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ADIPONEKTINEMIA IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND VAR IOUS STAGES OF NON-ALCOHOLIC FAT LIVER DISEASE

Abstract. *One of the urgent problems of Endocrinology is the study of the mechanisms of formation and progression of nonalcoholic fatty liver disease (NAFLD) in patients with diabetes mellitus (DM) type 2. Prognosis depends on the stage of NAFLD in which the changes occur and secretion of adipocytokines in the first place, adiponectin. The aim of the study was to: define adiponectin levels in the circulation of patients with type 2 diabetes at different stages of NAFLD and its impact on the detection of metabolic changes, the establishment of linkages with carbohydrate, lipid, protein metabolism, and liver function. The study determined the relevance of the study of pathogenetic mechanisms of NAFLD in the future, because the violation of metabolic control and reduce the protective properties of adipocytokines in patients with comorbid disorders is of great interest to further improve diagnostic capabilities, prevent complications and improve the quality of life of these patients. It was found that in patients with type 2 diabetes with NAFLD, along with manifestations of IR, there is a violation of lipid storage in adipose tissue, which contributes to the development of NAFLD. In parallel with the progression of changes in the liver tissue*

(steatosis - NASH - fibrosis) and the start of a cascade of metabolic events with the formation of dyslipidemia, adiponectin expression is impaired, manifested by a decrease in insulin sensitivity at the NASH stage and the liver's ability to deposit lipids at the fibrosis stage. The level of adiponectin makes it possible to predict not only the development of cardiovascular complications, but also the stage of development of NAFLD.

Keywords: *adiponectin, type 2 diabetes mellitus, non-alcoholic fatty liver disease, cardiovascular diseases.*

Non-alcoholic fatty liver disease (NAFLD) is a common chronic disease, the main symptom of which is the accumulation of fat in hepatocytes, which is not associated with alcohol consumption and combines various clinical and morphological changes in the liver: steatosis, non-alcoholic steatohepatitis (NASH) and fibrosis with a possible transition to cirrhosis and hepatocellular carcinoma. Most researchers isolate NAFLD as part of metabolic syndrome (MS) or insulin resistance syndrome (IR) [1-4]. According to various authors, NAFLD occurs in 50-80% of patients with type 2 diabetes mellitus (DM) and aggravates the course of the underlying disease [5, 6].

The pathogenesis of NAFLD in patients with type 2 diabetes is rather complex and continues to be studied. Thus, according to a number of studies, the leading factors of the staged course of NAFLD from the stage of steatosis to cirrhosis in patients with type 2 diabetes are obesity, especially of the abdominal type, IR and hypertriglyceridemia [7]. Oxidative stress (OS) and obesity-associated changes in the secretion of adipocytokines, primarily adiponectin, play an important role in the pathogenesis of NAFLD [8, 9].

Until now, there are practically no studies in the literature comparing clinical and laboratory changes in NAFLD at different stages of its course, depending on changes in the protective, immune properties of adipose tissue hormones, in particular, adiponectin, changes in secretion, which are different depending on the stages of NAFLD.

Adiponectin, known as Acrp30 (adipocytes complement-related protein of 30 kDa), AdipoQ, GBP28 (gelatin binding protein of 28 kDa), and apM1 (adipose most abundant gene transcript 1), is an oligomerized glycoprotein with varying molecular

weight and spatial the structure of the fraction (trimers, hexamers and multimers), the main biological role of which is mainly in the regulation of tissue sensitivity to insulin and inhibition of differentiation of preadipocytes [10, 11]. At the same time, adiponectin has immunomodulatory, mitotic, antiatherogenic, antiapoptic, metabolic and properties, the severity of which depends on the type of tissue [12]. Adiponectin reduces damage to the vascular endothelium, stimulates the production of nitric oxide, inhibits the adhesion of monocytes,

Hypoadiponectinemia is a key etiological factor responsible for many pathological conditions associated with obesity [13]. As a factor that increases insulin sensitivity, adiponectin prevents metabolic disorders in obesity, including hypertension, atherosclerosis, NAFLD, NASH, heart failure, inflammation, and some cancers [14, 15].

The aim of the study was: determination of adiponectin levels in circulation in patients with type 2 diabetes at various stages of NAFLD and revealing its effect on metabolic changes, establishing relationships with indicators of carbohydrate, lipid, protein metabolism and the functional state of the liver.

Materials and methods. Inclusion criteria: patients with type 2 diabetes with NAFLD (men and women) aged 33 to 80 years. The number of examined patients was 122 people who were divided into 3 groups depending on the stage of NAFLD, namely: group I included 69 patients with type 2 diabetes with NAFLD at the stage of steatosis; Group II consisted of 35 type 2 diabetes patients with NAFLD at the stage of NASH; Group III included 18 type 2 diabetes patients with NAFLD at the fibrosis stage.

Exclusion criteria: patients with type 1 diabetes, morbid and secondary obesity, severe physical and mental disorders, alcohol abuse, use of hepatotoxic drugs, viral hepatitis, chronic diseases of the gastrointestinal tract, provoked by malabsorption.

During the examination, complaints, medical history were collected, including to exclude alcoholic and drug-induced liver damage. Anthropometric parameters were determined (height, weight, waist circumference (WC), hip circumference (HC) and their ratio (WC/HC), body mass index (BMI) (Table 1).

Table 1

Clinical and anthropometric indicators of patients with type 2 diabetes with NAFLD, depending on its stage,(±)$\bar{x}S_{\bar{x}}$

Index	Group, number of examined		
	I (stage of steatosis), n = 69	II (stage of NASH), n = 35	III (stage of fibrosis), n = 18
Age years	58.54 ± 1.14	55.17 ± 1.65	52.44 ± 1.80
Height, cm	169.33 ± 1.27	167.54 ± 1.44	167.89 ± 2.00
Body weight, kg	89.86 ± 2.41	91.99 ± 2.59	92.44 ± 5.60
WC, cm	101.17 ± 2.21	104.63 ± 2.49	102.67 ± 5.40
HC, cm	104.42 ± 1.98	106.77 ± 1.77	103.11 ± 4.20
WC/HC	0.97 ± 0.01	0.98 ± 0.02	0.99 ± 0.02
BMI, kg / m2	30.88 ± 0.86	32.52 ± 0.79	32.55 ± 1.20
Duration of type 2 diabetes, years	7.93 ± 0.62	6.59 ± 0.74	10.47 ± 2.23

All patients included in the study confirmed alcohol abuse (less than 40 grams of ethanol per day for men and 20 grams for women).

Patients with type 2 diabetes with NAFLD were divided into subgroups depending on ultrasound data and biochemical parameters according to the World gastroenterology organization global guidelines (Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis), 2012, i.e. the stage of steatosis includes patients with the following ultrasound criteria: increased liver echogenicity; reduced sound conductivity of the ultrasonic signal; deterioration in visualization of the branches of the portal and hepatic veins; an increase in the size of the liver. The patients who had a combination of ultrasound signs of fatty degeneration of the liver and laboratory signs of hepatitis were referred to the NASH stage. The stage of fibrosis was recorded on the basis of ultrasound criteria: an increase in the density of the liver tissue, liver vessels and bile ducts, and biochemical parameters.

Glycemic parameters during the day, including fasting blood glucose (FBG), postprandial blood glucose (PBG) were determined by the glucose oxidase method using the BIOSENC-LINE ECF Diagnostic apparatus (laboratory norm 3.8-6.2 mmol/l), was also carried out Calculation of average daily blood glucose (ADBG), glycemic amplitude, glycosylated hemoglobin (HbA1c) by colorimetric method.

Determination of the blood lipid spectrum included the study of: total cholesterol (TC), high density lipoprotein cholesterol (HDL cholesterol), triglycerides (TG) by the enzymatic method using the sets of LLC NPP "Filisit-Diagnostika", Ukraine, "Cholesterol-Novo cholesterol", Russia; beta-lipoprotein (β-LP) – by the turbodimetric method; total bilirubin - by Jendrashik's method on devices Fluorat-02-AVLF-T and photometer Solar PM 2111. Calculation of the content of low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) in blood, as well as the atherogenic coefficient (CA) was carried out according to generally accepted formulas.

The study of thymol test in blood serum was carried out according to the McLagan method; the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the blood serum was determined by the Reitman-Frenkel method using the Fluorat-02-AVLF-T apparatus and the Solar PM 2111 photometer. The coefficient where Ritis is calculated, the value of which is less than 0.91 indicates liver damage.

The total protein in the blood serum was determined by the biuret method, the fractional composition of the blood serum proteins was determined by the turbodimetric method.

In patients with enzyme immunoassay, the level of adiponectin in the blood plasma was studied using the EA2500-1 kit, human Adiponectin ELISA (laboratory norm 4-16 ng/ml) on a Stat Fax 3200 biochemical analyzer.

Statistical processing of the obtained results of the normal distribution of quantitative variables was determined using the Kolmogorov-Smirnov test. To compare the indicators that are characterized by a normal distribution, the unpaired Student's t-test was used. Testing of null statistical hypotheses regarding the absence of differences between groups was carried out at a significance level of 0.05. All calculations were carried out on a Pentium computer in the Windows-XP environment using the Excel XP, STATISTICA-6.0 software.

Results and its discussion. As a result of the study, it was found that all examined patients were in a state of subcompensation of carbohydrate metabolism, however, the level of SCR was significantly higher in type 2 diabetes patients with

NAFLD in the fibrosis stage compared with type 2 diabetes patients in the NASH stage, which indicates a deterioration in carbohydrate metabolism. with the development of connective tissue, which is represented by the hepatocyte at this stage of NAFLD. Indicators of carbohydrate metabolism in the examined patients are presented in table. 2.

Table 2

Indicators of carbohydrate metabolism in patients with type 2 diabetes with NAFLD in depending on its stage, (±)$\bar{x}S_{\bar{x}}$

Index	Group, number of examined			P
	I (stage of steatosis), n = 69	II (stage of NASH), n = 35	III (stage of fibrosis), n = 18	
FBG, mmol / l	8.54 ± 0.31	8.59 ± 0.41	9.80 ± 0.86	
PBG, mmol / l	8.85 ± 0.34	9.36 ± 0.49	10.89 ± 1.17	
ADBG, mmol / l	8.32 ± 0.32	8.13 ± 0.36	9.71 ± 0.71	p_{2-3} <0.05
Ampl. glitch.	4.41 ± 0.37	3.91 ± 0.32	3.67 ± 0.50	
HbA1c,%	7.19 ± 0.17	7.22 ± 0.19	7.67 ± 0.50	

Note. p - the significance of differences when comparing indicators among the examined groups of patients by Student's t-test

A study of lipid metabolism indicators in patients with type 2 diabetes with NAFLD at various stages revealed a significant increase in the level of total cholesterol, thymol test, triglycerides, β-LP at the NASH stage compared with NAFLD patients at the stage of steatosis and the greatest increase in these indicators at the stage of fibrosis. The highest level of VLDL cholesterol was recorded at the NASH stage: (0.83 ± 0.05); (1.19 ± 0.08) and (1.03 ± 0.24) mmol/L, respectively, p<0.001. These changes indicate progressive inflammatory processes in the liver with a tendency to chronicity of the process against the background of general metabolic changes in the body of patients with type 2 diabetes, and the presence of NAFLD contributes to the deterioration of metabolic control, which significantly worsens in conditions of concomitant pathology prone to progression.

Based on the determination of the functional state of the liver in the late stages of NAFLD, progression of the activity of the inflammatory process at the stages of NASH and fibrosis was established according to the data of transaminases and the coefficient where Ritis (Table 3).

Table 3

Indicators of the functional state of the liver in the examined patients with type 2 diabetes with NAFLI, depending on its stage, (±)$\bar{x}S_{\bar{x}}$

Index	Group, number of examined			P
	I (stage of steatosis), n = 69	II (stage of NASH), n = 35	III (stage of fibrosis), n = 18	
ASA, mmol/l	0.51 ± 0.04	0.70 ± 0.04	1.13 ± 0.07	p_{1-2} <0.001 p_{1-3} <0.001 p_{2-3} <0.001
ALA, mmol/l	0.71 ± 0.11	1.06 ± 0.05	2.04 ± 0.16	p_{1-2} <0.01 p_{1-3} <0.001 p_{2-3} <0.001
ASA/ALT	0.83 ± 0.04	0.69 ± 0.04	0.57 ± 0.04	p_{1-2} <0.05 p_{1-3} <0.001 p_{2-3} <0.05

Note. p - the significance of differences when comparing indicators among the examined groups of patients by Student's t-test

The level of adiponectin significantly decreased in the examined patients with type 2 diabetes with NAFLD with the progression of the latter from the stage of steatosis to the stage of fibrosis (Table 4).

Table 4

The level of adiponectin in patients with type 2 diabetes with NAFLI, depending on its stage, (±)$\bar{x}S_{\bar{x}}$

Index	Group, number of examined			P
	I (stage of steatosis), n = 69	II (stage of NASH), n = 35	III (stage of fibrosis), n = 18	
Adiponectin, μg/ml	5.14 ± 0.24	4.30 ± 0.28	3.52 ± 0.28	p_{1-2} <0.05 p_{1-3} <0.001 p_{2-3} <0.05

Note. p - the significance of differences when comparing indicators among the examined groups of patients by Student's t-test

Thus, pronounced violations of metabolic control at various stages of NAFLD in patients with type 2 diabetes were revealed, along with a decrease in the level of the protective hormone of adipose tissue, adiponectin. The next stage of the work was the study of the correlation relationships of various metabolic parameters with the level of adiponectin in the circulation in order to understand the pathogenetic disorders in comorbid pathology.

Informative data were obtained in the study of the correlations between the levels of adiponectin and triglycerides at various stages of NAFLD. Thus, a negative correlation was found between the level of adiponectin and TG with a pronounced progression of NAFLD from the stage of steatosis to NASH, which does not occur at the stage of fibrosis, where a positive correlation is registered. A significant decrease in the level of adiponectin was noted at the stages of NASH and fibrosis, and a correlation analysis of the levels of adiponectin and TG revealed a significant decrease in the level of TG along with a decrease in the level of adiponectin. The data obtained can be explained by a decrease in the protective properties of the studied hormone and the inclusion of adaptive mechanisms in the pathogenesis of NAFLD development by reducing the intake of FFAs and their main source, TG, into the hepatocyte, by reducing the mass and size of functioning hepatocytes. At the stage of steatosis and NASH, on the contrary, there is an excessive formation of TGs in the hepatocyte in the composition of VLDL, which contribute to the accumulation of products of their peroxidation with a damaging mechanism of action of the hepatocyte, as evidenced by the positive correlation between the levels of adiponectin and TG at the stage of fibrosis – $r = 0.5$, $p < 0.02$.

Thus, with a decrease in the level of adiponectin, metabolic control is disturbed, which is manifested by an increase in the level of VLDL cholesterol at the stage of steatosis; an increase in the level of thymol test, alkaline phosphatase at the stage of NASH, and at the stage of fibrosis there is a decrease in the formation of triglycerides in the hepatocyte in the composition of VLDL, which indicates the depletion of hepatocyte reserves with a decrease in their synthesizing properties (Table 5).

As a result of the study, the relevance of studying the pathogenetic mechanisms of NAFLD development in the future was determined, since violations of metabolic control and a decrease in the protective properties of adiponectin in patients with comorbid pathology are of great interest for further improving diagnostic capabilities, preventing complications and improving the quality of life of this category of patients.

Table 5

Correlation relationships between the adiponectin level and lipid profile and some indicators of the functional state of the liver at various stages of NAFLD in patients with type 2 diabetes (according to Pearson)

Index	I (stage of steatosis), n = 69	II (stage of NASH), n = 35	III (stage of fibrosis), n = 18
Total cholesterol, mmol/l	-0.21 *	-0.005	-0.15
VLDL cholesterol, mmol/l	-0.34 *	-0.3 *	0.52 *
TG, mmol/l	-0.12	-0.01	0.38 *
Thymol test, U	-0.04	-0.34 *	-0.14
ALP, nmol/s-l	-0.06	-0.46 *	-0.28

*Note. * - Significance of differences when comparing indicators ($p \leq 0.05$)*

Conclusions:

It was found that in patients with type 2 diabetes with NAFLD, along with manifestations of IR, there is a violation of lipid storage in adipose tissue, which contributes to the development of NAFLD. In parallel with the progression of changes in the liver tissue (steatosis - NASH - fibrosis) and the start of a cascade of metabolic events with the formation of dyslipidemia, adiponectin expression is impaired, manifested by a decrease in insulin sensitivity at the NASH stage and the liver's ability to deposit lipids at the fibrosis stage. The level of adiponectin makes it possible to predict not only the development of cardiovascular complications, but also the stage of development of NAFLD

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