METABOLIC MARKERS OF CARDIOVASCULAR DISEASE AND TYPE 2 DIABETES MELLITUS IN VARIOUS CLINICAL AND MORPHOLOGICAL FORMS OF NONALCOHOLIC FATTY LIVER DISEASE IN PATIENTS WITH ABDOMINAL OBESITY

Kseniya Komshilova[*], **Natalya Mazurina**[*,1], **Ekaterina Troshina**[*], **Teona Shvangiradze**[*], **Pavel Bogomolov**[**], **Galina Melnichenko**[*] **and Ivan Dedov**[*]

[*]Endocrinology Research Centre, Dmitry Ulyanov str. 11, Moscow, Russia., [**]Moscow Regional Research Clinical Institute of M.F.Vladimirsky ("MONIKI"), Shchepkina str. 61/2, Moscow, Russia.

ABSTRACT Objective: Nonalcoholic fatty liver disease (NAFLD) is closely associated with obesity and metabolic syndrome (MS). Liver biopsy can verify the histological form of NAFLD. The study aimed to evaluate the relationship between histological indicators of liver damage and metabolic parameters. **Material and methods:** 80 patients, median age 45 [41.5; 47.5], with abdominal obesity and US signs of NAFLD were included in the study. All patients underwent percutaneous liver biopsy. Glucose levels during OGTT, total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TG), ALT, and AST were measured. Fasting insulin, serum adiponectin, C-reactive protein (CRP) and active plasminogen antigen activator inhibitor-1 (PAI-1) were determined. **Results:**NAFLD was confirmed in 77 patients (96.3%), NASH – in 64 cases (80%), steatosis - in 11 cases (13.8%). In patients with steatosis, 72.7% had dyslipidemia, 63.6% had impaired glucose metabolism, 63.6% had insulin resistance (IR); with NASH – in 93.8%, 78.1% and 100%, respectively. Adiponectin level correlated negatively with IR indicators: HOMA-IR ($r = -0.45$; $p = 0.023$) and fasting insulin ($r = -0.35$; $p = 0.024$). NASH patients had significantly higher levels of CRP than those with steatosis: 3.8 [2.1; 6.7] mg/L vs 1,1 [1.0; 1.7] mg/L, ($p < 0.0001$). PAI-1 activity correlated positively with anthropometric parameters, IR, the level of CRP, and negatively with adiponectin concentration. **Conclusions:**Metabolic disorders in patients with NAFLD are dependent upon the severity of the liver damage and the morphological stage of the disease.

KEYWORDS: Nonalcoholic fatty liver disease, liver biopsy, abdominal obesity, cardiovascular risk factors, type 2 diabetes mellitus risk factors

DOI:10.5455/ijsm.ct-scan-children-mild-head-injury
First Received: July 13, 2018
Accepted: September 01, 2018
Manuscript Associate Editor: Georgi Baitchev (BG)
Editor-in Chief: Ivan Inkov (BG)
Reviewers: Onur Öztürk (TR), Poongkodi Karunakaran (IN)
[1]Natalya Mazurina Address: 117036, Dmitry Uliyanov Street 11, Endocrinology Research Center, Moscow, Russian Federation Telephone number: +7 903 199 49 83 Fax: +7 495 124 35 02 E-mail: natalyamazurina@mail.ru

Introduction:

Nonalcoholic fatty liver disease (NAFLD) is a chronic condition that is not associated with excessive alcohol consumption and is characterized by the excessive fat accumulation in the liver, exceeding 5% of its weight. NAFLD refers to a wide spectrum of liver damage that ranges from nonalcoholic fatty liver (or simple steatosis) to nonalcoholic steatohepatitis (NASH) with the possible outcome in fibrosis and cirrhosis. NAFLD is closely related to abdominal obesity, type 2 diabetes mellitus (T2DM),

and dyslipidemia, with a prevalence ranging from 50% to 90% in these patient subgroups [1-3] and correlates with life expectancy [4-6]. NAFLD, especially at the stage of steatosis progresses very slowly; however, liver cirrhosis and hepatocellular carcinoma can occur as the result of its evolution [4, 7]. Patients with NAFLD have increased overall mortality compared to the general population, not only due to cirrhosis and hepatocellular carcinoma but also because of CVD [5, 6]. Obesity, hypertriglyceridemia, and insulin resistance (IR) have been identified as the most significant factors contributing to the development of NAFLD and its progression [8, 9]. At the same time, mechanisms associated with abdominal obesity and IR such as oxidative stress, endothelial dysfunction, chronic inflammation, and the dysregulation of adipokines, especially lack adiponectin secretion, play a crucial role in the NAFLD development and progression [8, 10, 11]. Usually, NAFLD is diagnosed incidentally by elevated levels of aminotransferases, or during liver ultrasound (US) [12, 13]. Clinical symptoms are non-specific and usually do not correlate with morphological changes of the liver structure. Liver ultrasound (US), which is widely used in routine clinical practice for verification of NAFLD, has certain diagnostic value, but the "gold standard" of NAFLD diagnosis is a needle biopsy of the liver [12, 13]. Liver biopsy verifies the histological form of NAFLD and can estimate inflammation activity and fibrosis score [12-14]. There are very few studies comparing clinical, biochemical and morphological data in patients with NAFLD. Our research aimed to evaluate the relationship between histological signs of liver damage and risk factors of T2DM and CVD.

Material and Method:

Subjects and study design

Patients were recruited from the outpatient clinic of the Endocrinology Research Center. After applying the exclusion criteria described below, patients with abdominal obesity were examined in the Therapeutic Endocrinology Department. We included 80 adolescents of both sexes between 30 and 50 years of age (median of age 45 [41.5; 47.5]). Inclusion criteria were: abdominal obesity (waist circumference $\geq$ 80 cm in women and $\geq$ 94 cm in men, according to the criteria of MS IDF, 2006) [15] and signs of NAFLD (based on liver US). Before enrollment, all patients confirmed the absence of excessive alcohol consumption (alcohol consumption less than 40g of ethanol per day for men and 20g for women) [16]. Exclusion criteria were: type 1 or type 2 diabetes mellitus; BMI > 40 kg/m^2, secondary obesity; severe somatic and mental illnesses; alcohol abuse; use of hepatotoxic drugs; viral hepatitis; and chronic diseases of the gastrointestinal tract, accompanied by malabsorption. None of the patients received glucocorticoids, statins, fibrates, aspirin, metformin or PPARγ agonists. Some patients were on angiotensin receptor blockers, ACE-inhibitors, diuretics orβ-blockers for their arterial hypertension. This study was approved by the Ethical Committee of the Endocrinology Research Centre. Informed consent was obtained from all the participants of the study.

Anthropometric measurements

Weight, height and waist circumference (WC) were measured according to the international standards, and Body Mass Index (BMI) was calculated (kg/m^2). The median of body weight was 90.0 [82; 100] kg, BMI - 32.1 [29.4; 35.3] kg/m^-, and waist circumference - 102 [95.5; 110]. Abdominal obesity was diagnosed by IDF criteria (2006) [15].

Liver biopsy

All patients underwent a percutaneous liver biopsy in the Hepatology centre of the Moscow Regional Research Clinical Institute. Histological examination of biopsy samples of biopsy samples was carried out in the Pathology department of the Moscow Regional Research Clinical Institute. Comprehensive assessment of morphological features in the liver tissue was based on the NAS scale (NAFLD activity score, Kleiner D., 2005). The severity of steatosis, intralobular inflammatory infiltration, hepatocyte ballooning degeneration and fibrosis were estimated [14]. Histological activity index (HAI) which considers the severity of necrotic and inflammatory lesions in the liver and the stage of fibrosis were also assessed [17, 18].

Biochemical tests

Blood samples were taken from the cubital vein in the morning after at least 12 hours of fasting. Glucose, total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TG), ALT, AST were measured using Architect (Abbott) automatic biochemical analyser. Standard oral glucose tolerance test with 75g of glucose (OGTT) was conducted to determine the disorders of carbohydrate metabolism. Disorders of carbohydrate metabolism were diagnosed by WHO criteria (1999-2006). Impaired fasting glucose (IFG) was diagnosed if the level of venous plasma glucose was more than or equal to 6.1 mmol/l but less than 7.0 mmol/l fasting and less than 7.8 mmol/l at the 120th minute of OGTT. The fasting glucose level lower than 7.0 mmol/l, with an increase to 7.8 mmol/l or higher but lower than 11.1 mmol/l at the 120th minute of OGTT was regarded as impaired glucose tolerance (IGT).

Immunoreactive insulin was measured by electrochemiluminescent immunoassay (ECLA) using automatic analyser Cobas 601 (Roche). The level of insulin resistance was assessed using a mathematical model based on the values of IRI and fasting plasma glucose (FPG) with the calculation of the HOMA-IR (homeostasis model assessment of insulin resistance) index: fasting glucose (mmol/l) * fasting insulin (U/L) / 22.5. The values of HOMA-IR <2.77 were considered as normal insulin sensitivity (i.e. absence of insulin resistance).

The level of serum adiponectin was determined using BioVendor analyser (reference values 18-39 mg/ml). Serum high-sensitivity C-reactive protein (CRP) was measured by solid phase ELISA using 4000 Architect (Abbott) analyser (reference values for hs-CRP: 0,03-5,00 mg/l). The level of active plasminogen antigen activator inhibitor-1 in plasma was measured by ELISA using Technoclone PAI-1 kits (reference interval - 7-43 ng/ml).

Statistical analysis

The statistical analysis was performed using STATISTICA version 8.0 (StatSoft Inc, USA). Data is presented as median values, interquartile ranges and frequency indicators (%). Spearman rank correlation coefficients were calculated to investigate the association between morphological signs of liver damage and biochemical parameters. A P value of less than 0.05 was considered statistically significant.

Results:

As a result of the liver biopsy, NAFLD was confirmed in 77 patients (96.3%). NASH was diagnosed in the majority of patients

- 64 cases (80%), and 11 patients (13.8%) had steatosis. Mild to moderate fibrotic lesions of the liver tissue was revealed in all 77 patients with NAFLD. Liver cirrhosis was diagnosed in 2 cases (2.5%). Comparison of the liver biopsy and liver US results confirmed the high diagnostic value of liver US for NAFLD verification. Most of the patients with abdominal obesity and NAFLD had metabolic disorders: 96.6% of patients had dyslipidemia, 76.6% - carbohydrate metabolism disorders (IFG or IGT), and 94.8% had hyperinsulinemia and IR. The majority of patients (95.7%) had two or more components of metabolic syndrome. Results of the liver tissue assessment according to the NAS scale (NAFLD activity score, Kleiner D., 2005) in patients with abdominal obesity and NAFLD (n=80) are presented in Table 1.

Biochemical characteristics of the patients with abdominal obesity and NAFLD are presented in Table 2. Average total cholesterol level was 6.2 [5.9; 7.0] mg / dL, LDL - 3.9 [3.4; 4.4] mg / dL, HDL - 1.1 [1; 1,3] mmol /l, triglycerides - 2.1 [1.8; 2,5] mmol/l. In general, dyslipidemia was detected in 96.6% of patients with NAFLD and obesity. In 90.9% cases total cholesterol level was$\geq$ 5.2 mmol /l, in 96.6% cases TG level was$\geq$ 1.7 mmol/l. Decreased concentration of HDL ($\leq$ 1.03 mmol/l in men and $\leq$ 1.29 mmol/l in women) were revealed in 74% patients. 79.2% of patients showed an increase in LDL$\geq$ 3.37 mmol/l. Figure 1 depicts the relationship between TG concentrations and the severity of hepatic steatosis. The severity of hepatic steatosis in patients with abdominal obesity and NAFLD was directly related to TG concentration.

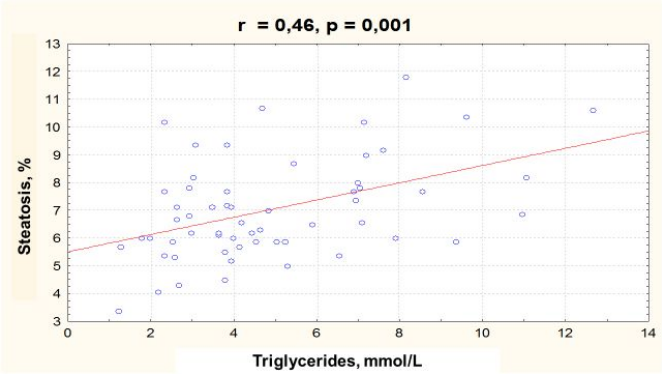

Figure 1: The relationship between plasma triglyceride concentration and steatosis severity (NAS score) in patients with abdominal obesity and NAFLD.

The median fasting plasma glucose level in the group was 6.0 [5.5; 6,3] mmol/l, after 120 min of glucose intake - 7.0 [5.9; 8.2] mmol/l. We diagnosed impaired glucose metabolism during OGTT in 76.6% of patients with NAFLD (50 patients): IFG – in 74% of cases, IGT – in 33.8% of cases. 31.2% of patients had the combination of IFG and IGT. Moreover, the incidence of IGT and IFG was significantly increased in patients with NASH (p = 0,008) in comparison with liver steatosis. Fasting insulin level was 18.7 [14; 23.7] U/l, HOMA-IR - 4.7 [3.6; 6.6]. 94.8% of patients with NAFLD had IR evidenced by values of fasting insulin and HOMA-IR. In patients with NASH, basal insulin levels and HOMA-IR index were significantly higher if compared with those who had hepatic steatosis (p = 0.005, p = 0.02, respectively).

Patients with different morphological stages of NAFLD presented different clinical results of the carbohydrate metabolism and lipid profile parameters. 72.7% persons with liver steatosis had dyslipidemia, 63.6% - glucose metabolism disorders, 63.6% - IR. 93.8%, 78.1% and 100% of patients with NASH had dyslipidemia, impaired glucose metabolism and IR respectively. In all patients with liver cirrhosis, we identified dyslipidemia, IFG, IGT and IR.

There was a direct relationship between fasting insulin level, HOMA-IR and the severity of liver biopsy lesions. For example, fasting serum insulin level (r = 0.36; p = 0.026) and HOMA-IR index (r = 0.35; p = 0.035) correlated positively with the HAI (Figure 2 and Figure 3). There was no significant correlation between insulin level, HOMA-IR index and the severity of liver fibrosis.

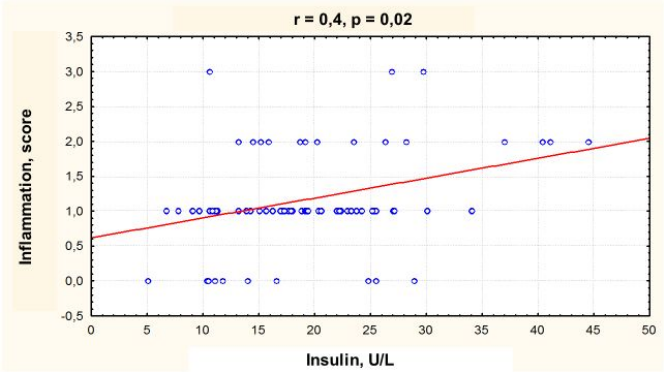

Figure 2: Relationship between plasma Insulin concentration and liver inflammation (NAS score) in patients with abdominal obesity and NAFLD.

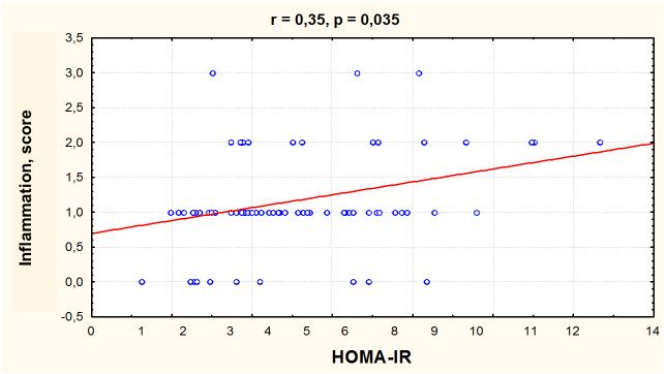

Figure 3: Relationship between HOMA-IR and liver inflammation score (NAS score) in patients with abdominal obesity and NAFLD.

Increased aminotransferases activity, especially ALT, was detected in patients with NASH more commonly than in patients with steatosis (76.6% vs 63.6%, respectively), and usually, aminotransferases levels did not exceed two standards. We identified the significant correlation of ALT and AST levels with morphological signs of liver lesions. ALT levels positively correlated with the severity of steatosis (r = 0.45; p = 0.005) and with intralobular inflammatory infiltration (r = 0.3; p = 0.019). Also, we found a positive correlation between AST levels and the severity of liver fibrosis (IFA, Ishak) (r = 0.6; p = 0.001).

Table 1 Results of liver tissue morphological assessment according to scale NAS (NAFLD activity score, Kleiner D., 2005) in patients with abdominal obesity and NAFLD

Item	Definition	Score/code	Number of patients
	NAS		
Steatosis	Low- to the medium-power evaluation of parenchymal involvement by steatosis		
	<5%	0	0
	5–33%	1	58
	>33–66%	2	17
	>66%	3	5
Lobular inflammation	Overall assessment of all inflammatory foci		
	No foci	0	6
	<2 foci per × 200 field	1	15
	2–4 foci per × 200 field	2	44
	>4 foci per × 200 field	3	15
Hepatocellular ballooning	None	0	6
	Few balloon cells	1	59
	Many cells/prominent ballooning	2	15
	Fibrosis		
Stage	None	0	0
	Perisinusoidal or periportal	1	64
	Mild, zone 3, perisinusoidal	1A	
	Moderate, zone 3, perisinusoidal	1B	
	Portal/periportal	1C	
	Perisinusoidal and portal/periportal	2	14
	Bridging fibrosis	3	0
	Cirrhosis	4	2

Table 2 Biochemical parameters and parameters of surrogate insulin resistance in patients with different morphological forms of NAFLD

Characteristics Me [25; 75]	**NAFLD (n=77)**	**Steatosis (n=11)**	**NASH (n=64)**	**Cirrhosis (n=2)**	**No NAFLD (n=3)**
Total cholesterol, mmol/L	6,2 [5,9; 7,0]	6,4 [4,7; 7,3]	6,4 [5,9; 7,1]	5,9 [5,9; 6,0]	6,4 [5,0; 9,3]
HDL, mmol/L	1,1 [1,0; 1,3]	1,3 [1,0; 1,4]	1,1 [1,0; 1,2]	1,1 [0,7; 1,6]	1,0 [1,0; 1,4]
LDL, mmol/L	3,9 [3,4; 4,4]	3,9 [2,9; 4,0]	3,9 [3,5; 4,6]	3,4 [2,6; 4,1]	4,0 [2,9; 4,4]
Triglycerides, mmol/L	2,1 [1,8; 2,5]	2,1 [1,8; 2,5]	2,5 [2,2; 2,8]	2,1 [2,0; 2,3]	2,2 [1,9; 2,4]
Glucose 0 min, mmol/L	6,0 [5,5; 6,3]	5,7 [5,5; 5,9]	6,1 [5,7; 6,4]	5,9 [5,0; 6,8]	5,2 [5,2; 5,3]
Glucose 120 min (OGTT), mmol/L	7,0 [5,9; 8,2]	6,0 [5,7; 6,8]	7,1 [5,9; 8,2]	10,0 [8,9; 11,0]	6,0 [5,0; 6,1]
Insulin, U/L	18,7 [14,0; 23,7]	14,0 [10,5; 22,2]	18,7 [14,7; 23,6]	28,3 [26,9; 29,7]	7,8 [7,4; 9,7]
HOMA-IR	4,7 [3,6; 6,6]	3,6 [2,5; 4,2]	5,1 [3,8; 6,9]	7,4 [6,6; 8,1]	1,8 [1,7; 2,2]
ALT, U/L	47,0 [36,1; 59,5]	37,0 [32,0; 45,3]	50,8 [37,5; 61,0]	46,7 [36,0; 57,4]	35,0 [29,0; 43,0]
AST, U/L	32,0 [26,0; 41,8]	25,0 [24,0; 34,5]	33,0 [27,4; 42,4]	39,4 [37,0; 41,8]	25,0 [24,0; 32,0]
Adiponectin, μ g/ml	6,1 [4,5; 9,1]	10,1 [7,4; 10,9]	5,7 [4,3;8,3]	5,4 [5,3;5,5]	15,7 [9,1; 17,5]
CRP, mg/L	3.2 [1.7;6,0]	1,1 [1,0;1,7]	3.8 [2.1;6,7]	4,7 [1,8; 7,7]	0,7 [0,5; 0,9]
PAI-1, ng/ml	103,1 [77,9; 112,0]	70,2 [59,0; 90,4]	104,5 [85,4; 115,6]	113,1 [109,4; 116,8]	81,8 [72,1; 86,5]

Adiponectin level was found to be below normal range in 88.3% (n = 68) of patients with abdominal obesity and NAFLD, confirmed by liver biopsy. Only 45.5% of patients with hepatic steatosis had low adiponectin level, while it was decreased in 89.1% of patients with NASH. Moreover, adiponectin concentration decreased with the progression of NAFLD: adiponectin level in patients with liver steatosis was 10.1 [7.4; 10.9] g/ml, in patients with NASH - 5.7 [4.3; 8.3] g/ml, in patients with cirrhosis - 5.4 [5.3; 5.5] g/ml.

Adiponectin correlated negatively with IR indicators: HOMA- IR index (r= -0.45; p= 0.023) and fasting insulin concentration (r= -0.35; p = 0.024), and positively- with serum HDL cholesterol (r= 0.45; p= 0.007). A negative correlations of adiponectin level with IGA (r= -0.56;p= 0.007) and intrabular inflammatory infiltration (r= -0.4;p= 0.013) were also observed. The correlation between the adiponectin level and the degree of liver inflammation in patients with abdominal obesity and NAFLD is presented in Figure 4.

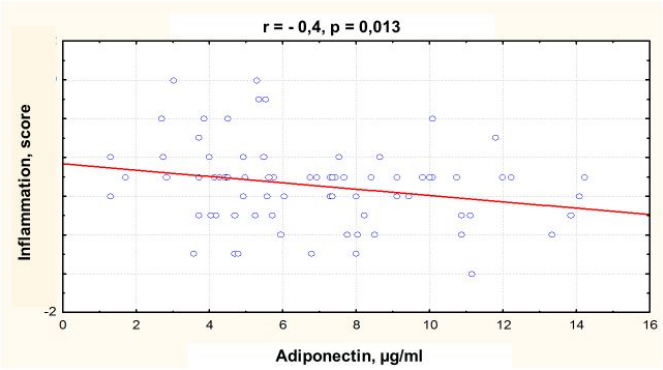

Figure 4: Relationship between serum adiponectin concentration and intralobular inflammatory infiltration (NAS score) in patients with abdominal obesity and NAFLD.

Regarding inflammatory parameters, 30% patients with NAFLD presented values of CRP above normal range values (upper limit 5.0 mg/l) considered as subclinical inflammation: median level was 3.2 [1.7; 6.0] mg/L. NASH patients had significantly higher levels of CRP than those with hepatic steatosis: 3.8 [2.1; 6.7] mg/L vs 1,1 [1.0; 1.7] mg/L, (p <0.0001). CRP correlated positively with all anthropometric measurements, as well as with fasting insulin concentration, HOMA-IR index and activity of PAI-1. CRP correlated negatively with the concentration of adiponectin. Assessment of the relationship between CRP and morphological changes in the examined patients with NAFLD demonstrated a high positive correlation between CRP levels and IGA (r = 0.4; p = 0.04).

PAI-1 activity exceeded the upper limit of the reference range (43 ng/ml) in 98.7% patients (n = 76). Moreover, in patients with NASH PAI-1 activity in plasma was 104.5 [85.4; 115.6] ng/ml and was higher than patients with hepatic steatosis (p = 0.002). PAI-1 activity correlated positively with anthropometric parameters, HOMA-IR as a measure of IR, the level of CRP, and negatively with the concentration of adiponectin. There were no statistically significant relationships between PAI-1 and morphological changes in the liver.

Discussion:

This study was carried out to evaluate the relationship between histological signs of liver damage and metabolic parameters in patients with abdominal obesity and NAFLD. In our study, most patients had NASH (80%) confirmed by liver biopsy. Our findings are not consistent with the results of cohort studies reporting the prevalence of clinical forms of NAFLD in obese people [4, 7, 19]. According to previous publications, the prevalence of NASH in this category of patients is 18.5-26%. Those discrepancies can be explained by patient selection: all patients in our study had abdominal obesity with waist circumference$\geq$ 80 cm in women and$\geq$ 94 cm in men, while in population studies, obesity is usually confirmed by BMI, disregarding the type of fat distribution [4, 19].

It is well known that the combination of visceral obesity and hypertriglyceridemia is associated with high risk of CVD. Several studies have demonstrated that waist circumference$\geq$ 90 cm in both males and females over 40 years and hypertriglyceridemia over than 2.0 mmol/l, (so-called "hypertriglyceridemic waist"), are likely to indicate the presence of the MS [5,6]. In our study, 95.7% of patients older than 40 years with abdominal obesity and NAFLD (n = 67) had "hypertriglyceridemic waist". In the NASH group "hypertriglyceridemic waist" was diagnosed in 100% of patients, in steatosis group – in 70.0%. Thus, hypertriglyceridemia in abdominal obesity can be regarded as an indirect marker of the presence of NAFLD, NASH in particular.

As expected, TG level positively correlated with the severity of hepatic steatosis (r = 0.46; p = 0.001). Excessive hepatic TG accumulation due to increased transport of free fatty acids into the liver leads to the development of steatosis and secretion of the high amount of triglycerides as a part of LDL. Alterations of TG synthesis and transport eventually result in hepatocyte damage and necrosis [9, 10].

The liver plays a significant role in maintaining glucose homeostasis by balancing the production and storage of glucose. Our results are consistent with previous data about the frequency of glucose metabolism disorders in patients with NAFLD [5, 6, 9]. We revealed IFG, IGT or their combination in 76.6% of patients. Moreover, pathophysiological and metabolic consequences of the various stages of simple steatosis and NASH are different and dependent on the severity of NAFLD but are strongly linked to hepatic and peripheral IR.

Our findings confirm that the development and progression of IR appear to be the key mediator in the initiation and progression of NAFLD, primarily through changes in glucose, fatty acid, and lipoprotein metabolism. We found a close relationship between fasting insulin, HOMA-IR and inflammation and necrosis in the liver. These results indicate the importance of therapeutic interventions, resulting in the improvement of insulin sensitivity and preventing progression of the disease and, thus, the development of T2DM.

ALT levels have shown to be the best single biochemical indicator of hepatic steatosis. Nevertheless, up to 70% of NAFLD patients may have normal liver enzymes; ALT and AST levels do not distinguish between varying stages of NASH and can be normal in histologically severe disease [20]. Our results, also confirm that normal levels of transaminases do not exclude the existence of necrotic, inflammatory and fibrotic changes in the liver [21, 22]. In our study, patients with NAFLD had following laboratory data: ALT - 47 [36.1; 59.5] U/L, AST - 32 [26.0; 41,8] U/L. Thus, liver biopsy is necessary for the refinement of clinical and morphological forms of NAFLD, staging of the disease and process activity assessment.

Adiponectin is the best-studied adipokine in NAFLD. Plasma levels of adiponectin are markedly diminished in visceral obesity

and states of IR, atherosclerosis and type 2 diabetes mellitus [23]. Hypoadiponectinemia is primarily a feature of patients with NASH. Published data shows that the adiponectin level in patients with NAFLD and obesity is significantly lower than in patients with the same BMI, but without NAFLD [24, 25]. Also, adiponectin negatively correlates with the content of fat in the liver, which confirms the protective role of adiponectin about the development of steatosis, steatohepatitis and their progression [26, 27]. Our results confirm previously published data on the significant role of low adiponectin level in NASH development. We found a negative correlation between the adiponectin level with IGA and intralobular inflammatory infiltration, but in our study adiponectin was not associated with the severity of fibrosis and hepatic steatosis.

CRP is considered to be the best marker of chronic inflammation [28]. Chronic systemic inflammation is a significant risk factor for CVD and T2DM; the inflammatory state has been proposed to underlie increased risk for atherosclerotic disease [29, 30]. In abdominal obesity, inflammation results from obesity-induced dysregulation of visceral adipocytes with the production of inflammatory cytokines. In our study serum, CRP was elevated in 30 % of patients concerning values observed in a healthy population, in agreement with other results. Taking into account positive correlation of circulating CRP level with necrotic and inflammatory lesions of liver tissue we can confirm that CRP, which is primarily produced by the liver, is a marker of inflammation and an independent predictor of CVD.

PAI-1 is widely known as an endogenous inhibitor of plasminogen activation by tissue-type and urokinase-type plasminogen activator [31]. Increased PAI-1 activity has been associated with a higher risk of MS, CVD and tissue fibrosis [32, 33]. There is increasing evidence that implies that PAI-1 mediates the development of hepatic steatosis in MS [34]. Clinically, a steady elevation of plasma and hepatic PAI-1 levels correlates with the progression of NAFLD [35]. We found elevated PAI-1 activity in most of the patients with NAFLD that correlated with IR and CRP, but not with the morphological stage of liver disease. Our data suggest that the development of the fibrinolysis failure (and hence prothrombogenic state) is associated with chronic inflammation and IR in patients with abdominal obesity and NAFLD. It also indicates an increased risk of thrombosis in this category of patients.

Conclusions:

Some long-term prospective studies in patients with biopsy-proven NAFLD show significantly higher total mortality rates compared with a matched reference population—with CVD representing the primary mode of death [36, 37]. NAFLD contributes to the deterioration of life prognosis by a combination of hypertriglyceridemia with IR and the reduction of protective factors. Of note, only subjects with NASH rather than simple steatosis had significantly reduced survival [37].

The current study provides evidence that metabolic disorders in patients with abdominal obesity and NAFLD are directly related to the morphological stage of the disease. Due to the invasive nature and potentially risky procedure of liver biopsy, clinical and biochemical blood markers can be considered to allow improved, non-invasive detection of disease stage and activity. As NASH is associated with more metabolic abnormalities than simple hepatic steatosis, we may predict NASH with a high probability in patients with hypertriglyceridemia, impaired glucose tolerance and elevated CRP level.

Acknowledgements

We thank Doctor Alexander Ilyin and the staff of the Clinical Biochemistry Department (Endocrinology Research Centre) for performing biochemical and hormonal assays. We especially thank the staff of the Department of Pathology of Moscow Regional Research Clinical Institute of M.F.Vladimirsky for the morphological evaluation of liver tissue.

Disclosure

The authors have nothing to disclose. None of the authors had a conflict of interest.

Competing Interests

The authors declare no conflict of interest.

References

1. Ong J, Younossi Z. Epidemiology and natural history of NAFLD and NASH. Clin Liver Dis 2007; 11: 1-16, vii.

2. Leite N, Salles G, Araujo A, et al. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. Liver Int 2009; 29:113-119.

3. Assy N, Kaita K, Mymin D, et al. Fatty infiltration of liver in hyperlipidemic patients. Dig Dis Sci 2000; 45: 1929-1934.

4. Lazo M, Clark J. The epidemiology of nonalcoholic fatty liver disease: a global perspective. Semin Liver Dis 2008; 28(4): 339-50.

5. Misra V, Khashab M, Chalasani N. Nonalcoholic Fatty Liver Disease and Cardiovascular Risk. Current Gastroenterology Reports 2009, 11: 50–55.

6. Stefan N, Kantartzis K, Häring H. Causes and Metabolic Consequences of Fatty Liver. Endocrine Reviews 2008; 29(7): 939-60.

7. Musso G, Gambino R, Cassader M. Non-alcoholic fatty liver disease from pathogenesis to management: an update. Obesity Reviews 2010; 11(6): 430–45.

8. Bugianesi E, Gastaldelli A, Vanni E, et al. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. Diabetologia 2005; 48: 634-42.

9. Dowman J, Tomlinson J, Newsome P. Pathogenesis of non-alcoholic fatty liver disease. QJM: 2010; 103(2): 71-83.

10. Milner K, Van der Poorten D, Xu A, et al. Adipocyte fatty acid binding protein levels relate to inflammation and fibrosis in nonalcoholic fatty liver disease. Hepatology 2009; 49(6): 1926-34.

11. Tilg H, Diehl A, Li Z et al. Cytokines and the pathogenesis of non-alcoholic steatohepatitis. Gut 2005; 54: 303-306.

12. Chalasani N, Younossi Z, Lavine J.E, et al. The Diagnosis and Management of Non-alcoholic Fatty Liver Disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Am. J. Gastroenterol; 2012; 107:811–26.

13. EASL–EASD–EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD) and European Association for the Study of Obesity (EASO). Journal of Hepatology; June 2016; 64(6): 1388–1402.

14. Kleiner D, Brunt E, Van Natta M, et al. Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005; 41(6): 1313-1321.

15. International Diabetes Federation. Worldwide Definition of the Metabolic Syndrome.http://www.idf.org/e-library/consensus-statements/60-idfconsensus-worldwide-definitionof-the-metabolic-syndrome.html (Accessed 2006)

16. World Health Organization. Global Status Report on Alcohol 2004.http://www.who.int/substance_abuse/

publications/global_status_report_2004_overview.pdf

17. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995; 22: 696-699.

18. Knodell R, Ishak K, Black W, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981; 1:431-435.

19. Vernon G, Baranova A, Younossi Z. Systematic review: the epidemiology and natural history of non-alcoholic fatty iver disease and nonalcoholic steatohepatitis in adults. Aliment Pharmacol Ther 2011; 34: 274-285.

20. Szczepaniak L, Nurenberg P, Leonard D, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol Endocrinol Metab 2005; 288: E462–E468.

21. Kotronen A, Westerbacka J, Bergholm R, et al. Liver fat in the metabolic syndrome. J Clin Endocrinol Metab 2007; 92: 3490–3497.

22. Mofrad P, Contos M, Haque M, et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology 2003; 37: 1286–1292.

23. Adolph T., Grander C., Grabherr F., Tilg H. Adipokines and non-alcoholic fatty liver disease: multiple Interactions. Int J Mol Sci 2017; 18: 1649.

24. Polyzos S, Toulis K, Goulis D, et al. Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. Metabolism 2011; 60: 313–326.

25. Aygun C, Senturk O, Hulagu S, et al. Serum levels of hepatoprotective peptide adiponectin in non-alcoholic fatty liver disease. Eur J Gastroenterol Hepatol 2006; 18(2): 175-80.

26. Bugianesi E, Pagotto U, Manini R, et al. Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. J Clin Endocrinol Metab 2005; 90: 3498–504.

27. Cnop M, Havel P, Utzschneider K. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasms lipoproteins:evidence for independent roles of age and sex. Diabetologia 2003; 46: 459-69.

28. Bassuk S, Rifai N, Ridker P. High-sensitivity C-reactive protein: clinical importance. Curr Probl Cardiol 2004; 29: 439-493.

29. Jeppesen J, Hansen T, Olsen M, et al. C-reactive protein, insulin resistance and risk of cardiovascular disease: a population-based study. European Journal of Cardiovascular Prevention & Rehabilitation 2008; 15: 594-598.

30. Brea A, Mosquera D, Martin E, et al. Nonalcoholic Fatty Liver Disease Is Associated With Carotid Atherosclerosis: A Case-Control Study. Arterioscler Thromb Vasc Biol 2005; 25: 1045–1050.

31. Ghosh A, Vaughan D. PAI-1 in tissue fibrosis. J Cell Physiol. 2012; 227:493–507.

32. Skurk T, Hauner H. Obesity and impaired fibrinolysis: role of adipose production of plasminogen activator inhibitor-1. Int J Obes Relat Metab Disord 2004; 28(11): 1357–64.

33. Trost S, Pratley R, Sobel B. Impaired fibrinolysis and risk for cardiovascular disease in the metabolic syndrome and type II diabetes. Curr Diab Rep 2006; 6: 47–54.

34. Lee S, Dorotea D, Jung I, et al. TM5441, a plasminogen activator inhibitor-1 inhibitor, protects against high fat diet-induced non-alcoholic fatty liver disease. Oncotarget 2017; 8(52): 89746-89760

35. Verrijken A, Francque S, Mertens I, et al. Prothrombotic factors in histologically proven nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Hepatology 2014; 59:121–129.

36. Soderberg C, Stal P, Askling J, et al. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. Hepatology 2010; 51:595–602.

37. Ekstedt M, Franzen L, Mathiesen U, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 2006; 44:865–873.