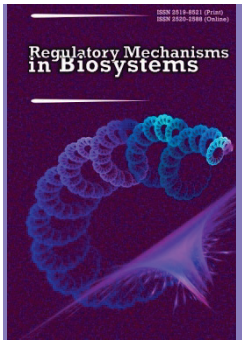

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Specificities of lipotoxicity of free fatty acids and cytokine profile in patients with chronic diffuse liver diseases

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Non-alcoholic fatty liver disease is an important cause of global liver disease characterized by diffuse hepatocytes with hepatocellular ballooning, intrahepatic inflammation and progressive fibrosis. A relevant task is the study of the relationship between content of free fatty acids and serum cytokine profile in patients with chronic diffuse liver diseases. A total of 74 people with chronic diffuse liver diseases were examined, including 32 patients with non-alcoholic fatty liver disease, 22 patients with alcoholic liver disease, 20 patients with toxic hepatitis. Chromatographic examination of free fatty acids (FFA) in blood serum was carried out using a Chromatek-Crystal 5000 gas chromatography system. Patients with chronic diffuse liver diseases had a significant increase in the level of unsaturated free fatty acids (USFA) in cases of toxic hepatitis (by 2.92 times, $P > 0.05$) and a decrease in the level of saturated free fatty acids (SFA) in cases of non-alcoholic fatty liver disease (by 1.52 times, $P > 0.05$) compared with the control group; the balance between omega-6 and omega-3 PUFA significantly changed due to increase in linoleic acid in patients with alcoholic liver disease and toxic hepatitis (by 1.91 and 2.11 times, respectively) and arachidonic acid in patients with toxic hepatitis (by 1.78 times). The level of interleukin (IL)-6, IL-10, tumor necrosis factor alpha (TNF-α) were determined. In patients suffering chronic diffuse liver diseases there were multidirectional changes in the composition of free fatty acids of blood serum: a significant increase in the level of USFA, levels IL-6 in toxic hepatitis; a decrease in the level of SFA, levels IL-6 and TNF-α during non-alcoholic fatty liver disease; increased TNF-α production, IL-6 during alcoholic liver disease compared with the control group. Significant change occurred in the balance between omega-6 and omega-3 PUFA due to increase in linoleic acid in cases of alcoholic liver disease and toxic hepatitis and arachidonic acid in cases of toxic hepatitis. The revealed correlations support the hypothesis that inflammation and lipotoxicity of FFA of blood serum contribute to the development and progression of structural changes in the liver. However, the pathomechanism of lipid metabolism and cytokine regulation with different etiological factors have their own characteristics, which should be taken into account when treating patients of these groups. Prospects for further research: these parameters may be used for serologic biomarkers of liver disease and development and implementation of the ratio between FFA and cytokines for the differential diagnosis of chronic diffuse liver disease in medical practice.

Keywords: alcoholic liver disease; toxic hepatitis; non-alcoholic fatty liver disease; saturated free fatty acids; unsaturated free fatty acids; interleukin.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is an important cause of global liver disease (Bellanti et al., 2018) characterized by diffuse hepatocytes with hepatocellular ballooning, intrahepatic inflammation and progressive fibrosis (Woods et al., 2017; Zhang et al., 2017; Younossi et al., 2018). Over the recent years, there has been a drastic increase in chronic diffuse liver diseases (CDLD). Among them, the most common and socially harmful is liver disease of viral etiology and metabolic origin, which makes the problem so relevant. NAFLD affects more than 40% of the global population. The progression of liver disease can be classified into simple steatosis, non-alcoholic steatohepatitis (NASH), fatty cirrhosis, cirrhosis and even liver cancer (Zavhorodnia et al., 2020). The mechanism for progression of NAFLD is considered to have at least two components. Accumulation of fat caused by lipid metabolism disorders is an inflammatory mechanism in hepatic steatosis, followed by immune cell activation and pro-inflammatory production of cytokines (Woods et al., 2017). It is proved that the pathogenesis of hepatic steatosis lies in abdominal obesity, insulin resistance, excessive production and circulation of free fatty acids, accumulation of triglycerides in hepatocytes, toxic bile acids. Increased lipolytic activity in adipose tissue leads to increased rate of fatty acid release into the plasma pool (Stepanov et al., 2018). The excess of free fatty acids (FFA) promotes the secretion of triglycerides in the form of very low-density lipoproteins and increases de novo lipogenesis in liver (Alves-Bezerra, 2018). Persistent excess of FFA not only participates in the pathogenesis of steatosis, but also leads to inflammatory reactions.

Insulin resistance, oxidative stress and inflammatory processes play a major role in the development of NAFLD. Day (1998) proposed a "two-hit model" that explains the pathogenesis of NAFLD and its progression to NASH. In this model, obesity, especially visceral, metabolic syndrome, type 2 diabetes mellitus (DM), increases the intake of FFA into the liver and develops hepatic steatosis. This is seen as "the first hit" at the same time, oxidative stress develops – "the second hit". The second hit requires the development of an inflammatory process, which is supported by a cytokine system with the secretion of large amounts of tumor necrosis factor-α (TNF-α). According to this view, the mechanism of NAFLD development is considered as a more complex process based on multiple and possibly simultaneous "hits" (multi–hit) that cause steatosis, oxidative stress and eventually apoptosis and hepatocellular fibrosis (Aitbaev, 2017; Fang, 2018). Among others, one of most important factor that affect the prognosis of NAFLD, the tactics and effectiveness of its treatment is the rate of progression of fibrous liver transformation (Sanyal, 2019). According to recent studies, the prevalence of liver fibrosis is increasing annually worldwide. Prevalence of NASH ranges from 1.5% to 6.5% (Caballería,

2018; Younossi, 2018). But the mechanisms that underlie the development of the disease and its progression need clarification.

The study of the initial mechanisms of liver lesion and inflammation resulting from toxic effects of excessive lipids is important. Recent data from around the world indicate that the total number of triglycerides preserving in liver cells are not a major contributor to lipotoxicity and that specific classes of lipids act as damaging agents. These lipotoxic species influence cell behaviour through a variety of mechanisms, including activation of death receptors, endoplasmic reticulum stress, modification of mitochondrial function and oxidative stress. FFA and glycerol are the end products of the breakdown of triglycerides. FFA are classified by the number of carbon atoms, the presence and position of different types of bindings that provide a specific biological activity and degree of pathogenicity. This concept has been confirmed by the fact that genetic or pharmacological inhibition of sterol-CoA-desaturase-1(SCD1), an enzyme that converts saturated fatty acids to monounsaturated fatty acids, is associated with hepatocyte apoptosis, lipotoxicity and the development of steatosis (Svegliati-Baroni G, 2019). Monounsaturated oleic acid may lead to liver steatosis, but it is less toxic than saturated FFA (SFA) such as palmitic and stearic acids. This confirms the claim that fat accumulation and lipotoxicity are not the same. In addition, monounsaturated lipids can prevent cell death by reducing the level of proaptotic proteins BIM (BCL2L11), PUMA (BBC3) and facilitating the sequestration of palmitic acid into triglycerides (Akazawa, 2010; Di Ciaula, 2021).

Normal constitutive generation of pro-inflammatory cytokines is absent or its level in the liver is minimal, whereas pathological stimuli (lipid accumulation) induce hepatic cells to produce these inflammatory molecules. Cytokines, especially TNF-α, play a central role in the development and progression of steatosis into NASH in NAFLD through the stimulation of hepatic inflammation, cell necrosis and apoptosis, as well as fibrosis induction (Fang, 2018; Raza, 2019). Promotion of lipid accumulation in hepatocytes induces persistent hepatic generation of pro-inflammatory cytokines (Tatarchuk et al., 2018), interleukin-6 (IL-6) concentration, which positively correlates with obesity, impaired glucose tolerance and insulin resistance (Fadeenko et al., 2019; Niederreiter & Til, 2018). TNF-α increases adipocyte lipolysis to released FFA. Conversely, FFA are an important factor that releases inflammatory cytokines from adipocytes (Lazebnik et al., 2019).

To date, there is fairly convincing evidence of the role of immune activation in the formation of the inflammatory process, both in the whole organism and in individual organs. The study of immunopathogenesis of inflammatory processes in the liver revealed impairment in the cellular link of immunity and a pronounced imbalance of cytokines at the whole body level (Gubergritz et al., 2019; Didenko et al., 2019). In the final stages of chronic diseases, elevated level of cytokines of different functional classes is associated with a damaging effect, namely, maintenance of local and systemic inflammation, apoptotic death of hepatocytes, the progression of fibrosis and the development of outside hepatic complications (Bedossa et al., 2017; Caballería et al., 2018).

Drug-induced liver injury accounts for 10% of all the side-reactions caused by the use of pharmacological drugs and it carries significant morbidity and mortality. The wide range of clinical manifestations, the lack of accurate diagnostic methods, often poor prognosis make drug-induced liver damage one of the biggest problems in clinical practice. In recent years, following the increasing expansion of the pharmaceutical market, there has been a clear upward trend in the number of toxic medication-associated hepatitis. One of the most common side effects associated with taking medications is hepatotoxicity (Sandhu, 2020). Direct hepatotoxicity occurs against the background of a hepatotoxic agent that causes death of necrosis or apoptosis. Most of the drugs that cause toxic damage are lipophilic and metabolize in the liver. Prolonged exposure to drugs can lead to hepatocyte dysfunction, necrosis and/or cell death. However, the mechanism of toxic damage involves a complex process involving the drug, its metabolites and the owner's immune system (EASL clinical practice guidelines, 2019). Drugs can interact with cytokines, resulting in cellular stress and liver damage (Hastings, 2020). *In vitro* studies have found synergy between cytokines and drugs that cause liver cell death. Many hepatitis-related drugs do not kill hepatocytes in clinically significant concentrations, but they become cytotoxic in the presence of non-toxic concentrations of TNFα or IFNγ (Maiuri, 2017; Hastings, 2020). Alcohol liver damage and its complications remain one of the most common causes of death in Europe and the USA, and the understanding of the epidemiology of alcoholic liver disease (ALD) as a global problem is still far from being solved. It is known that alcoholic liver damage is the result of a complex interaction between alcohol metabolism products, inflammatory and immune reactions, oxidative stress, fibrogenesis processes and hepatocyte regeneration disorders (Goldberg, 2017). In response to the influence of alcohol and its metabolites, a whole cascade of cytokines is released that triggers inflammation, fibrogenism and fibrolysis, resulting in excessive accumulation of extracellular collagen (Mannaa, 2016). The main cytokines that participate in the remodeling of the extracellular matrix are IL6 and TNF-α. They stimulate the formation of acute-phase proteins by hepatocytes, increase the expression of pro-inflammatory cytokines in macrophages, and cause infiltration by liver neutrophils (Niederreiter, 2018).

Study of specificities in the spectrum of FFA in patients with NAFLD, ALD, toxic hepatitis (TH) and imbalances in the profile of serum cytokines would reveal their role in the development and progression of the disease in patients with CDLD, as well as make it possible to recommend a treatment scheme for the identified disorders.

The objective of the study was the relationship between serum free fatty acids content and cytokine profile in patients with CDLD.

Materials and methods

The research was carried out following the bioethical norms according to the regulations of WMA, Helsinki Declaration of General Assembly of the World Medical Association (2013) – "Ethical principles for medical research involving human subjects", the current legislation of Ukraine, as confirmed by the Committee of Bioethics of the Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine. Informed consent was received from all the subjects of blood withdrawal. Blood was withdrawn at the same time in the morning from the vein of patients on an empty stomach in the amount of 10 mL; for this purpose, we used test tubes without anticoagulants, and blood samples from healthy donors were withdrawn in the same way.

74 patients with CDLD were examined, who were at the treatment hospital in the Department of the Liver and Pancreas Diseases at the Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine. All the patients were divided into 3 groups depending on nosology: I – NAFLD (32 patients); II – ALD (22 patients); III – TH (20 patients). Among the examined patients, 46% were women and 54% were men, their average age being (59.2 ± 2.4) years. The control group comprised 16 clinically healthy volunteer donors at the age of 25–52 years without history of liver diseases or other immune diseases. The diagnosis was verified on the basis of a thorough analysis of complaints, medical history, serological methods, ultrasound results, shear wave elastometry, liver steatometry and morphological studies, in accordance with the recommendations of international consensus.

Chromatographic examination of FFA in blood serum was carried out using a Chromatek-Crystal 5000 gas chromatography system according to the methods that have been described (Stepanov et al., 2018). Each free fatty acid fraction was identified according to the standards of methylated fatty acids from RESTEK. Over 20 fractions were identified in FFA blood serum spectrum. The content of saturated free fatty acids (SFA), unsaturated free fatty acids (USFA), polyunsaturated free fatty acids (PUFA), monounsaturated free fatty acids (MUFA), IAFFA of blood serum and ratios: k_1 – the ratio of stearic acid to oleic acid concentrations (C18:0/C18:1); k_2 – the ratio of palmitoleic acid to palmitic acid concentrations (C16:1/C16:0); k_3 – the ratio of stearic acid to palmitic acid concentrations (C18:0/C16:0) were calculated separately. Indicators were measured according to healthy people. IL-6, IL-10, TNF-α levels in the blood serum were determined by the immuno-enzyme method using Vector-BEST reagents, Russia. The studies were carried out according to the instructions for each test kit.

Statistical analysis of the results was performed using the software package Statistica 6.1 (StatSoft Inc., USA). The correspondence of the type of data distribution to the law of normal distribution was tested by

Shapiro-Wilk's method. To describe the extent of the central tendency of quantitative features, we used mean arithmetic (m) and standard error (SE), median (Me), 25% and 75% quartiles. To analyze the interrelation between the parameters, we used correlational analysis with consideration of coefficients of Spearman range correlation (r), correlation coefficient $P < 0.05$ indicates strong correlational relationship.

Results

The study of the spectrum of FFA in blood serum revealed changes in both the fractions and the content of individual fatty acids compared with the control. An increase in the content of fatty acids with the number of carbon atoms from 10 to 21 in the blood serum of patients with NAFLD was determined (C10:0–C21:0). It was found that the content of linoleic acid in patients of groups II and III increased by 1.91 and 2.11 times, respectively. There was a statistically significant increase in the content of acids, mainly saturated (palmitic) and monounsaturated (oleic) acids. This confirms the fact that these acids are easily deposited in the adipose tissue and are pathological fraction and markers of steatohepatitis in CDLD. Changes in spectrum of USFA were characterized by an accumulation of monoenic acids due to an increasing share of oleic acid (C18:1) and a decrease in the level of polyenic acids, which was probably due to the absence of arachidonic acid (C20:4) fraction in all groups of patients. There were multidirectional changes in the spectrum of FFA of blood serum, depending on the etiological factor. The development of CDLD was accompanied by a 1.37-fold decrease in content of FFA in group I; there was a tendency to decrease in group II; in group III there was a physiological norm compared with control (Table 1).

The observed changes were accompanied by a tendency towards decrease in the content of lipotoxic fractions, included in SFA: by 1.52 times in group I ($P > 0.05$); by 1.33 times in group II and by 1.34 – group III compared with the control group.

Increases in the content of USFA in blood serum were seen: 1.15 times ($P > 0.01$) in group I; 2.25 times in group II; 2.92 times ($P > 0.05$) in group III compared with the control group (Table 1).

Statistically significant increase in the content of MUFA in blood serum was observed in patients of group I and II: by 5.60 times ($P < 0.05$) and 9.65 times ($P < 0.05$), in patients of group III – by 10.57 times ($P < 0.05$) in comparison with control (Table 1). Due to the revealed imbalance in the spectrum of FFA, IAFFA was 1.66 times higher in patients of group III compared with the control group, while in patients of groups I and II, the indicator was within the physiological norm.

Table 1

Spectrum of free fatty acids of blood serum in patients with chronic diffuse liver diseases (x ± SE, g/L)

Biochemical indicator/acid	Groups			
	control indicator (n = 16)	I (n = 32)	II (n = 22)	III (n = 20)
FFA	3.53 ± 0.46	2.58 ± 0.30	3.38 ± 0.33	3.60 ± 0.50
∑ MUFA	0.079 ± 0.005[a]	0.442 ± 0.090[b]	0.762± 0.053[b]	0.835 ± 0.302[b]
∑ SFA	3.10 ± 0.45	2.04 ± 0.31	2.33 ± 0.29	2.31 ± 0.36
∑ USFA	0.473 ± 0.170	0.542 ± 0.100	1.062 ± 0.372	1.379 ± 0.391
∑ PUFA	0.397 ± 0.160	0.100 ± 0.039	0.300 ± 0.172	0.545 ± 0.130
IA_{FFA}	0.281 ± 0.081	0.291 ± 0.072	0.299 ± 0.067	0.466 ± 0.032
Palmitic acid (C16:0)	0.0019 ± 0,0010	0.026 ± 0.003	0.016 ± 0.005	0.011 ± 0.003
Oleic acid (C18:1)	0.007 ± 0.002	0.057 ± 0.019	0.077 ± 0.032	0.061 ± 0.027
Linoleic acid (C18:2)	0.0152 ± 0.0041	0.0070 ± 0.0001	0.0291 ± 0.0013	0.0320 ± 0.0021
Linolenic acid (C18:3)	0.137 ± 0.071	0.035 ± 0.025	0.042 ± 0.001	0.074 ± 0.004
Arachidonic acid (C20:0)	0.167 ± 0.114	0.132 ± 0.022	0.129 ± 0.031	0.297 ± 0.049

Note: different letters indicate values that differed one from another reliably within one line of the table according to the results of comparison using the Tukey test with Bonferroni correction.

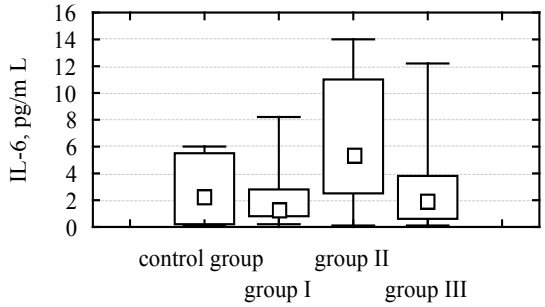

Fig. 1. Concentrations IL-6 in blood serum of the groups of healthy donors (control) and patients with chronic diffuse liver diseases: group I – patients with non-alcoholic fatty liver disease (NAFLD); group II – patients with alcoholic liver disease (ALD); group III – patients with toxic hepatitis (TH); small square – median, upper and lower borders of the rectangle – 25% and 75% quartiles, vertical line – minimum and maximum values, N = 74

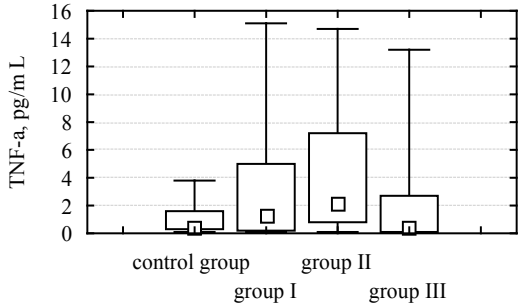

Fig. 2. Concentrations of TNF in blood serum of the groups of healthy donors (control) and patients with chronic diffuse liver diseases: group I – patients with non-alcoholic fatty liver disease (NAFLD); group II – patients with alcoholic liver disease (ALD); group III – patients with toxic hepatitis (TH); small square – median, upper and lower borders of the rectangle – 25% and 75% quartiles, vertical line – minimum and maximum values, N = 74

The development of CDLD was accompanied by an increased production of pro-inflammatory cytokines in blood serum (Fig. 1). For example, the IL-6 content in patients of group II significantly increased to 5.3 pg/mL compared with control values of 2.4 pg/mL. In addition, its content was significantly higher (by 3.13 times and 2.59 times, $P < 0.05$) than in patients of groups I and III, respectively. Similarly, the TNF-α level was the highest in patients of group II – 2.2 pg/mL (Fig. 2), being significantly higher (by 4.40 times and 4.89 times, $P < 0.05$) than the level in the control group and patients of group III, respectively.

Also, in patients of group I, the TNF-α concentration in blood serum was significantly higher (by 2.61 times and 2.89 times, $P < 0.05$) compared with control group and patients of group III, respectively. A correlation between the level of IL-6 and TNF-α ($r = 0.551$; $P < 0.01$), IL-10 – ($r = 0.416$; $P < 0.01$) was determined. Due to a certain imbalance in the level of pro-inflammatory and anti-inflammatory cytokines, the coefficient TNF-α/IL-10 was 8.67 times higher ($P < 0.05$) in group I patients and 10.86 times higher ($P < 0.05$) in group II patients compared with the control group (Fig. 3). In addition, TNF-α/IL-10 was significantly higher by 3.06 times and by 3.77 times in patients of groups I and II ($P < 0.05$) com-

pared with the values of group III. In patients with CDLD, disproportion in the ratio of TNF-α/IL-10 pro-inflammatory to anti-inflammatory cytokines was associated with an increase in toxic damage (r =0.390; P < 0.05), metabolic disorders (r = 0.41; P < 0.01).

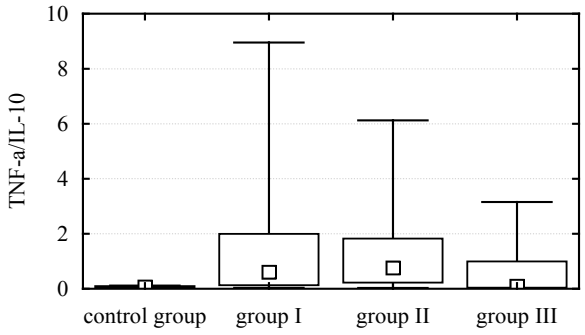

Fig. 3. The TNF-α/IL-10 ratio in blood serum of the groups of healthy donors (control) and patients with chronic diffuse liver diseases: group I – patients with non-alcoholic fatty liver disease (NAFLD); group II – patients with alcoholic liver disease (ALD); group III – patients with toxic hepatitis (TH); small square – median, upper and lower borders of the rectangle – 25% and 75% quartiles, vertical line – minimum and maximum values, N = 74

There was found a direct high correlation C20:0 with TNF-α (r = 0.886; P < 0.01), the ratios of TNF-α/IL-10 (r = 0.428; P < 0.05) and IL-6 (r = 0.645; P < 0.01) in patients with NAFLD, imbalance and disproportion of the ratio in the composition of serum FFA were closely associated with an increase in pro-inflammatory cytokines (Table 2). In patients with ALD a close correlation was found between an increased content of linolenic acid (C18:3), which is a member of the omega-3 PUFA family, a precursor for the synthesis of anti-inflammatory cytokines and IL-10 that play a protective role in liver damage mechanisms (r = 0.439; P < 0.05; Table 3).

It was found to have a direct correlation between linoleic acid (C18:2) and the ratio of TNF-α/IL-10 (r = 0.777; P < 0.01) in patients with TH. The IL-6 level also positively correlated with the TNF-α level, indicating their joint participation in the inflammatory process in patients with NAFLD, ALD, TH (Table 4).

The observed changes in individual FFA fractions are directly related to an increase in pro-inflammatory cytokines in patients with CDLD.

Based on correlation analysis in patients with NAFLD, the disproportion in ratios of FFA of blood serum had a close relationship with an increase in pro-inflammatory cytokines, in particular a direct relationship between arachidonic acid (C20:0) (which is a substrate for the production of eicosanoids), TNF-α (r = 0.886; P < 0.01) and IL-6 (r = 0.645; P < 0.01) was found (Fig. 4).

In our opinion, it is quite interesting that there was a correlation found in patients with ALD between increased level of linoleic acid (C18:2) and IL-10, which plays a protective role in the mechanisms of liver damage (r = 0.496; P < 0.05).

Table 2
Correlation relationship between free fatty acids fractions and cytokine profile in patients with non-alcoholic fatty liver disease (NAFLD, n = 32)

Indicator/acid	IL-6		IL-10		TNF-α		TNF-α /IL-10	
	r	P	*r*	P	*r*	P	*r*	P
Decanoic *(C10:0)*	–0.094	0.608	–0.092	0.617	0.014	0.940	0.392	0.027
Dodecanoic *(C12:0)*	0.404	0.022	0.106	0.565	0.409	0.020	0.374	0.013
Pentadecanoic *(C15:0)*	0.445	0.014	0.087	0.635	0.616	0.01	0.244	0.179
Palmitic *(C16:0)*	0.599	0.010	–0.010	0.959	0.260	0.151	0.490	0.004
Linolenic (*C18:3)*	0.619	0.011	0.201	0.269	0.804	0.01	0.435	0.037
Arachidonic *(C20:0)*	0.645	0.012	0.183	0.317	0.886	0.012	0.428	0.015

Table 3
Correlation relationship between free fatty acids fractions and cytokine profile in patients with alcoholic liver disease (ALD, n = 22)

Indicator/acid	IL-6		IL-10		TNF-α		TNF-α /IL-10	
	r	P	*r*	P	*r*	P	*r*	P
Myristic *(C14:0)*	–0.274	0.217	–0.187	0.404	0.009	0.968	0.634	0.002
Myristoleic *(C14:1cis-9)*	–0.148	0.512	–0.176	0.433	0.136	0.546	0.729	0.011
Pentadecanoic *(C15:0)*	–0.217	0.332	–0.026	0.908	0.478	0.025	0.680	0.010
Heptadecanoic *(C17:0 cis-10)*	0.247	0.268	–0.219	0.327	0.222	0.321	0.751	0.012
Oleic *(C18:1 cis-9)*	–0.014	0.951	0.206	0.357	0.667	0.001	0.355	0.105
Linolenic *(C18:3 cis-9)*	0.054	0.813	0.439	0.041	0.439	0.041	0.366	0.094
FFA	0.303	0.170	–0.003	0.989	0.656	0.001	0.284	0.204
USFA	0.094	0.678	0.010	0.965	0.532	0.011	0.420	0.052
k_2 *(C16:1/C16:0)*	0.298	0.245	0.605	0.010	0.267	0.303	–0.167	0.522

Table 4
Correlation relationship between free fatty acids fractions and cytokine profile in patients with toxic hepatitis (TH, n = 20)

Indicator/acid	IL-6		IL-10		TNF-α		TNF-α /IL-10	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Decanoic *(C10:0)*	0.308	0.119	0.135	0.582	0.567	0.011	–0.009	0.972
Undecanoic *(C11:0)*	–0.114	0.643	–0.150	0.583	0.593	0.007	0.138	0.572
Myristic *(C14:0)*	0.298	0.215	0.132	0.590	0.564	0.012	–0.015	0.951
Pentadecanoic *(C15:0)*	–0.156	0.523	–0.210	0.388	0.259	0.284	0.603	0.006
Palmitoleic *(C16:1cis-9)*	0.110	0.663	0.164	0.515	0.588	0.010	0.028	0.911
Heptadecanoic *(C17:0)*	0.434	0.063	0.022	0.928	0.629	0.040	0.038	0.876
Linolenic *(C18:3)*	–0.106	0.665	0.226	0.153	0.583	0.009	0.050	0.862
Linoleic *(C18:2)*	0.102	0.667	0.425	0.115	0.222	0.128	0.777	0.011
Arachidonic *(C20:0)*	–0.146	0.550	–0.196	0.422	0.053	0.875	0.088	0.720
SFA	–0.146	0.055	–0.532	0.019	0.289	0.230	0.521	0.022
PUFA	–0.134	0.557	0.100	0.693	–0.076	0.765	–0.132	0.602
k_1	0.184	0.450	–0.302	0.209	0.535	0.018	0.225	0.355

In TH, direct correlations were found between TNF-α/IL-10 ratio and contents of linoleic acid pentadecanoic acid, which is a member of the omega-3 family, and overall FFA (Fig. 5).

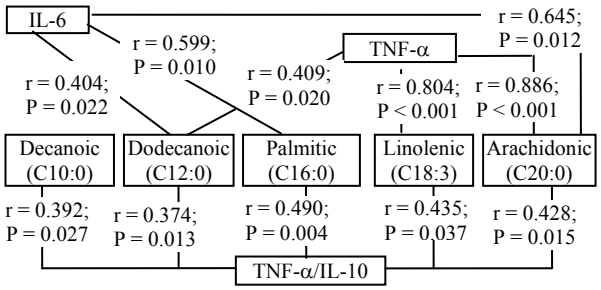

Fig. 4. Correlation relationship between free fatty acids fractions and cytokine profile in patients with non-alcoholic fatty liver disease (NAFLD)

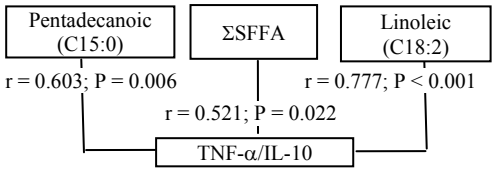

Fig. 5. Correlation relationship between free fatty acid and cytokine profile in patients with toxic hepatitis (TH)

Discussion

Pathogenesis of NAFLD in most cases is abdominal obesity, insulin resistance, and the excess circulation of free fatty acids with deposition of triglycerides in hepatocytes – all of these symptoms were observed in our patients. In fact, it is believed that the metabolic syndrome in patients with NAFLD is a condition of chronic inflammation with a continuously elevated level of pro-inflammatory cytokines. (Sandhu, 2020). Progression of CDLD chronic diffuse liver disease of any origin is characterized by a gradual substitution of a healthy parenchyma by connective tissue. Decrease in the number of functionally active cells leads to disorders of the blood biochemical composition, while accumulation of collagen and elastin changes the hepatic hemodynamics, hepatic stiffness and progression of fibrosis. In our study, we compared the lipid metabolism and cytokine profile in patients with chronic diffuse liver disease of various etiologies. (Didenko, 2017).

Thus, the absorption and impairment of fatty acids synthesis can affect lipid metabolism in the liver (Di Ciaula, 2021). The significant increase in pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β) contributed to the progression of the pathological process. (Boutari, 2018; Raza, 2019; Hastings, 2020). The use of gas chromatography today is the main method to determine the fatty acid composition of biological substrates. The advantage of this method is the speed of execution and high sensitivity of the method, so it is possible to detect the lowest concentrations of compounds in the test sample. Determination of the content of free fatty acids using a chromatograph allows detection of liver disease at an early stage of development and the detected violation to be corrected. Fatty acid composition of serum of patients with NAFLD is characterized by decrease in the total content of FFA due to deficiency of unsaturated fatty acids. In combination with morphological research methods, the use of long-chain FFA to diagnose diseases associated with impaired structure, function and viscosity of hepatocytes, opens the possibility to reveal the pathogenesis of CDLD in order to increase the differential diagnosis and the effectiveness of treatment of identified disorders (Perez-Cornago, 2014; Chen, 2015; Copaci, 2015). It can be seen that FFA play a key role in formation of NAFLD: on the one hand, fats are fully hydrolyzed, releasing fatty acids, approximately 20% of which get to the liver (Engin et al., 2017; Tatarchuk et al., 2018); on the other hand, accumulation of FFA in hepatocytes may cause lipotoxicity, leading to inflammatory reactions and damage to hepatocytes (Svegliati-Baroni, 2019). To achieve balance between liver and blood circulation, the liver absorbs more fatty acids into hepatocytes, which places an additional strain on liver function.

To date, quite convincing data have been obtained on the role of immune activation in the formation of the inflammatory process both at the level of the whole organism and in individual organs. The study of the immunopathogenesis of inflammatory processes in the liver revealed an impairment of the cellular immune system and a pronounced imbalance of cytokines at the level of the whole organism (Reccia, 2017; Fang, 2018). It is established that TNF-α is synthesized not only by macrophages, but also by adipocytes and stroma cells. The prevailing opinion is that TNF-α realizes its action mainly by the auto- and paracrine way. Its concentration in the tissues is hundreds of times higher than in blood. Its local effects are – reduction of adipose tissue sensitivity to insulin, stimulation of lipogenesis and growth of adipocytes. The highest level of TNF-α indicates significant changes in the cytokine balance for this disease, as it is known that the degree of increase in the content of this cytokine in blood serum correlates with the severity of this disease. In addition, the level of pathomorphological activity and biochemical transformations taking place in the body during an inflammatory process in the liver parenchyma should also be taken into account. (Reccia, 2017; Fang, 2018) If the result is favourable, the TNF-α concentration decreases, so the study of the concentration of this cytokine in blood serum in the dynamics can be used to assess the prognosis in combination with other indicators. (Boutari, 2018; Fang, 2018).

Increasing TNF-α level for a long time inhibits the activity of type 1 T-helpers (Th1) and, consequently, cell immune response. On the other hand, TNF-α is necessary for the proliferation of hepatocytes, prevents their apoptosis during liver regeneration, and on the other hand, it mediates hepatotoxicity during bacterial, viral and toxic influences. In addition, TNF-α can exert systemic effects by activating the synthesis of fatty acids and increasing their concentration in the blood, by inhibiting the secretion of adiponectin and regulating IL-6 production. Up to 30% of circulating IL-6 is synthesized by fat cells. Its secretion in visceral adipose tissue is several times higher than in the subcutaneous (Boutari, 2018; Fang, 2018; Hastings, 2020). Increased TNF-α production is supposed to play a particular role in pathogenesis of insulin resistance in the liver. Many researchers consider TNF-α as a mediator of IR in obesity. Involvement of the liver in immune responses, inflammation is an important aspect of the pathophysiology of NASH. It has been shown that TNF-α is able to enhance IR, activate lipogenesis in the liver and increase serum triglyceride levels, that is, to contribute to the main pathogenetic mechanisms of NAFLD/NASH (Engin, 2017).

It has been suggested that TH arises as a result of activation of immune T-cells, which leads to the secretion of cytokines with T-helpers CD4 and infiltration of T-cells CD8 (Sandhu, 2020). Recent evidence has shown that molecular mediators of the immune response, such as TNF-α, IL-1β and IL-6, cause acute and chronic liver damage (Mannaa, 2016; Caballería, 2018). These cytokines are released into the bloodstream from both the liver and distal sites during toxic liver damage. Moreover, the decrease in liver damage was accompanied by a significant decrease in the level of pro-inflammatory liver cytokines (TNF-α, IL-1β i IL-18), NF-κB, NLRP3, caspase-1 and proapoptic protein Bax (Ibrahim, 2020).

In patients with ALD, a significant increase in the level of IL-6 was found, which is known to be a pro-inflammatory mediator and hepatocyte growth regulator. This indicates an inflammatory process and contributes to steatohepatitis and fibrosis in patients with CDLD. The IL-6 level also positively correlated with the TNF-α level, indicating their joint participation in the inflammatory process in patients with NAFLD, ALD, TH. Consequently, an increased level of pro-inflammatory cytokines (IL-6 and TNF-α) in patients' blood does not induce the secretion of anti-inflammatory cytokines (IL-10), which leads to excessive activation of macrophages, maintenance of the inflammatory process and progression of CDLD. During the course of CDLD, serum lipid imbalance was revealed, which was expressed in increased SFA and decreased FFA, some specificities in the content of certain fractions in blood serum of patients with NAFLD, ALD, TH. Arachidonic acid (C20:0) is a substrate for the production of eicosanoids, pro-inflammatory cytokines. An imbalance in FFA content was closely related to increase in cytokines, as evidenced by the correlations described above. Thus, the pathomechanism for each disease is different.

Conclusions

The patients suffering CDLD had multidirectional changes in the composition of free fatty acids of blood serum: significant increase in the level of USFA in TH (by 2.92 times, $P < 0.05$) and decrease in the level of SFA in NAFLD (by 1.52 times, $P < 0.05$) compared with the control group; significantly changed balance between omega-6 and omega-3 PUFA as a result of increase in linoleic acid in ALD and TH (by 1.91 and 2.11 times, respectively) and arachidonic acid in TH (by 1.78 times).

The study revealed the activation of the pro-inflammatory cytokines synthesis in the form of increased TNF-α production against the background of dyslipidemia, which indicates the severity of the course of the disease. The TNF-α level was significantly higher than control values in NAFLD (by 2.61 times, $P < 0.05$) and ALD (by 4.40 times, $P < 0.05$). In blood serum, the levels of pro-inflammatory mediator and hepatocyte growth regulator IL-6 – significantly increased by 2.21 times ($P < 0.05$) in patients with ALD, by 3.13 times ($P < 0.05$) in patients with NAFLD and by 2.59 times ($P < 0.05$) in patients with TH compared with the control group.

Correlation analysis conducted in groups of patients with CDLD, depending on nosology, showed the presence of significant relationships between arachidonic acid and TNF-α ($r = 0.886$; $P < 0.01$); between an increased content of linolenic acid and IL-10 ($r = 0.496$; $P < 0.05$) in patients with ALD; while in patients with TH, its direct correlation with the TNF-α/IL-10 ratio ($r = 0.777$; $P < 0.01$) was found. The level of IL-6 also positively correlated with the level of TNF-α, indicating their joint participation in the inflammatory process in patients with NAFLD, ALD and TH.

The revealed correlations support the hypothesis that inflammation and lipotoxicity of FFA of blood serum contribute to the development and progression of structural changes in the liver. However, the pathomechanism of lipid metabolism and cytokine regulation with different etiological factors have their own characteristics, which should be taken into account when treating patients of these groups.

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