CHANGES IN LIPIDS IN PATIENTS WITH ALCOHOLIC CIRRHOSIS OF THE LIVER ASSOCIATED WITH NON-ALCOHOLIC FATTY LIVER DISEASE DEPENDING ON THE STAGE OF DECOMPENSATION

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Abstract. With the global growth of obesity, fatty liver, which is characteristic to alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD), becomes one of the most common among hepatic pathological changes throughout the world. Both ALD and NAFLD are associated with a lipid metabolism disorder. There are three main sources of excessive accumulation of lipids in the liver: increased lipolysis of visceral adipose tissue, accompanied by excessive intake of free fatty acids (FFA) from adipose tissue (59%), activation of de novo liver lipogenesis (26%) and high calorie and/or fat content in the diet (15%). Excessive input of FFA in adipose tissue leads to “overloading” of fat cells that are no longer able to contain such an amount of FFA and the accumulation of fat in other tissues of the body that is not adapted for such function – in the liver, pancreas, muscles, etc. Such ectopia and large amount of FFA in the body result in a decrease in insulin sensitivity and the development of lipotoxicity. The consequence of these processes is a disorder of the synthesis of adipokines.

The purpose of the research was to study the changes of lipids in patients with alcoholic liver cirrhosis (ALC) associated with NAFLD depending on the stage of decompensation. The study included 204 patients. Among them, 78 patients (Gr. I) had ALC and 126 patients (Gr. II) had a combination of ALC with NAFLD. Patients were subgrouped according to compensation classes by the Child-Pugh score (A, B, C). Diagnosis was verified using clinical and laboratory-instrumental methods in accordance with the order of the Ministry of Health of Ukraine No. 826 dated November 6, 2014, adapted clinical guidelines "Non-Alcoholic Fatty Liver Disease", 2014, adapted clinical guidelines "Alcoholic Liver Disease", 2014, adapted clinical guidelines "Liver Cirrhosis, 2017 (State Expert Centre of the Ministry of Health of Ukraine, Ukrainian Gastroenterology Association, Kyiv), recommendations of the European Association for the Study of Liver, Diabetes and Obesity (EASL-EASD-EASO, 2016).

Higher levels of total cholesterol, lipoprotein cholesterol of low and very low density, atherogenic coefficient and triacylglycerides were in patients of classes A and B. Patients of group II had higher rates than those in group I (p<0.05). The content of lipoprotein cholesterol of high density in patients of group II was significantly lower in comparison with patients in group I (p<0.05). With the progression of the liver cirrhosis the level of leptin decreased, while the levels of adiponectin increased. The higher level of leptin in patients of classes A and B is accompanied not only by the impaired liver function, but also by its increased release from adipose tissue. In patients of class C fat depot is exhausted, therefore the level of leptin decreases. Moreover, this decrease correlates with the severity of the disease and the prognostic MELD score. The level of adiponectin was lowered in class A patients and increased in patients with more severe course and correlated with severity of the disease and MELD score. The revealed correlation between the levels of leptin and adiponectin with the degree of severity of the liver cirrhosis and the prognostic MELD score allows considering their changes for assessment of the severity of the liver cirrhosis and predicting the course of the disease.

Keywords: alcoholic liver disease; non-alcoholic fatty liver disease; cirrhosis; leptin; adiponectin.

Introduction. With the global growth of obesity, fatty liver, which is characteristic to alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD), becomes one of the most common among hepatic pathological changes throughout the world [1]. Both diseases have a stage – from simple steatosis (accumulation of fat in hepatocytes) to steatohepatitis (inflammation with balloon dystrophy of liver cells that initiates fibrosis) which can progress to fibrosis, cirrhosis, liver failure or hepatocellular carcinoma [2]. Staging of the pathological condition is the result of complex interaction, which involves the population of the liver cells (parenchymal and nonparenchymal), and pathological signals coming from other organs such as fatty tissue and the gastrointestinal tract. Such stimuli include the death of hepatocytes, biologically active substances and intestinal pathogens secreted by adipose tissue which contribute to inflammation and fibrogenesis by activating macrophages (Kupffer cells), which, in turn, activate leukocytes and star cells (Ito cells, lipocytes) with subsequent excessive production of the extracellular matrix components [3].

Both ALD and NAFLD are associated with a lipid metabolism disorder. There are three main sources of...
excessive accumulation of lipids in the liver: increased lipolysis of visceral adipose tissue, accompanied by excessive intake of free fatty acids (FFA) from adipose tissue (59%), activation of de novo liver lipogenesis (26%) and high calorie and/or fat content in the diet (15%) [4]. Excessive input of FFA in adipose tissue leads to “overloading” of fat cells that are no longer able to contain such an amount of FFA and the accumulation of fat in other tissues of the body that is not adapted for such function – in the liver, pancreas, muscles, etc. [5]. Such ectopia and large amount of FFA in the body result in a decrease in insulin sensitivity and the development of lipotoxicity. The consequence of these processes is a disorder of the synthesis of adipokines [6].

Adiponectin and leptin are the most described adipokines. According to the literature, in patients with NAFLD the level of adiponectin decreases, and the level of leptin on the contrary – increases, which is due to metabolic processes in the adipose tissue [7]. Adiponectin is secreted entirely by adipose tissue and, to a lesser extent, by the placenta and it circulates in various isoforms: low molecular weight trimers, medium molecular weight hexamers and high molecular weight multimers [8, 9]. Known links of adiponectin influence are the stimulation of lipid oxidation in the liver, induction of receptor of proliferation activation of periloxisomes, inhibition of lipogenesis and transformation of macrophages into foam cells, regulation of catabolism of fatty acids, cleavage of fatty acids with further reducing of triacylglycerides, inhibition of preadipocytes differentiation, anti-inflammatory and antiatherosclerotic (regulation of calcification of arteries) properties, the effect on the cells of the hypothalamus with subsequent decrease in body weight, decrease in the synthesis of glucose by liver cells, increasing the sensitivity of cells to insulin; antioncogenic action is described [10, 11].

Leptin is also excreted primarily by adipose tissue, though its low levels are found in the placenta, skeletal muscles, epithelium of the stomach and mammary glands, in the brain, and, affecting the hypothalamus it suppresses the feeling of hunger, and thus controls body weight. However, in obesity and high leptinemia, there is a resistance of the hypothalamus to leptin [12, 13]. Hyperleptinemia is accompanied by the development of inflammation in the vascular wall by affecting the activation of cellular immunity and the production of proinflammatory cytokines, which is accompanied by oxidative stress in endothelial cells and leads to the development of systemic hemostasis disorders [14, 15, 16].

Thus, the role of adiponectin and leptin in the development and progression of diseases accompanied by lipid disorders is ambiguous and is still the subject of scientific research.

The purpose of the research was to study the lipids changes in patients with ALD associated with NAFLD depending on the stage of decompensation.

Materials and methods. 204 patients with diagnosed liver cirrhosis (LC) participated in the study; they underwent inpatient treatment in the gastroenterology department of the Ivano-Frankivsk Regional Clinical Hospital. Among them, 78 patients were diagnosed with ALD at the stage of the LC (group I) and 126 patients had a combination of alcoholic liver cirrhosis (ALC) and NAFLD (group II). Among the patients in group I, there were 24 women and 54 men (53.2±11.4) years old and average duration of the disease (5.9±2.1) years; among patients of group II there were 22 women and 104 men (47.8±9.4) years old and average duration of the disease (4.2±2.7) years. Patients of groups I and II were subgrouped according to the compensation classes of LC by Child-Pugh score: IА (17 persons), IВ (38 persons), IC (23 persons); IIA (44 persons), IIB (48 persons), IIC (34 persons). Diagnosis was verified using clinical and laboratory-instrumental methods in accordance with the order of the Ministry of Health of Ukraine No. 826 dated November 6, 2014, adapted clinical guidelines "Non-Alcoholic Fatty Liver Disease", 2014, adapted clinical guidelines "Alcoholic Liver Disease", 2014, adapted clinical guidelines " Liver Cirrhosis, 2017 (State Expert Centre of the Ministry of Health of Ukraine, Ukrainian Gastroenterology Association, Kyiv), recommendations of the European Association for the Study of Liver, Diabetes and Obesity (EASL-EASD-EASO, 2016).

A general-clinical examination, ultrasound examination of the abdominal cavity and esophagogastro-duodenoscopy were performed. To detect the alcoholic aetiology of the disease, according to the recommendations of the World Health Organization, more than 2 doses of alcohol (1 standard dose = 10 g of ethyl alcohol) per day for women and more than 4 doses for men, were taken into account. CAGE (Cut, Annoyed, Guilty, Eye-opener), AUDIT (Alcohol Use Disorders Identification Test, 1989), the PAS questionnaire (post-alcohol syndrome developed by P.P. Opgurtsv, A.B. Pokrovsky, A.E. Uspensky), LeGo (P.M LeGo 1976) in the modification of O.B. Zharkov, 2000), ANI index (Alcoholic liver disease/non-alcoholic fatty liver disease index, 2006) were used. The control group included 20 practically healthy persons.

Exclusion criteria were liver cirrhosis of the viral, toxic and autoimmune genesis, metabolic diseases of the liver, oncological diseases, and the lack of individual consent of the patient to conduct the study. All patients were matched according to age and sex. The research was carried out in accordance with the ethical principles of conducting scientific research and principles of the Helsinki Declaration.

The state of the fat depot was assessed on the basis of the triceps skin fold thickness (TSFT) measurement which was determined with the caliper. Body mass index (BMI) was calculated according to the formula: BMI = weight (kg) / height (m²). The ratio of waist and hips circumference was not performed because of the ascites in some patients. The lipid spectrum of blood was estimated by the indices of total cholesterol (TC), lipoprotein cholesterol of high, low and very low density (HDLC, LDLC, VLDLC), atherogenic coefficient (AC), triacylglycerides (TG). The severity of the LC was assessed using the Child-Pugh score and the MELD score (Mayo Endstage Liver Disease, 2001). The level of leptin and adiponectin was determined by immunoasay using Human Leptin ELISA (Biovendor, Czech Republic) and Human Adiponectin ELISA kit (Biovendor, Czech Republic) respectively.
Statistical processing of the obtained results was carried out using the software package Statistica v. 12.0, StatSoft, USA and Microsoft Excel. The following data of parametric statistics were used: the arithmetic mean (M) and the standard deviation (SD). To determine the significance of the differences between groups in the distribution, close to normal, t-criterion Student was used. For the analysis of dependencies, a method of correlation analysis with determining the Spearman rank correlation coefficient was used. Statistically significant differences were considered at p<0.05.

Results. Analyzing the data of the clinical examination, it was found that the symptoms of asthenoviril, painful, dyspeptic, hepatorenal, hepatopulmonary syndromes, jaundice, medically uncontrolled ascites, signs of hepatic encephalopathy were more common in patients of group II of the corresponding classes, accompanied by changes in the Child-Pugh score and MELD score. In patients of group II, they were higher compared to those in group I at 22.74% and 31.18%, 21.06% and 17.78%, 13.72% and 15.98% of classes A, B, C respectively (Table 1).

The BMI in patients of IA, IB, IC and IIA, IIB, IIC groups was (22.61±2.15), (21.04±1.52), (19.21±1.63), and (34.56±4.67), (30.83±2.87), (21.35±1.63) kg/m² respectively. The analysis of the BMI values showed a significant difference between the indicators in groups I and II, depending on the stage of compensation (p<0.05). Numerical values of TSFT are reducing from IA and IIA groups of patients to IB, IC and IIB and IIC groups respectively. With the development of decompensation, there is a significant difference between the indices in the groups of stages A and B (p<0.05), the differences between the indices of groups IC and IIC were not found (p>0.05).

Changes in the biochemical parameters of the lipid spectrum were manifested by an increase in blood levels of TC, LDL, HDL, VLDL, AC and TG in patients with a stage of compensation compared with control group. In patients of group II of each class by Child-Pugh, such indicators were higher than in patients of group I of the corresponding class (p<0.05). With increasing decompensation, they decreased proportionally. HDLC in the blood of patients of both groups decreased with increasing decompensation; in patients of group II, the indicator was significantly lower compared to patients in group I (p<0.05). Such changes in the lipid spectrum are associated with an increase in liver function disorders and correlate with the prognostic MELD criteria. The most obvious association was found in patients of group II of the class C: TC = r = -0.72, LDLC = r = -0.54, VLDLC = r = -0.63, AC = r = 0.67, TG = r = -0.56, HDLC = r = -0.69.

Table 1

<table>
<thead>
<tr>
<th>Characteristics of lipid metabolism in patients with alcoholic liver cirrhosis associated with non-alcoholic liver disease</th>
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<tr>
<td>Indicators</td>
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<td>n=17</td>
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<td>BMI, kg/m²</td>
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<td>TSFT, mm</td>
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<td>TC, mmol/l</td>
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<td>Child-Pugh</td>
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<td>MELD Index</td>
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Notes:
1) * - the probability of differences between groups IA and IIA (p<0.05);
2) • - the probability of differences between groups IB and IIB (p<0.05);
3) # - the probability of differences between groups IC and IIC (p<0.05);
4) ▲ - the probability of differences between groups IA and IB (p<0.05);
5) ● - the probability of differences between groups IB and IC (p<0.05);
6) ■ - the probability of differences between groups IIA and IIB (p<0.05);
7) □ - the probability of differences between groups IIB and IIC (p<0.05).
The imbalance of adipocytokines was more obvious in patients suffering from ALC with concomitant NAFLD (Table 2). In particular, the content of leptin in the blood of patients of group II was higher compared to those in patients of group I of class A by Child-Pugh at 2.26 and 1.74 times respectively (p<0.05). In patients of both groups of class C by Child-Pugh, the level of leptin did not differ significantly (p>0.05). Adiponectin content in patients of group II was lower in comparison with patients of group I of A and B class by Child-Pugh at 1.6 and 1.56 times respectively (p<0.05). The significant difference between adiponectin levels in patients of both groups of class C was not found (p>0.05). The content of leptin was the highest in patients of both groups in stage A. With an increase in decompensation, this indicator decreased in both groups. Adiponectin content was the lowest in persons of both groups of class A and with increasing decompensation it decreased. In people of group II, these changes significantly differed from those of patients in group I (p<0.05).

### Table 2: Characteristics of adipocytokines levels in patients with alcoholic liver cirrhosis associated with non-alcoholic liver disease

<table>
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<tr>
<th>Indicators</th>
<th>Control, n=20</th>
<th>Class of LC by Child-Pugh score</th>
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<td></td>
<td>Gr. IA</td>
<td>Gr. IIA</td>
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<tr>
<td>Adiponectin µg/ml</td>
<td>8.46±0.11</td>
<td>4.73±0.26</td>
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<tr>
<td>Leptin ng/ml</td>
<td>7.92±0.28</td>
<td>9.49±0.51</td>
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**Notes:**
1) * – the probability of differences between groups IA and IIA (p<0.05);
2) ● – the probability of differences between groups IB and IIB (p<0.05);
3) # – the probability of differences between groups IC and IIC (p<0.05);
4) ▲ – the probability of differences between groups IA and IB (p<0.05);
5) ■ – the probability of differences between groups IB and IC (p<0.05);
6) ○ – the probability of differences between groups IIA and IIB (p<0.05);
7) □ – the probability of differences between groups IIB and IIC (p<0.05).

Changes in the levels of leptin and adiponectin in both groups are associated with lipid imbalance. Correlation between levels of adipocytokines and indices of lipid metabolism in group II was more obvious. The correlation between lipid metabolism and leptin level in patients of group II was as follows: for TC – 0.84, 0.79 and 0.67 for classes A, B and C respectively; for HDLc – 0.71, 0.56 and 0.48 for classes A, B and C respectively; for LDLc – 0.47, 0.42 and 0.39 for classes A, B and C respectively; for VLDLc – 0.52, 0.38 and 0.33 for classes A, B and C respectively; for AC – 0.73, 0.64 and 0.53 for classes A, B and C respectively; for TG – 0.76, 0.62 and 0.59 for classes A, B and C respectively; for adiponectin – 0.72, -0.65 and -0.61 for classes A, B and C respectively. The correlation between the adiponectin level and lipid metabolism indices in patients of group II was: for TC – 0.65, -0.58 and -0.48 for classes A, B and C respectively; for HDLc – 0.48, -0.51 and -0.36 for A, B and C classes respectively; for LDLc – 0.47, -0.42 and -0.33 for classes A, B and C respectively; for VLDLc – 0.24, -0.21 and -0.17 for classes A, B and C respectively; for AC – -0.46, -0.39 and -0.37 for classes A, B and C respectively; for TG – -0.38, -0.33 and -0.30 for classes A, B and C respectively. The correlation between the level of adiponectin, the severity of the disease and the MELD index was more obvious in patients of group II.

Correlation analysis between the levels of leptin and adiponectin and the indices of the Child-Pugh score and the MELD score revealed a stronger connection among people in group II with an increase in decompensation. Thus, between the level of leptin and the indicator of the severity of the disease by Child-Pugh score and the MELD score, the correlation was as follows: for the Child-Pugh score – r=-0.72, r=-0.58, r=-0.44, and for the MELD score – r=-0.66, r=-0.61, r=-0.68 for classes A, B, C respectively. The relation between the level of adiponectin, the severity of disease and the MELD score was as follows for the Child-Pugh score – r=0.69, r=-0.49, r=0.67, and for the MELD score – r=0.73, r=0.52, r=0.34 for classes A, B, C respectively.

**Discussion.** Thus, in patients with ALC associated with NAFLD, the course of the disease is more severe, it is accompanied by more severe clinical signs and disorders of lipid metabolism. Patients in group II had a higher BMI than patients in group I. We found a significant difference between the indicators of BMI in groups I and II depending on the stage of compensation (p<0.05). Measurement of the TSFT revealed that it was decreasing with the development of decompensation. There was a significant difference between the parameters in groups I and II of stages A and B (p<0.05), however, there was no significant difference between the parameters in groups IC and IIC (p>0.05), which indicates the depot fat depletion with progression of the disease.
As for the biochemical parameters of the lipid spectrum, higher levels of TC, LDLc, VLDLc, AC and TG were in patients of classes A and B compared to patients of class C. Patients of group II had higher rates than those in group I (p<0.05). The content of HDLC in patients of group II was significantly lower in comparison with patients in group I (p<0.05). Such changes, in our opinion, are associated with the progression of dysfunction of the liver as an organ that plays a central role in the regulation of the synthesis, degradation and deposition of cholesterol and lipoproteins. According to the literature, lowering TG levels in serum is associated with a decrease in their synthesis and a decrease in the processes of esterification; low levels of VLDLC and LDLc is associated with a deficiency of microsomal triglyceride transferase protein and inhibition of cholesterol synthesis; a decrease in HDLC is associated with a decrease in the synthesis of apolipoprotein AI.

The peculiarity of the adipocytokines was that with the progression of the LC, the level of leptin decreased, while the levels of adiponectin increased. Resistance to leptin is associated with fatty tissue as an endocrine organ and is characteristic for overweight patients, which is confirmed by higher levels of leptin in patients of group II. The higher content of leptin in patients of classes A and B is accompanied not only by the impaired liver function, but also by its increased release from adipose tissue. In patients of class C fat depot is exhausted, therefore the level of leptin decreases. Moreover, this decrease correlates with the severity of the disease and the prognostic MELD score. The level of adiponectin was lowered in class A patients and increased in patients with more severe course and correlated with severity of the disease and MELD score. Considering the hepatoprotective effect of adiponectin, some scientists believe that its elevated level reflects the anti-inflammatory response to liver damage, which depends on the severity of the disease.

The revealed correlation between the levels of leptin and adiponectin with the degree of severity of the LC and the prognostic MELD score allows considering their changes for assessment of the severity of the LC and predicting the course of the disease.

Conclusions:

1. Progression of liver cirrhosis in patients with ALC associated with NAFLD is accompanied by more severe clinical and laboratory manifestations.
2. Lipid metabolism in patients with ALC associated with NAFLD in the stage of subcompensation and decompensation is characterized by a decrease in levels of TC, HDLC, LDLc, VLDLC, and TG.
3. Levels of leptin and adiponectin in patients with ALC associated with NAFLD correlate with changes in lipid metabolism, the severity of the LC, and prognostic score MELD, which allow their use in assessment of the severity and prediction of ALD associated with NAFLD.

References:

15. Jamali R, Razavizade M, Arj A, Aarabi Mh. Serum adipokines might predict liver histology findings in non-
Резюме. Целью работы было изучение изменений липидов у больных алкогольным циррозом печени (АЦП) при сочетании с неалкогольной болезнью печени (НАЖХП) в зависимости от стадии декомпенсации. Обследовано 204 пациента. Среди них у 78 человек (I гр.) диагностирован АЦП и в 126 лиц (II гр.) было сочетание АЦП с НАЖХП. Пациентов поделили на подгруппы в зависимости от класса декомпенсации по критериям Чайльд-Пью (А, В, С). Высшие уровни в крови общего холестерина, холестерина липопротеидов низкой и очень низкой плотности, коэффициента атерогенности и триацилглицеридов были у лиц класса А и В. У больных II гр. такие показатели были выше, чем у больных I гр. (p<0,05). Содержание в крови холестерина липопротеидов высокой плотности у пациентов II гр. было ниже по сравнению с больными I гр. (p<0,05). С прогрессированием цирроза печени уровень лептина уменьшался, а уровень адипонектина возрастал. Высший уровень лептина был у пациентов II гр. У пациентов класса С жировые депо истощены, поэтому уровень лептина снижается. Причем это снижение коррелирует со степенью тяжести заболевания и прогностическим индексом MELD. Уровень адипонектина был снижен у лиц класса А и повышался у пациентов с более тяжелым течением, а также коррелировал со степенью тяжести заболевания и индексом MELD. В результате анализа связи между уровнями лептина, адипонектина и показателями углеводного обмена установлена, что сильнее корреляционная связь наблюдалась у пациентов с АЦП при сочетании с НАЖХП. Выявленные корреляционные связи уровней лептина и адипонектина со степенью тяжести цирроза печени, а также с прогностическим индексом MELD позволяют рассматривать их изменения для оценки степени тяжести цирроза печени и прогнозирования течения заболевания.

Ключевые слова: алкогольная болезнь печени; неалкогольная жировая болезнь печени; цирроз; лептин; адипонектин.