No 11 (11) (2017)

P.1

The scientific heritage

(Budapest, Hungary)

The journal is registered and published in Hungary.

The journal publishes scientific studies, reports and reports about achievements in different scientific fields. Journal is published in English, Hungarian, Polish, Russian, Ukrainian, German and French.

Articles are accepted each month. Frequency: 12 issues per year. Format - A4

ISSN 9215 — 0365

All articles are reviewed

Free access to the electronic version of journal

«The scientific heritage»

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E-mail: public@tsh-journal.com

Web: www.tsh-journal.com

CONTENT

MEDICAL SCIENCES

Bilovol O.M., Bobronnikova L.R.

IMBALANCE OF ADIPOCYTOKINS IN PATIENTS WITH COMBINED NON-ALCOHOLIC FATTY LIVER OF LIVER DISEASE AND DIABETES MELLITUS 2 TYPE.....23

Ermakova M.K.

QUALITY OF LIFE AND PSYCHOEMOTIONAL STATUS IN OBSTRUCTIVE RESPIRATORY DISEASES IN CHILDREN AND ADULTS 27

Kravchenko A.I.

ERECTILE DYSFUNCTION AS A PREDICTOR OF CORONARY ARTERY DISEASE 36

Magrlamova K.G., Kravchenko A.I.

NEW EFFECTIVE MEDICAL TEACHING FOR STUDENTS.....38

Kravchenko A.I., Magrlamova K.G.

NEW HEALTH SYSTEM AND MODERN MEDICAL EDUCATION 41

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IMBALANCE OF ADIPOCYTOKINS IN PATIENTS WITH COMBINED NON-ALCOHOLIC FATTY LIVER OF LIVER DISEASE AND DIABETES MELLITUS 2 TYPE

ABSTRACT

The aim of our research was to study the peculiarities of metabolic disorders in an interrelationship with the imbalance of adipocytokines in patients with comorbid non-alcoholic fatty liver disease (NAFLD) and diabetes mellitus (DM) type 2. Decrease in omentin level and increase in serum level of RBP -4 in patients with NAFLD and DM type 2 should be considered as a biomarker for the formation of metabolic syndrome and activation of the liver fibrogenesis.

Keywords: non-alcoholic fatty liver disease, diabetes mellitus type 2, metabolic disorders, pathogenetic relationships, adipocytokines.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a common chronic disease and often occurs in comorbidity with diabetes mellitus (DM) type 2, insulin resistance (IR), increased body weight, hyperlipidemia and atherosclerosis, and is considered as one of the manifestations of the metabolic syndrome [12]. NAFLD is diagnosed in 80-90% of obese individuals, in 30-50% of patients suffering from diabetes mellitus and in 90% of surveyed with hyperlipidemia [2, 5]. The combination of DM type 2 and NAFLD increases the risk of developing liver cirrhosis and

hepatocellular carcinoma by 2-2.5 times and ranks number 4 among the causes of lethality of DM [3]. The weight gain and immediate abdominal obesity play an important role both in the pathogenesis of NAFLD and associated metabolic disorders and in DM type 2, and IR, according to according to the data of some authors, is the cause of fat accumulation in the liver [6, 12]. This process is due to the peculiarities of visceral adipocytes, which are characterized by a reduced sensitivity to the anti-lipolytic action of insulin and an increased sensitivity to the lipolytic action of catecholamines [7, 11]. It should be noted that a low concentration of insulin receptors in the abdominal adipose tissue contributes to an increase in the production of fatty acids, an increase in the level of triglycerides in the blood and the process of atherogenesis [11]. It is assumed that the significant role in the development of IR is played by the change in the production of adipocytokines, biologically active proteins that are formed in adipose tissues, such as omentin, retinol-binding protein-4 (RBP-4), tumor necrosis factor alpha (TNF-α) and others. [8,10,13,15]. Progressing with excess body weight, adipocytokine imbalance is a risk factor for lipid and carbohydrate metabolism disorders, increasing blood pressure and, as a consequence, leads to the formation of metabolic syndrome components [2]. Progression and variants of NAFLD course depends on the activity of the inflammatory process and the intensity of fibrosis in the liver tissue, however, the mechanisms and cause-effect relationships between the progression of liver fibrosis and the disorders of carbohydrate metabolism, as well as the influence of fatty tissue hormones during comorbid NAFLD and DM type 2 have not been studied enough.

The aim of the research was to study the peculiarities of metabolic disorders in intercurrent with imbalance of adipocytokines in patients with comorbid course of NAFLD and DM type 2.

Materials and methods. In the study, 92 participated patients with NAFLD and DM type 2 (subcomputed): the 1st group consisted of 48 patients with combined course of NAFLD and DM type 2, and the 2nd group consisted of 44 patients with NAFLD. The control group (n = 20) was maximally comparable in age and sex to surveyed patients. The average age of the patients was 49.6 ± 5.7 years.

Diagnosis of DM type 2 and MS was performed according to the criteria of the International Diabetes Federation (IDF, 2013). Clinical examination of the patients included the analysis of complaints, the collection of anamnesis, physical monitoring and anthropometric evaluation – was determined the height, body weight, waist size (WS), hip size (HS), calculated the ratio of WS/HS and body mass index (BMI). The presence of abdominal obesity (AO) was diagnosed at a waist size (WS) of 94 cm in men or more and 80 cm or more in women.

Biochemical and instrumental methods of research were used to verify the diagnosis of NAFLD and to assess the functional state of the liver. The study of the hepatobiliary system was carried out according to the standard procedure on the ultrasound diagnostic system Philips HDI-11. Reliable criteria for fatty liver infiltration in ultrasound were hepatomegaly, medium-grained structure transformation, parenchymal hyperechogenicity, and dorsal echo attenuation. The functional state of the liver was assessed using biochemical methods: the content of bilirubin and its fractions (the Endrashik-Cleghorn-Grof method), the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Reitman-Frenzel method), γ-glutamate transpeptidase (GGT) (unified methodology by using a standard set of reagents). The AST/ALT ratio was determined, which is an indicator of the liver fibrosis progression. To assess the presence and activity of the inflammatory process in the liver, the level of the C-reactive peptide (CRP) in the blood serum was determined by an enzyme immunoassay. The concentration of fasting blood glucose in the serum (FBG) was determined by the glucose oxidative method, glucose tolerance was also determined. The serum lipid profile (total cholesterol (TC), triglycerides (TG)) was determined by the enzymatic colorimetric method using «Human» kits (Germany). The low-density lipoprotein cholesterol (LDL-C) was calculated according to the W.T. formula: Friedwald: LDL-C = TC - (TG / 2,2 + HDL-C). The insulin resistance level was assessed using HOMA (homeostasis model assesment), a homeostasis model with the calculation of the insulin resistance index (HOMA-IR). The HOMA-IR index was determined by the calculation method according to the formula D.R. Matthews et al. (1985). At value HOMA-IR greater than 2.77 were diagnosed the insulin resistance. The blood content of the C-peptide was determined by an enzyme

immunoassay (Finland). The serum level of TNF-α was determined by the method of enzyme immunoassay (ELISA) using the "Protein contour" kits (St. Petersburg). The concentration of fibronectin was determined by the method of solid-phase enzyme immunoassay ("NVO Immunotech", Moscow). The content of platelets in the blood serum was determined using a photoelectric colorimeter KFK-2. The concentration in the serum of venous blood of adipocytokines was defined: omentin - by EIA using the kit ("Bio Vendor", Czech Republic); Retinol-binding protein -4 (RBP-4) by EIA using the kit ("DRG" USA). Statistical processing of research results was carried out by the Statistica-8.0 application software package using Student's t-test.

Results and discussion. In assessing the trophic status in patients with combined course of NAFLD and DM type 2, the mean of BMI was 34.26 ± 4.2 kg / m^2, with 1 degree of obesity diagnosed in 47.3%, 2 grade of the obesity in 29.0% and 3 degree of obesity in 22.4% of patients ($p < 0.05$). In half of patients with AO, the obesity duration was more than 7 years, and in 15.4% - since childhood ($p < 0.05$). The body mass index in patients with combined course of NAFLD and DM type 2 was by 1.2 times higher than that of patients with NAFLD ($p < 0.05$) (Table 1), and the WS/HS index was higher by1.7 times ($p < 0.05$). In patients of the 2nd group, overweight ($p < 0.05$) was diagnosed in 54.4% of cases. The established increase of BMI in patients with concomitant course of NAFLD and DM type 2 is consistent with the literature data on the large specific weight of non-alcoholic steatosis in overweight patients [1, 12]. In this case, the liver receives an excessive amount of fats and carbohydrates, which are converted to fatty acids, which are a substrate for the synthesis of triglycerides, accumulating in hepatocytes [1, 12].

Table 1

Peculiarities of the clinical and biochemical parameters of blood serum in the surveyed patients (M ± m)

Index, measurement unit	Control group (n=20)	NAFLD (n=48)	NAFLD +DM (n=44)
BMI	24,6±1,6	27,5± 3,5$^{*/\#}$	35,24± 4,26$^{*/\#\#}$
AST, mmol\l	0,42±0,03	0,87±0,07$^{*/\#}$	1,44±0,16$^{*/\#\#}$

ALT, mmol\l	0,54±0,06	0,96±0,06*/#	1,52±0,7*/##
AST\ALT μkmol/l	0,64±0,4	0,75±0,6*/#	0,97±0,8*/##
GGT, IU/L	43,75±12,74	57,2±15,2*/#	63,9±19,7*/##
FBG, mmol\l	4,03±0,6	6,18±1,5 */#	7,9±1,7*/##
HbA1c,%	4,2±0,4	5,1±0,5*	7,8 ±1,8*/##
HOMA-IR	1,4±1,5	3,6±1,8*/#	5,4±2,6*/##
CRP, n mmol\l	0,82±0,4	2,8±0,8*/#	3,2±1,2*/##
ТГ, mmol\l	1,8±0,6	4,4±1,6* / #	5,2±1,4*/##
ТС, mmol\l	4,2±0,4	4,8±0,8*/#	5,7±1,8*/##
CH LDL	5,3±0,5	4,5±0,7*	4,2±0,7* #
CH HDL	0,9±0,2	1,6±0,1*	1,9±0,4* #
Platelets, 109/l	227,0±24	209,0±36*/#	200,0±46*/##
Fibronectin, kg\ml	344,5±6,0	397,0±8,6*/#	485,0±10,5* */##

* p<0,05 – the reliability of differences in comparison with the control group;
# p<0,05 – the reliability of differences in comparison with patients of the first group;
p<0,05 – the reliability of differences in comparison with patients in the second group;

The level of systolic and diastolic BP in patients with combined course of NAFLD and DM type 2 was higher than in patients of the 2nd group 164.5 ± 0.07: 142.3 ± 1.05, respectively (p <0.05).

In studying the functional state of the liver (see Table 1), the activity indices of ALT, AST and GGT in patients of the 1st group were significantly higher in comparison with the parameters of patients of the 2nd group and control (p <0.05), which indicated the activity of inflammatory process in the liver. The ratio of AST/ALT was significantly higher in patients of the 1st group, than in patients of the 2nd group (p <0.05), which indicated a mutually disturbing metabolic disorder in the combined course of the disease and a high risk of fibrotic changes in the liver. In assessing the lipid profile, lipid metabolism disorders were significantly higher in patients with concomitant course of the disease, compared with patients of the 2nd group (93.6% and 43.4%, respectively, p <0.05). Hypercholesterolemia

was diagnosed in 79.6% of patients with combined course of NAFLD and DM type 2, in 22.9% of patients was increased CHLDL, and in 36.3% of patients was decreased CHLDL level ($p<0.05$). TC level in patients of this group was significantly higher than in the comparison and control group ($p<0.05$). The serum triglyceride level was by 1.5 times higher ($P<0.05$) in patients with NAFLD and DM type 2 than in the 2nd group and exceed 2.5 times in the control group ($p <0.05$), that is associated with the development of the so-called vicious circle, when the fatty liver intensively synthesizes triglycerides and very low density lipoproteins. From recent, due to increased activity of triglyceride synthetase and triglycerid-lipase, β-lipoproteins are synthesized [1]. The increase in the concentration of TC and TG in the 1st group is directly depended on BMI ($r = 0.64$, $p <0.05$, $r = 0.65$, $p <0.05$, respectively), which is associated with the progression of metabolic disorders in the liver, in particular, with excessive intake of fat and carbohydrates into the liver converting to fatty acids, which are a substrate for the synthesis of triglycerides, which accumulate in hepatocytes, that confirms the theory regarding of dyslipidemia effect on the progression of NAFLD [1, 12]. It has been established that a decrease in the level of CHHDL in patients with NAFLD and DM type 2 was significantly more likely than in the comparison group (56.2% and 22.0%, respectively, $p <0.05$). In obese patients observed a lower level of CHHDL compared with the value of this index in the group of patients with normal body weight ($p <0.05$). The patients of the 1st group had a significant increase in the fasting blood glucose (FBG) level relative to the control group ($p <0.05$), which can be explained by the presence of abdominal obesity, the highest level was observed in patients with NAFLD in combination with DM 2 type - 2.4 times ($p <0.05$). The impaired glucose tolerance (IGT) in patients with isolated course of NAFLD was identified in 10.5% of patients ($p <0.05$). A significant increase in HbA1c in patients with combined course of NAFLD and DM type 2 was found, indicating a negative effect of excess weight on carbohydrate metabolism ($p<0.05$). Decreased sensitivity of tissues to insulin by the HOMA-IR test was observed in 100% patients of the 1st group and in 87.5% patients of the 2nd group ($p<0.05$). It has been found that the level of insulin resistance correlated with the serum AST content ($r = 0.63$, $p <0.001$), which

confirms the hypothesis that insulin resistance is one of the factors that leads to the development and progression of inflammation in the liver. Correlation analysis revealed positive relation between the index of insulin resistance HOMA-IR and BMI ($r = 0.47$, $p < 0.001$), TG level ($r = 0.49$, $p < 0.001$). The identified patterns in the combined course of NAFLD and DM type 2 emphasize the systemic nature and regularity of metabolic disorders. In hyperglycemia, free oxygen radicals are formed directly from glucose, which triggers a cascade of reactions of lipid peroxidation and proteins with the development of hypoxia and leads to an increase in the atherogenic modified fraction of lipoproteins, inhibition of the enzymatic link of antioxidant protection, which leads to disruption of apoptosis and development of systemic metabolic changes [8]. The level of CRP in the serum exceeded the control values in both groups of patients ($p < 0.05$). The greatest increase (by 2.1 times) was observed in patients with combined course of NAFLD and DM type 2 ($p < 0.05$) correlated with BMI ($r = 0.49$; $p < 0.001$), FBG levels ($r = 0, 46$; $p < 0.001$), ALT ($r = 0,53$; $p < 0.001$, TG levels ($r = 0,44$; $p < 0.04$), index HOMA-IR ($r = 0,46$; $p < 0.001$) . Decreased sensitivity of tissues to insulin leads to compensatory hyperinsulinemia which enhances lipolysis in adipose depo, and release into the bloodstream of a large amount of free fatty acids, resulting in enhanced synthesis of TG [7]. Analyzing parameters of coagulation homeostasis increase was identified an increase in the serum of the fibronectin level by1.4 times in patients with combined course of NAFLD and DM type 2 compared with the control group ($p < 0.05$). Fibronectin is known as an extracellular matrix protein and as a severity marker of the mesenchymal-inflammatory syndrome [7]. The established changes indicate a hypercoagulable syndrome which contributes to the progression of the liver cell apoptosis; enhance insulin resistance, the development and deepening of hypoxia, activation of free radical lipid oxidation, destruction of cell membranes and the closure vicious circle pathogenesis of NAFLD [7]. A decrease in platelet content in the blood serum is the most pronounced in patients with concomitant disease course compared with the control group ($p < 0.05$), indicating that indirectly has pointed out fibrotic formation in the liver.

In the analysis on hormonal parameters in the groups of the surveyed patients, a significant increase of TNF-α level in the serum was observed in comparison with

the control group (p <0.05). The greatest increase of the index in 4.2 times (p <0.001) was observed with the combination of NAFLD and DM 2 type (Table 2).

Table 2

Adipocytokine blood profile in the examined patients

(M± m)

Index, measurement unit	Control group (n=20)	Groups of patients (n=92)	
		II group (n=44)	I group (n=48)
TNF-α, pg/ml	41,9 ±5,0	72,2±2,4*	109,2±3,4*#
CRP, pg/ml	3,3±1,2	3,7±1,7	4,3±1,5
Omentine, ng /ml	452,63 ± 2,16	415,21±1,24*	348,46±1,58*#
RBP-4, μg /ml	31,2 ± 6,2	38,9 ± 7,9*	34,02±0,4*#

*– p<0,001 - the likelihood of differences in comparison with the control group;
#– p<0,001 – t he likelihood of differences in comparison with patients of the first group

The established correlation between TNF-α and ALT indices (r = 0.42, p <0.001), as well as GGT (r = 0.43, p <0.001), testified to the role of TNF-α in the development of cytolytic syndrome in patients with comorbid course of NAFLD and type 2 diabetes. In 83.4% of patients in the 1st group, the fasting C-peptide level was increased in comparison with the patients of the 2nd group and with control (p<0.001), which is explained by increased lipolysis with insulin resistance and subsequent accumulation of lipids in the liver [7].

In the surveyed patients, the role of omentin imbalance and RBP-4 on the progression of metabolic disorders in the liver was analyzed. It is known that omentin belongs to protective adipocytokines, since it has antidiabetic, anti-inflammatory, anti-atherogenic and cardioprotective effects [16].

Several clinical studies have shown that an elevated level of RBP-4 is a risk factor associated with NAFLD [14,15]. In singular clinical studies, it has been established that levels of serum RBP-4 have a positive association with the degree of fat accumulation in the liver and liver enzymes, including serum ALT, AST and γ-glutamyl transpeptidase [16].

The level of omentin in patients with concomitant course was lower than in patients of the 2nd group and with control (p<0.001) and correlated with BMI (p <0.001) and obesity (p<0.001). Negative connections between the level of

omenthin and BMI (r = -0.46, p <0.001), WS (r =-0.52, p <0.001) the ratio of WS/HS (r = -0.48, p <0.001) (R = -0.46, p <0.001), TG level (r=-0,52; p<0,001) and CRP (r = -0.44, p <0.001), as well as positive connections between the level of omentinos and CHHDL (r = 0.44, p <0.001). An inverse relationship was established between the level of omentin and glucose (r = -0.48, p <0.001), HOMA-IR (r = -0.42, p <0.001). The level of serum RBP4 was significantly higher in patients with combined course of NAFLD and DM type 2, compared with patients of the 1st group and control (p <0.001).

The level of RBP-4 positively correlated with the BMI (r = 0.44, p <0.001), WS (r = 0.42, p <0.001), HOMA-IR (r = 0.45, p <0.001), CRP (R = 0.44, p <0.001), and negatively correlated with CHHDL (r = 0.46, p <0.001) and the level of omentin (r = 0.44, p <0.001). The results obtained are consistent with the literature data, that point to RBP-4 as a marker for the development of IR, atherogenic dyslipidemia, and directly for NAFLD [13].

The study on the relationship between the content of fatty tissue hormones and the individual components of the metabolic syndrome in patients with NAFLD and DM type 2 confirm the possibility of these hormones participating in the formation of the metabolic syndrome and its components.

Conclusions. The causal relationships of the progression of metabolic disorders and adipocytokines imbalance in patients with comorbid course of NAFLD and DM type 2 are considered. It was established that the progression of IR, atherogenic dyslipidemia, systemic inflammation, which are most pronounced in patients with obesity, is characteristic for the combined course of this pathology. Decrease in omentin level and increase in serum level of RBP-4 in patients with NAFLD and DM type 2 should be considered as a biomarker for the formation of metabolic syndrome and activation of the liver fibrogenesis.

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