

IL-1 β AND IL-10: DIAGNOSTIC AND PROGNOSTIC POTENTIAL OF CYTOKINES IN THE ASSESSMENT OF PROGRESSION OF NON-ALCOHOLIC FATTY LIVER DISEASE IN PATIENTS WITH HYPERTENSION

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Abstract

Non-alcoholic fatty liver disease (NAFLD) affects about a quarter of the world's population and it is closely linked to hypertension (HT). Pro-inflammatory and anti-inflammatory cytokines play a key role in the pathology progression, and the search for non-invasive biomarkers for the diagnosis of NAFLD remains an important issue.

The aim of the study was to determine the diagnostic and prognostic value of IL-1 β and IL-10 in assessing the progression of liver parenchyma changes in patients with NAFLD and HT comorbidity.

Materials and methods. A study of 115 patients with non-alcoholic steatohepatitis (NASH) was performed. The main group consisted of 63 patients with NASH and HT, 52 patients with isolated NAFLD represented the comparison group. Clinical and laboratory parameters were evaluated, IL-10 and IL-1 β levels were measured by ELISA method, ultrasound steatometry and elastography were performed in all patients.

Results. The attenuation coefficient and median liver stiffness in NAFLD and HT group significantly exceeded the results in the isolated NAFLD group and in the control group. The IL-1 β level in NAFLD and HT group was 17.55 pg/ml, and in isolated NAFLD group the indicator averaged 15.72 pg/ml, which exceeded the control values (8.26 pg/ml). IL-10 level was 12.69 pg/ml and 14.34 pg/ml in patients with comorbid and isolated NAFLD, respectively, while control results averaged 16.19 pg/ml. It were found strong relationship between IL-1 β , IL-10 and CRP levels in patients with NAFLD and HT ($r=0.61$, $p=0.024$, and $r=-0.69$, $p=0.036$, respectively). Inverse correlations were also found between the cytokines IL-1 β and IL-10 in NAFLD patients with and without HT ($r=-0.61$, $p<0.001$, and $r=-0.57$, $p<0.001$, respectively). Changes in the cytokine status of patients with NAFLD at different stages of steatosis and liver fibrosis had been identified.

Conclusions. The presence of concomitant HT in patients with NAFLD is associated with greater severity of liver parenchyma changes. NAFLD manifestation is accompanied by increase of IL-1 β and decrease of IL-10 levels, and deepening of these deviations were found in patients with comorbidity of NAFLD and HT.

Interleukins IL-1 β and IL-10 can be defined as biomarkers of NAFLD progression both in its isolated course and in its comorbidity with HT. The possibility of using biomarkers as an independent non-invasive test of diagnosing NAFLD requires further study.

Keywords: kallistatin, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, hypertension, IL-10, IL-1 β .

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1. Introduction

Non-alcoholic fatty liver disease is the most common cause of chronic liver dysfunction. The prevalence of the disease worldwide averages about 25 % with wide variations depending on the region [1, 2]. The disease covers a wide range of histological pathologies from simple steatosis to non-alcoholic steatohepatitis (NASH), which affects 1.5–6.45 % of the population, liver fibrosis that develops in more than 40 % of patients with NASH, liver cirrhosis and hepatocellular carcinoma [3].

The probable role of NAFLD as a risk factor for cardiovascular disease remains a debatable question, so special attention is paid to the comorbidities of fatty liver and disorders of the cardiovascular system [1, 4]. In particular, the combination of NAFLD and hypertension (HT) is

an important issue, as NAFLD affects about 50 % of patients with high blood pressure [5]. The results of studies indicate a bidirectional relationship between HT and fatty liver infiltration through common pathogenetic links, such as systemic inflammation, obesity, disorders of lipid and carbohydrate metabolism and oxidative stress. These data form the basis for considering the disease as equal and interdependent components of the metabolic syndrome [6, 7].

NAFLD is known to be associated with inflammatory processes in the liver, and cytokines play an important role in its pathogenesis. In recent years, the importance of interleukin-1 β (IL-1 β) in the development of NAFLD has been actively studied. It is known that its activity increases in patients with NASH and correlates with the severity of fatty infiltration of the liver [8–10].

At the same time, there is evidence that interleukin-10 (IL-10) is involved in various biochemical processes as an anti-inflammatory cytokine. It is produced to protect against excessive immune response, limits organ damage, in particular, plays a protective role in liver fibrogenesis and prevents the progression of hepatic steatosis. [11–13]. In the liver, IL-10 was detected in hepatocytes, stellate cells, and Kupffer cells, but only a few studies have been performed to investigate the role of endogenous IL-10 in the progression of NAFLD [14]. Cytokine IL-10 levels have been shown to be significantly reduced in patients with metabolic syndrome and chronic liver disease [14, 15].

Characteristic changes in cytokine levels are also observed in patients with persistently elevated blood pressure. Research suggests that hypertension is associated with increased production of pro-inflammatory cytokines, including IL-1, which may be considered a potential mediator of inflammatory complications of hypertension [16]. In the meanwhile, IL-10 exhibits protective activity under conditions of hypertension, as it counteracts both the pressor activity of Angiotensin II-mediated reactions and vascular dysfunction associated with hypertension [17].

Various studies suggest that patients with NAFLD have an imbalance of pro-inflammatory and anti-inflammatory cytokines, and its severity increases with the progression of changes in the liver parenchyma [11]. Therefore, the determination of pro-inflammatory and anti-inflammatory interleukins activity can play a significant role in the diagnosis of NAFLD progression, in particular, in combination of NAFLD and HT.

The aim of our study was to determine the diagnostic and prognostic value of IL-1 β and IL-10 in assessing the progression of liver parenchyma changes in patients with NAFLD and HT comorbidity.

2. Materials and methods

The research was conducted on the basis of the Department of Internal Medicine No. 1 of Kharkiv National Medical University from September 1, 2018 to January 31, 2020. The study was approved by the Ethics and Bioethics Commission of Kharkiv National Medical University (protocol No. 8 on October 03, 2018). The experimental part of this study respects the ethical standards in the Helsinki Declaration of 1975, as well as the principles of Good Clinical Practice (GCP) and the national law. All patients voluntarily participated in the study and signed the Consent to Participate in a Research Study.

We observed 115 patients with NAFLD at the stage of non-alcoholic steatohepatitis (NASH). Among the examined patients were 57 men and 58 women aged 38 to 59 years ($M = 48.4$, 95 % CI [47.4, 49.3]). Patients were divided into two groups depending on the presence of concomitant hypertension. The main group ($n = 63$) consisted of 32 men and 31 women aged 38 to 59 years ($M = 48.4$, 95 % CI [47.2, 49.6]) with NAFLD on the background of HT, and the comparison group ($n = 52$) consisted of 25 men and 27 women aged 39 to 59 years ($M = 48.3$, 95 % CI [46.8, 49.8]) with isolated NAFLD. The groups had no differences in age ($p = 0.908$), gender composition ($p = 0.772$).

The control group ($n = 20$) consisted of 12 women and 8 men aged 38 to 56 years ($M = 47.1$, 95 % CI [45.1, 49.1]), that were almost healthy volunteers, randomized by age ($p = 0.394$; $p = 0.555$) and sex ($p = 0.400$; $p = 0.538$).

The NASH was diagnosed in previous stages of the study based on laboratory and instrumental survey data according to the guidelines EASL-EASD-EASO, 2016. The diagnosis of HT was also confirmed in previous stages of the study based on the criteria of clinical guidelines for hypertension (ESH/ESC) 2018).

All patients were calculated body mass index (BMI) according to the conventional formula: BMI = body weight (kg)/height (m²), and the values of office systolic (CAT) and diastolic (DAT) blood pressure were determined using a BP tonometer AG1-40 (Microlife AG, Switzerland) in accordance with current recommendations (ESH/ESC).

Plasma levels of interleukin-1 β and interleukin-10 were determined by enzyme-linked immunosorbent assay using the Human IL-1 β (Interleukin 1 Beta) ELISA Kit (Elabscience, USA) and the Human IL-10 (Interleukin 10) ELISA Kit (Elabscience, USA) in accordance. The level of the marker of the acute phase of inflammation of C-reactive protein (CRP) was determined using a highly sensitive method (hs-CRP ELISA) (Biomerica, USA).

Evaluation of steatosis and liver fibrosis was performed using an ultrasound scanning system Soneus P7 (Ultrasign, Ukraine). The ultrasound steatometry was performed using the «AC» mode, the liver fat was quantified by measuring the value of the linear attenuation coefficient (AC; dB/cm) of ultrasound in the liver parenchyma with the following staging of fatty hepatosis.

The degree of fatty infiltration of the liver was evaluated on a validated scale (NAS – NAFLD activity score) and the Soneus P7 correspondence table (Table 1).

Table 1

Correspondence of Soneus P7 measurement results and steatosis stage according to NAS

Attenuation coefficient (AC-Medin, dB/cm)	NAS	Steatosis stage	Hepatocytes with macrovesicular steatosis, %
1.0–2.19	S0	No steatosis	from 0 to 5
2.2–2.29	S1	Mild steatosis	> 5 to 33
2.3–2.9	S2	Moderate steatosis	> 33
2.9–3.5	S3	Severe steatosis	> 66

Shear wave elastometry and elastography were performed in the «SE» mode using a convex sensor 2–5 MHz with concordance determination of the liver stiffness (LS; kPa or m/s) to the fibrosis and cirrhosis stages according to the METAVIR scale (Castera et al.) [18] and the Soneus P7 correspondence table (Table 2).

Table 2

Correspondence of Soneus P7 measurement results and liver fibrosis stages according to METAVIR scale

The median liver stiffness (LS-Median, kPa)	METAVIR score	Fibrosis stage	Morphologic features
2.5–6.0	F0	No fibrosis	No fibrosis
6.0–7.0	F1	Weak fibrosis	Star-shaped expansion of portal tracts by fibrosis without septal formation
7.0–9.5	F2	Mild fibrosis	Portal tracts expansion with single porto-portal septa (> 1 sept)
9.5–12.5	F3	Severe fibrosis	Numerous port-central septa
12.5–60	F4	Cirrhosis	Cirrhosis

The obtained results were statistically processed using computer programs «Excel 2019» (Microsoft), «STATISTICA 8.0» (StatSoft Inc.). Continuous values were presented as mean (M) or median (Me) according to the sample of the law of normal distribution and confidence intervals (CI) with a given reliability $\gamma = 0.95$ (95 % CI). The statistical significance of the differences between the numbers of results in the groups was confirmed by Pearson's χ^2 test. Significant differences between the levels of parameters in the two groups were determined using the Mann-Whitney U-test.

The relationships between the results were determined using Spearman's rank correlation coefficient (r), and the correlation was assessed according to the Evans scale [19]. The results of all statistical analysis procedures were considered reliable at a significance level of $p < 0.05$.

3. Results

Anthropometric examination of patients showed that BMI in the groups with NAFLD and HT comorbidity and isolated NAFLD had no significant differences, but in both groups significantly exceeded similar control values. Blood pressure values differed significantly between groups (**Table 3**).

Table 3
Clinical and laboratory characteristics of the examined patients

Indicator	NAFLD+HT (n = 63)	NAFLD (n = 52)	Control group (n = 20)	Reliability between groups
Age, years	48.4, 95 % CI [47.2, 49.6]	48.3 95 % CI 46.8, 49.8)	47.1, 95 % CI [45.1, 49.1]	$p_{1-2} = 0.908$ $p_{1-3} = 0.394$ $p_{2-3} = 0.555$
BMI, kg/m ²	26.9, 95 % CI [24.45, 29.34]	25.1, 95 % CI [25.38, 26.56]	22.7, 95 % CI [22.41, 23.46]	$p_{1-2} = 0.477$ $p_{1-3} < 0.001$ $p_{2-3} < 0.001$
SBP, mm Hg	140, 95 % CI [137.86, 140.55]	120, 95 % CI [120.83, 122.24]	122.50, 95 % CI [121.94, 126.56]	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} = 0.059$
DBP, mm Hg	85, 95 % CI [82.72, 86.17]	70, 95 % CI [70.54, 73.30]	75, 95 % CI [73.75, 79.25]	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} = 0.004$
IL-1 β , pg/ml	17.55, 95 % CI [17.06, 19.73]	15.72, 95 % CI [15.25, 17.44]	8.26, 95 % CI [7.79, 8.46]	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} < 0.001$
IL-10, pg/ml	12.69, 95 % CI [11.93, 12.95]	14.34, 95 % CI [13.27, 14.34]	16.19, 95 % CI [15.15, 17.74]	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} < 0.001$
CRP, mg/l	7.90, 95 % CI [7.96, 8.75]	6.55, 95 % CI [6.47, 7.57]	2.07, 95 % CI [1.83, 2.85]	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} < 0.001$

Note: $p < 0.05$ – the difference is statistically significant between groups; p_{1-2} – the difference between the NAFLD+HT group and the isolated NAFLD group; p_{1-3} – the difference between the NAFLD+HT group and the control group; p_{2-3} – the difference between the isolated NAFLD group and the control group

During the study of cytokine status, it was found that IL-1 β levels in patients with comorbidity of NAFLD on the background of HT significantly exceeded the corresponding values in the group with isolated NAFLD, and in general the level of inflammatory marker in patients of the main group and comparison group exceeded control values 2.1 and 1.9 times, respectively. At the same time, the level of the anti-inflammatory biomarker IL-10 in patients with isolated NAFLD significantly exceeded similar indicator of patients with a combination of NAFLD and HT, and anti-inflammatory activity of cytokine was higher in the control group in comparison with all patients with NAFLD (**Table 3**).

Significant differences in the indicators of steatosis and liver fibrosis in patients with comorbidity and isolated course of NAFLD were revealed, as well as the indicators differed significantly from the control results.

According to the results of ultrasound steatometry, the attenuation coefficient in NAFLD and HT group averaged 2.55 dV/cm (95 % CI [2.50, 2.65]), that was 1.13 and 1.4 times greater, than in isolated NAFLD patients (2.24 dV/cm (95 % CI [2.31, 2.43], $p < 0.001$) and in the control group (1.82 dV/cm (95 % CI [1.72, 1.90], $p < 0.001$), respectively (**Fig. 1**).

The average liver stiffness in patients with comorbidity of NAFLD and HT was 7.24 kPa (95 % CI [7.09, 7.84], which significantly exceeded the results in the isolated NAFLD group (5.97 kPa (95 % CI [5.61, 6.53], $p < 0.001$) and in the control group (5.02 kPa (95 % CI [4.94, 5.21], $p < 0.001$) (**Fig. 2**).

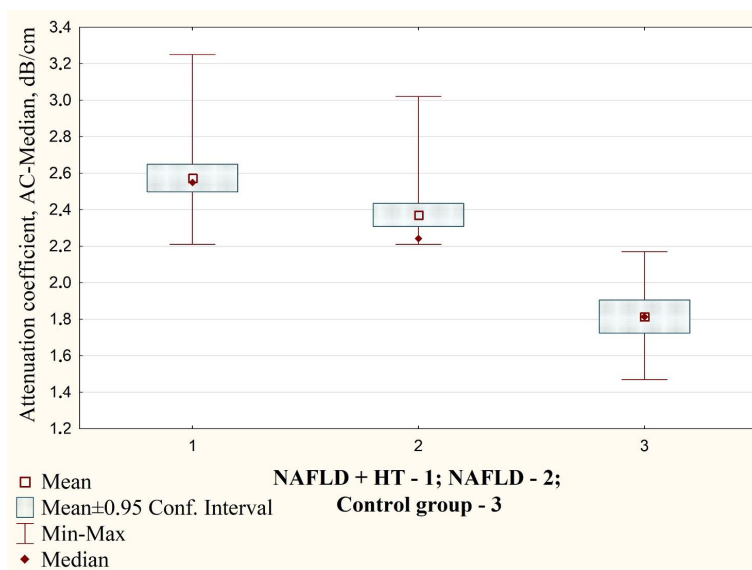


Fig. 1. The results of ultrasound steatometry: the value of the linear attenuation coefficient in the examined patients

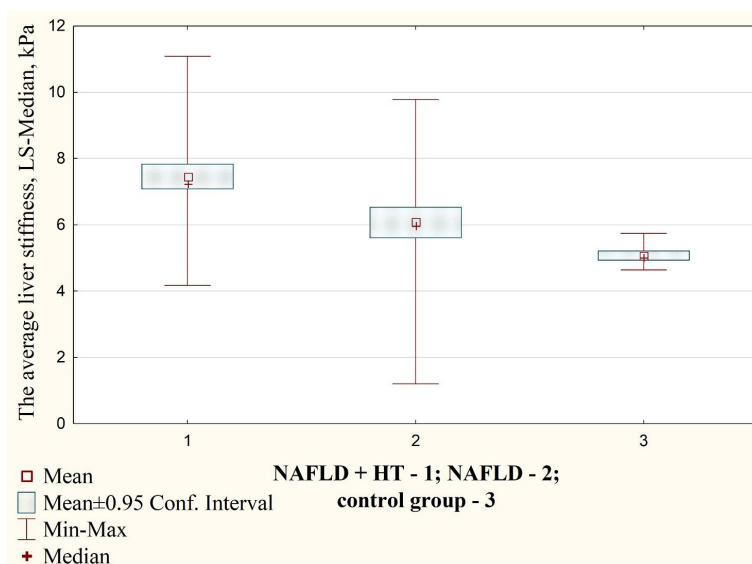


Fig. 2. The results of shear wave elastometry: the value of the average liver stiffness in the examined patients

Ranging of liver steatosis and fibrosis in the examined patients according to the NAS and METAVIR scales revealed that severity of fatty infiltration of liver parenchyma ($p = 0.005$) and fibrotic liver changes severity ($p < 0.001$) increases in conditions of accession HT to NAFLD. However, the number of patients at the stage of F3 fibrosis in both groups did not differ, which indicates an inessential effect of high blood pressure on severe liver fibrosis development (**Table 4**).

Evaluation of the correlations between cytokines and the marker of acute inflammation revealed a strong direct relationship between the content of IL-1 β and CRP and a strong inverse relationship between the level of IL-10 and CRP in patients with NAFLD and HT ($r = 0.61$, $p = 0.024$ and $r = -0.69$, $p = 0.036$, respectively) (**Fig. 3**).

In the group with isolated NAFLD, inverse correlations between IL-10 and CRP were considered as weak ($r = -0.30$, $p = 0.029$), and no statistically significant interaction between IL-1 β and CRP was found ($p = 0.295$), which proves the significance of concomitant hypertension in processes of systemic and local inflammation in patients with NAFLD.

Table 4

Distribution of examined patients according to the stages of steatosis and liver fibrosis

Steatosis and fibrosis stages	NAFLD and HT (n = 63)		NAFLD (n = 52)		Reliability of difference (Pearson's χ^2 test results)	
	n	%	n	%		
Steatosis stages						
S1	20	31.75	29	55.77	$p = 0.010$	$p = 0.005$
S2	27	42.86	20	38.46	$p = 0.634$	
S3	16	25.40	3	5.77	$p = 0.005$	
Fibrosis stages						
F0	4	6.35	21	40.38	$p < 0.001$	$p < 0.001$
F1	20	31.75	18	34.61	$p = 0.745$	
F2	33	52.38	12	23.07	$p = 0.002$	
F3	6	9.52	1	1.92	$p = 0.090$	

Note: $p < 0.05$ – the difference is statistically significant between groups

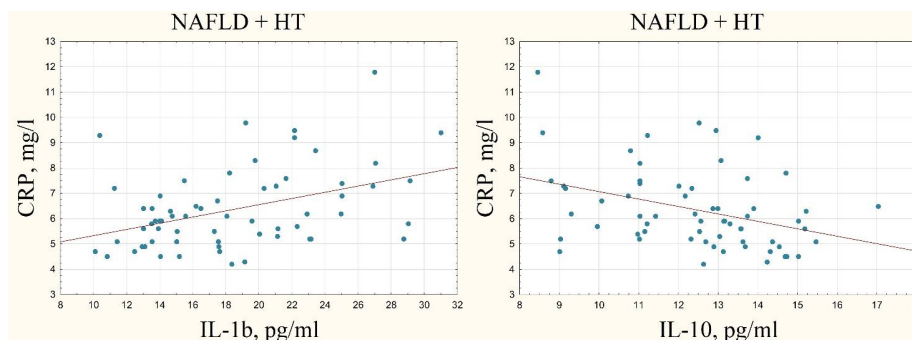


Fig. 3. Correlations between cytokine levels and C-reactive protein content in patients with NAFLD and HT

Strong and moderate inverse correlations were also found between the cytokines IL-1 β and IL-10 in NAFLD patients with and without concomitant hypertension ($r = -0.61$, $p < 0.001$, and $r = -0.57$, $p < 0.001$, respectively) (Fig. 4).

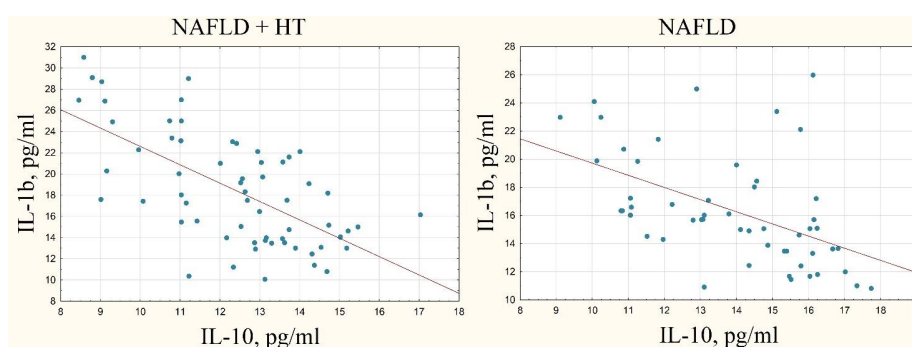


Fig. 4. Correlations between cytokine levels in both groups of patients with NAFLD

Analysis of the biomarkers levels by stages of hepatic steatosis among the examined patients revealed that pro-inflammatory activity of IL-1 β was significantly increased with the progression of steatosis in patients with a combination of NAFLD and HT. However, the IL-1 β level increased statistically in patients with isolated NAFLD only during the progression of steatosis from stage S1 to S2 (Table 5).

IL-10 levels also decreased significantly with increasing fatty liver infiltration severity in patients with NAFLD on the background of HT, which indicates a decrease in anti-inflammatory

activity of plasma proteins in these conditions. The obtained results suggest a decrease in anti-inflammatory activity with a simultaneous increase in the pro-inflammatory effect of plasma proteins with the progression of hepatic steatosis, especially with the additional effect of HT on the NAFLD course.

Ranging of patients by liver fibrosis stages in the analysis of plasma biomarker levels revealed an increase in the IL-1 β activity with a sharp decrease in the content of IL-10 starting from stage F2 by METAVIR classification. In the group of isolated NAFLD patients, statistically significant differences were observed only between the stages of liver fibrosis F2 and F3, which may indicate an additional effect of HT on the severity of anti-inflammatory and pro-inflammatory activity of biomarkers in NAFLD (Table 6).

Table 5

Biomarker levels in the examined patients depending on the hepatic steatosis stage according to the NAS classification

Indicator	Steatosis stage	NAFLD and HT (n = 63)	Reliability between groups	Steatosis stage	NAFLD (n = 52)	Reliability between groups
IL-1 β , pg/ml	S1 (n = 20)	14.35, 95 % CI [14.09, 17.45]	$p_{1-2} = 0.041$ $p_{1-3} < 0.001$	S1 (n = 29)	14.62, 95 % CI [13.74, 16.34]	$p_{1-2} = 0.006$ $p_{1-3} = 0.196$
	S2 (n = 27)	18.21, 95 % CI [15.92, 19.73]	$p_{2-3} = 0.014$	S2 (n = 30)	16.49, 95 % CI [16.06, 19.21]	$p_{2-3} = 0.523$
	S3 (n = 16)	21.96, 95 % CI [19.71, 25.60]		S3 (n = 3)	23.00, 95 % CI [2.04, 38.63]	
IL-10, pg/ml	S1 (n = 20)	13.60, 95 % CI [12.57, 14.19]	$p_{1-2} = 0.030$ $p_{1-3} = 0.002$	S1 (n = 29)	15.32, 95 % CI [14.23, 15.54]	$p_{1-2} < 0.001$ $p_{1-3} = 0.800$
	S2 (n = 27)	12.51, 95 % CI [11.82, 13.19]	$p_{2-3} = 0.036$	S2 (n = 30)	11.74, 95 % CI [11.54, 13.39]	$p_{2-3} = 0.390$
	S3 (n = 16)	11.12, 95 % CI [10.03, 12.18]		S3 (n = 3)	16.12, 95 % CI [3.33, 24.83]	

Note: $p < 0.05$ – the difference is statistically significant between groups; p_{1-2} – the difference between patients with hepatic steatosis in stages S1 and S2; p_{1-3} – the difference between patients with hepatic steatosis in stages S1 and S3; p_{2-3} – the difference between patients with hepatic steatosis in stages S2 and S3

Table 6

Interleukin levels in the examined patients depending on the liver fibrosis stage according to METAVIR

Indicator	Fibrosis stage	NAFLD and HT (n = 63)	Reliability between groups	Fibrosis stage	NAFLD (n = 52)	Reliability between groups
IL-1 β , pg/ml	F0 (n = 4)	13.26, 95 % CI [6.78, 23.72]	$p_{1-2} = 0.561$ $p_{1-3} = 0.179$	F0 (n = 21)	15.02, 95 % CI [13.60, 16.96]	$p_{1-2} = 0.757$ $p_{1-3} = 0.009$
	F1 (n = 20)	14.34, 95 % CI [13.88, 17.69]	$p_{1-4} = 0.014$ $p_{2-3} = 0.011$	F1 (n = 18)	15.00, 95 % CI [13.94, 16.85]	$p_{1-4} = 1.000$ $p_{2-3} = 0.014$
	F2 (n = 33)	18.34, 95 % CI [17.10, 20.14]	$p_{2-4} < 0.001$ $p_{3-4} < 0.001$	F2 (n = 12)	18.25, 95 % CI [16.39, 21.57]	$p_{2-4} = 1.000$ $p_{3-4} = 1.000$
	F3 (n = 6)	27.89, 95 % CI [25.77, 30.18]		F3 (n = 1)	24.11, 95 % CI [24.11, 24.11]	
IL-10, pg/ml	F0 (n = 4)	14.34, 95 % CI [12.14, 15.87]	$p_{1-2} = 0.333$ $p_{1-3} = 0.087$	F0 (n = 21)	14.75, 95 % CI [13.88, 15.61]	$p_{1-2} = 0.382$ $p_{1-3} = 0.013$
	F1 (n = 20)	13.43, 95 % CI [12.51, 14.07]	$p_{1-4} = 0.014$ $p_{2-3} = 0.045$	F1 (n = 18)	14.73, 95 % CI [13.36, 15.12]	$p_{1-4} = 1.000$ $p_{2-3} = 0.024$
	F2 (n = 33)	12.56, 95 % CI [11.70, 12.90]	$p_{2-4} < 0.001$ $p_{3-4} < 0.001$	F2 (n = 12)	11.07, 95 % CI [10.61, 13.92]	$p_{2-4} = 1.000$ $p_{3-4} = 1.000$
	F3 (n = 6)	8.91, 95 % CI [8.20, 10.19]		F3 (n = 1)	10.05, 95 % CI [10.05, 10.05]	

Note: $p < 0.05$ – the difference is statistically significant between groups; p_{1-2} – the difference between patients with F0 and F1 liver fibrosis; p_{1-3} – the difference between patients with F0 and F2 liver fibrosis; p_{1-4} – the difference between patients with F0 and F3 liver fibrosis; p_{2-3} – the difference between patients with F1 and F2 liver fibrosis; p_{2-4} – the difference between patients with F1 and F3 liver fibrosis; p_{3-4} – the difference between patients with F2 and F3 liver fibrosis

Determination of the relationship between cytokines and indicators of the liver parenchyma in patients with comorbid NAFLD showed direct moderate correlations between IL-1 β and AC-Median ($r = 0.53, p < 0.001$) and the LS-Median ($r = 0.53, p < 0.001$), while in the group with isolated NAFLD the corresponding correlations were weak ($r = 0.39, p = 0.005$, and $r = 0.30, p = 0.029$, respectively). At the same time, the correlations between the anti-inflammatory cytokine IL-10 and the indicators of liver parenchyma changes were inverse and moderate in patients with NAFLD and HT ($r = -0.43, p < 0.001$ with AC-Median and $r = -0.58, p < 0.001$ with LS-Median), and in the isolated NAFLD group they were less pronounced ($r = -0.44, p = 0.01$ and $r = -0.39, p = 0.04$, respectively).

4. Discussion

Our findings on the relationship between cytokines and liver parenchymal status confirm the data of Nelson J., Bocsan, I. et al. that pro-inflammatory cytokines levels, such as IL-1 β , are elevated in non-alcoholic steatohepatitis [9, 14, 20, 21]. The literature also suggests that its high levels contribute to the development of hepatic steatosis and fibrosis of the liver parenchyma, and low levels of IL-10 allow to produce pro-inflammatory cytokines and cause chronic inflammation [8, 9, 11].

The data indicate that the cytokine IL-10 levels decreases with deepening of steatosis changes according to NAS classification in the comorbidity of NAFLD and HT group. However, in patients with isolated NAFLD IL-10 decreases only in the transition of steatosis from stage S1 to S2, and even slightly increases in patients with stage S3 steatosis.

These ambiguous results regarding the anti-inflammatory activity of IL-10 among the examined patients partially confirm the opinion of different authors that the cytokine IL-10 exhibits different phenotypic effects.

Thus, Saraiva M. et al. investigated that its antifibrotic activity is detected only at the beginning of the disease, and with the progression of NAFLD this cytokine can stimulate irreversible changes in the liver parenchyma due to the complexity of the fibrogenesis process and different effects of IL-10 [15]. Steen E. H. et al. also concluded that the anti-inflammatory effects of IL-10 are more pronounced in acute inflammation, and during chronic processes the cytokine can lead to tissue damage and deepen inflammatory changes [13]. These data confirm our results, which are associated with an unexpected increase in IL-10 in patients with NAFLD at the stage of steatosis S3.

Study limitations. Patients with other diffuse and focal liver diseases (alcoholic fatty liver disease, viral hepatitis, liver cirrhosis, etc.), HT III stage, 3 grade, symptomatic HT, coronary heart disease, diabetes mellitus and other endocrine pathologies, oncological diseases, systemic connective tissue diseases, acute and chronic internal inflammatory diseases were not included in study. We also didn't study the characteristics of cytokines in patients with NAFLD at the stage of severe fibrosis (F4), cirrhosis and hepatocellular carcinoma.

Prospects for further research. Therefore, despite certain inverse correlations between IL-10 and acute inflammatory markers and indicators of the liver parenchyma conditions, the cytokine anti-inflammatory effect requires further more detailed study depending on the NAFLD history and other links of pathogenetic influence on metabolism in this category of patients.

5. Conclusions

The presence of concomitant HT in patients with NAFLD is associated with significantly greater severity of fatty infiltration and fibrotic changes in the liver parenchyma. This is evidenced by a significant decrease in the mild steatosis (S1) and fibrosis (F0) incidence with a corresponding increase in the number of cases represented by significant fatty (S3) and fibrotic (F2) changes in the liver in patients with NAFLD on the background of HT.

NAFLD manifestation is accompanied by a significant increased pro-inflammatory cytokine IL-1 β content and a decrease in the anti-inflammatory IL-10 concentration. In the meanwhile, the comorbidity of NAFLD and HT lead to a statistically significant deepening of these deviations.

The depth of change in cytokine status of patients with NAFLD and HT was also correlated with an increase in CRP concentration, which additionally emphasizes the importance of low-grade systemic inflammation in pathogenesis of the comorbid course of NAFLD.

The obtained data allow us to consider the interleukins IL-1 β and IL-10 as biomarkers of NAFLD progression both in its isolated course and in its comorbidity with HT. However, due to the complexity of pathogenetic processes, in particular, the bidirectional effect of anti-inflammatory cytokine IL-10, the possibility of using biomarkers as an independent non-invasive test of diagnosing NAFLD requires further study.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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