66±0,21	0,92±0,12	0,001
Cholesterol LDL (mmol / l)	3,26±0,07	3,95±0,41	4,62±0,12	0,005
Cholesterol HDL (mmol / L)	1,32±0,04	0,95±0,05	0,82±0,08	0,001
Triglycerides (g / L)	0,93±0,02	1,76±0,21	1,97±0,18	0,001
Atherogenic coefficient (CA)	2,72±0,04	5,6±0,82	7,79±0,83	0,03

Dyslipidemia in NAFLD was characterized by an increase in the level of triglycerides more than 1.9 mmol / L and in which the level of HDL cholesterol is <1 mmol / L. These disorders turned out to be more noticeable, which indicated more severe disorders of lipid metabolism. Lipid metabolism indicators are presented in the table. Judging by the data in Table 3, in patients with NAFLD at the stage of steatosis and hepatic steatohepatitis, significant changes in lipid metabolism were revealed towards an increase in cholesterol ($p = 0.005$), VLDL cholesterol ($p = 0.001$), LDL cholesterol ($p = 0.001$), TG ($p = 0.001$), CA ($p = 0.03$) and a decrease in HDL ($p = 0.001$). The results obtained indicate the presence of atherogenic dyslipidemia in NAFLD patients at the stage of steatosis and steatohepatitis. Atherogenicity is a concept that reflects the relationship between bad and good fats. The blood serum from the vein is examined, the indicators for calculating the coefficient are determined by the colorimetric photometric method [13]. Normally, the value ranges from 2.2 to 3.5. When calculating the coefficient of atherogenicity, experts use a simple formula:

Atherogenic coefficient (Atherogenic index) = (Total cholesterol - HDL) / HDL

According to our data, severe coronary artery (more than 6 mmol / L) was more often recorded. The atherogenic index markedly exceeded the permissible values in all examined patients. In order to assess the functional state of the liver in NAFLD at the stage of fatty hepatosis and steatohepatitis, the parameters of pigment metabolism, cytolysis and cholestasis were studied (Table 4). Biochemical studies were carried out to determine the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGTP), alkaline phosphatase (ALP), the content of total bilirubin and its fractions.

Table 4.
Indicators of transaminase levels in the group of examined

Index	CG (n=24)	HS (n=67).1	SG. (n=31).2	P_{1-2}
Total protein (g / l)	65,22±0,21	75,2±3,2	78,2±3,8	> 0,05
Albumin g / l	53,2±1,0	45,2±2,2	44,2±2,2	> 0,05
Total bilirubin μmol / l	10,6±0,2	13,6±6,2	19,2±5,2	0,01
Binding bilirubin μmol / l	3,5±0,5	3,8±0,8	4,1±1,6	0,02
ALT (unit / l)	17,6±0,96	27,6±8,7	88,6±31,7	0,001
AST (unit / l)	20,9±1,1	20,9±7,7	48,2±23,7	0,001
alkaline phosphatase ALF (unit / l)	121,9±5,9	132,9±21,9	150,0±28,8	0,02
γ-ГТТП (unit)	24,9±1,1	34,9±12,7	71,9±41,7	0,001
Glucose (mmol / l)	4,3±0,8	5,9±0,9	6,45±0,65	> 0,05

The level of bilirubin was significantly increased relative to the indicators of the control group. The activity of HS cytolysis indices, the AST level reached 20.9, the ALT - 27.6. With steatohepatitis, there are higher ALT values 88.6 and AST 48.2 than in healthy individuals and patients with hepatic steatosis, so ALT in NASH exceeds 6-8 norms, AST exceeds 3-4 norms, with HS ALT exceeds 1- 2 norms, AST does not change significantly. The ALP activity in the SP was 132.9 U / L, which corresponded to the standard values (Table 4). The increase in alkaline phosphatase activity is 1.5-2.5 higher in patients with SH. Indicators of carbohydrate metabolism: the level of glucose in the blood serum was significantly increased ($p > 0.05$) in the patients we observed, since in the observation group in 25 patients (25.51%), among the comorbidities, there was a violation of tolerance to carbohydrates. In order to determine the degree of compensatoryness of the increased insulin level in NAFLD patients at the stage of fatty hepatosis and steatohepatitis, the HOMA-IR index was determined. The HOMA-IR score is a homeostasis assessment model for insulin resistance. Normally, the HOMA index does not exceed 2.7, and this indicator is the same for men and women, and after 18 years it does not depend on age either. During adolescence, the HOMA index slightly increases due to physiological insulin resistance at this age. Insulin resistance is a decrease in the susceptibility of insulin-sensitive tissues to the action of insulin when its concentration in the blood is sufficient. Insulin resistance has no specific symptoms. Insulin resistance can appear even in a person without obesity and diabetes - this happens in about 25% of cases. The indicator was calculated by the formula: [fasting insulin (IU / ml) × fasting glucose (mmol / l)] / 22.5. An indicator of less than 2 is considered normal [8,13]. In our study, the HOMA-IR insulin resistance index in patients was significantly increased ($p = 0.01$) in comparison with the control (Table 5).

Table 5.

Dynamics of indicators of the level of hormones in blood serum in patients with NAFLD

Hormone	CG (n=24)	HS (n=67).1	SG. (n=31). 2	P_{1-2}
Insulin MCTB / ml	11,53±1,46	15,12±1,42	18,22±1,61	0,001
Cortisol (nmol / L)	355,62±32,3	401,2±31,21	519,2±22,31	0,001
HOMA-IR	2,2±0,56	5,58±0,9	7,68±1,1	0,02

The basal insulin level, according to the results of our studies, in individuals with NAFLD was significantly increased ($p = 0.001$) (Table 5).

Subsequent investigations examined serum cytokines (FNO-α, IL-6, IL-10) in order to assess the level of inflammation in NAFLD. In the main group of patients with NAFLD included in the research group, the amount of cytokines in the blood was significantly increased compared to the indicators of the control group (Table 6).

Table 6

Serum levels of inflammatory and anti-inflammatory cytokines in NAFLD

Serum cytokine levels (pg/ml)	CG (n=70)	HS (n=67).1	SG. (n=31). 2	P_{1-2}
TNF-α	4,5(1,3;7,0)	41,75 (19,4; 70,2)	46,44(23,33; 89,5)	< 0,001
IL-6	4,16 (1,6; 6,5)	31,46 (11; 62,2)	37,2(22,2; 71,1)	< 0,001
IL-10	5,28 (2,1;12)	48,15 (33,1; 79,4)	54,6(38,1; 97,6)	= 0,02

The level of TNF-α indicators in HS was 41.75 (19.4; 70.2), in steatohepatitis it was higher than in healthy and steatosis 46.44 (23.33; 89.5) and IL-6 in HS was 31 .46 (11; 62.2), and in SG this indicator was 37.2 (22.2; 71.1). Anti-inflammatory cytokine IL-10 was 48.15 (33.1; 79.4) in HS and 54.6 (38.1; 97.6) in SG. The results of the obtained cytokine analysis confirm that the activity of liver inflammation level in SG compared to HS in NAFLD was assessed and substantiated. Cytokines studied were found to be unrelated to levels of liver fibrosis. To assess the functional state of the liver, it was proved that cytolysis and cholestasis parameters are in a correct correlation (TNF-α and ALT).

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