



## Obesity and metabolic syndrome as risk factors for the development of non-alcoholic fatty liver disease as diagnosed by ultrasound

Gojaznost i metabolički sindrom kao faktori rizika od razvoja nealkoholne masne bolesti jetre dijagnostikovane ultrazvukom

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### Abstract

**Background/Aim.** Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease of a broad histological spectrum, characterized by the accumulation of triglycerides in more than 5% of hepatocytes in the absence of consuming alcohol in quantities harmful to the liver. The aim of our study was to determine the importance of anthropometric and laboratory parameters as well as metabolic syndrome for the diagnosis of NAFLD and to estimate their influence on the degree of liver steatosis as evaluated by ultrasound. **Methods.** The study included 86 participants, 55 of whom had fatty liver diagnosed by ultrasound and they comprised the study group. The control group consisted of 31 subjects with no liver diseases. During the course of hospitalization at the Clinic of Gastroenterology and Hepatology, Clinical Centre Niš, the patients had their anamnesis taken, and anthropometric measurements as well as biochemical blood analyses and abdominal ultrasound were performed. **Results.** The patients with NAFLD had statistically higher values of body mass index (BMI), waist circumference (WC), systolic (SBP) and diastolic blood pressure (DBP), levels of alanine and aspartate aminotransferase (ALT, AST), gamma-glutamyl transpeptidase (GGT) ( $p < 0.001$ ), low-density lipoprotein cholesterol (LDL), total bilirubin (TBIL) ( $p < 0.05$ ), total cholesterol ( $p < 0.01$ ), triglycerides (TGL), urates, C-reactive protein (CRP), ferritin, fibrinogenes, fasting blood glucose (FBG), insulin and Homeostasis Model Assessment (HOMA-IR) ( $p < 0.001$ ) compared to the control group, whereas the levels of high-density lipo-

protein cholesterol (HDL) were higher in the control than in the study group ( $p < 0.05$ ). In the NAFLD group, there were statistically significantly more patients with hypertension (72.73% vs 12.90%,  $p < 0.001$ ) and type 2 diabetes mellitus (DM) (47.27%). Metabolic syndrome was established in 48 (87.27%) patients of the study group. The equal number of patients, *ie* 16 (29.09%), had 3, 4 and 5 components of MS. In the NAFLD group there were 17 (30.91%) overweight (BMI from 25 kg/m<sup>2</sup> to 29.9 kg/m<sup>2</sup>) and 38 (69.09%) obese patients (BMI  $\geq$  30.0 kg/m<sup>2</sup>). The largest number of patients in the obesity group, 22 (40.00%) of them, had the first degree obesity (BMI from 30 kg/m<sup>2</sup> to 34.99 kg/m<sup>2</sup>). The largest number of the NAFLD group patients, 23 (41.82%), had an ultrasound finding of grade 3 fatty liver, 20 (36.36%) patients had grade 2 and 12 (21.82%) grade 1 fatty liver. Kruskal-Wallis test and ANOVA analysis showed statistically significant differences between groups with different US grade for insulin, LDL-cholesterol, WC, BMI ( $p < 0.05$ ), as well as HOMA-IR and body weight (BW) ( $p < 0.01$ ). Metabolic syndrome was statistically more present in patients with US finding grades 2 and 3 ( $p < 0.01$ ) in relation to US finding grade 1, as well as obesity, hypertension and DM type 2 ( $p < 0.05$ ). **Conclusion.** The results of our study confirm that a high percentage of patients with high risk factors (DM, MS, dyslipidemia, hypertension) have NAFLD.

**Key words:** obesity; metabolic syndrome x; ultrasonography; diagnosis; non-alcoholic fatty liver disease; risk factors.

### Apstrakt

**Uvod/Cilj.** Bolest nealkoholne masne jetre (NAFLD) je hronično oboljenje jetre, širokog histološkog spektra, koje karakteriše akumulacija triglicerida u više od 5% hepatocita u odsustvu konzumiranja alkohola u količinama štetnim za jetru. Cilj našeg istraživanja bio je da se utvdi značaj antropometrijskih, laboratorijskih parametara i metaboličkog sin-

droma u dijagnozi NAFLD i da se proceni njihov uticaj na stepen steatoze jetre procenjene ultrazvukom. **Metode.** Istraživanjem je bilo obuhvaćeno 86 ispitanika, od kojih je 55 imalo masnu jetru, dokazanu ultrazvukom, i oni su činili studijsku grupu. Kontrolnu grupu činio je 31 ispitanik bez bilo kakve bolesti jetre. Prilikom hospitalizacije u Klinici za gastroenterologiju i hepatologiju Kliničkog centra Niš, ispitanicima je uzeta anamneza, izvršena su antropometrijska

merjenja, biohemijske analize krvi i ultrazvučni pregled abdomena. **Rezultati.** Bolesnici sa NAFLD imali su statistički značajno povećan *body mass index* (BMI), obim struka (OS), sistolni i dijastolni krvni pritisak, vrednosti aspartat i alanin aminotransferaze (AST, ALT), gama-glutamil transpeptidaze (GGT) ( $p < 0,001$ ), lipoproteina niske gustine (LDL), ukupnog bilirubina ( $p < 0,05$ ), ukupnog holesterola ( $p < 0,01$ ), triglicerida, urata, C reaktivnog proteina (CRP), feritina, fibrinogena, glikernije našte, insulina i *Homeostasis Model Assessment* (HOMA-IR) ( $p < 0,001$ ), dok je vrednost lipoproteina visoke gustine (HDL) bila veća u kontrolnoj grupi ( $p < 0,05$ ). U NAFLD grupi bilo je statistički značajno više bolesnika sa hipertenzijom (72,73% vs 12,90%,  $p < 0,001$ ) i sa diabetesom melitusom (DM) tipa 2 (47,27%). Metabolički sindrom utvrđen je kod 48 (87,27%) bolesnika studijske grupe. Podjednak broj bolesnika, 16 (29,09%), imao je 3, 4 i 5 komponenti MS. U NAFLD grupi bilo je 17 (30,91%) bolesnika sa predgojaznošću (BMI od 25 kg/m<sup>2</sup> do 29,9 kg/m<sup>2</sup>) i 38 (69,09%) gojaznih (BMI  $\geq$  30,0 kg/m<sup>2</sup>) bolesnika. Najveći broj bolesnika u

grupi gojaznih, 22 (40,00%), bio je sa prvim stepenom gojaznosti (BMI od 30 kg/m<sup>2</sup> do 34,99 kg/m<sup>2</sup>). Najveći broj bolesnika NAFLD grupe, 23 (41,82%), imao je ultrazvučni nalaz masne jetre gradusa 3, 20 (36,36%) gradusa 2 i 12 (21,82%) gradusa 1. Kruskal-Wallis test i ANOVA analiza pokazali su da postoje statistički značajne razlike između grupa sa različitim ultrazvučnim gradusom za insulin, LDL holesterol, OS, BMI ( $p < 0,05$ ), kao i HOMA-IR i telesnu masu (TM) ( $p < 0,01$ ). Metabolički sindrom bio je statistički zastupljeniji kod ispitanika sa UZ gradusom 2 i 3 ( $p < 0,01$ ) u odnosu na UZ gradus 1, kao i gojaznost, hipertenzija i DM tip 2 ( $p < 0,05$ ). **Zaključak.** Rezultati naše studije potvrđuju da veliki procenat bolesnika sa faktorima visokog rizika (DM, MS, dislipidemija, hipertenzija) ima NAFLD.

#### Ključne reči:

**gojaznost; metabolički sindrom x; ultrasonografija; dijagnoza; jetra, masna infiltracija, nealkoholna; faktori rizika.**

## Introduction

Non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease in western countries in the past twenty years, with the prevalence of 20–30% in adults<sup>1</sup>. The prevalence of NAFLD increases parallelly with the epidemics of obesity and type 2 diabetes mellitus (DM), which are the risk factors for developing primary NAFLD<sup>2,3</sup>. The disease was first described as an entity in 1980 by Ludwig et al.<sup>4</sup> It is considered that primary NAFLD represents hepatic manifestation of metabolic syndrome (MS), and insulin resistance (IR) is the key pathophysiological mechanism<sup>5,6</sup>. Non-alcoholic fatty liver disease is histologically categorized into non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH)<sup>7</sup>. Non-alcoholic fatty liver is a benign, non-progressive liver disease, which is histologically characterized by the presence of macrovesicular steatosis in more than 5% of hepatocytes in the absence of consuming alcohol in quantities harmful to the liver, whereas NASH means the presence of hepatic steatosis, lobular inflammation (acute and/or chronic) with hepatocyte damage (ballooning degeneration), with or without accompanying fibrosis<sup>1,6</sup>.

Within NAFLD spectrum, only patients with NASH develop progressive liver damage. NASH progresses to cirrhosis in 10–20% of cases, or more rarely to hepatocellular carcinoma (HCC)<sup>8–10</sup>. NAFLD is usually asymptomatic, clinically silent disease. It is mostly diagnosed incidentally, during routine laboratory blood tests, when higher transaminase values are detected, particularly alanine aminotransferase (ALT) or when ultrasound (US) examination shows fatty liver<sup>3,6,11</sup>. More than 80% of patients with NAFLD have normal transaminase values which can remain unchanged even during disease progression<sup>12–14</sup>. Therefore, NAFLD should be suspected in patients with determined risk factors. In establishing the diagnosis of primary NAFLD, four criteria should be met: confirmation of liver steatosis by imaging methods or pathohistologically; absence of consuming alcohol in significant quantities (less than 21 alcohol units for

men and 14 units for women on a weekly basis); exclusion of other causes of liver steatosis, i.e. “secondary” NAFLD and exclusion of other etiological factors of chronic liver disease<sup>7</sup>.

The primary and mostly used diagnostic method for screening asymptomatic patients with higher aminotransferase values and suspicion of NAFLD is US<sup>15</sup>.

Ultrasound is a non-invasive, cheap, available method, with the sensitivity of 60–94%, and specificity of 66–97%<sup>16</sup>. Ultrasound changes in patients with NAFLD are characterized by hepatomegaly, hyperechogenicity of liver parenchyma (“bright” liver), hepatorenal contrast, attenuation of ultrasound waves in subcapsular regions, difficult visualization of the portal vein, gallbladder wall, liver capsule and blood vessels<sup>17,18</sup>. However, US has certain disadvantages. The sensitivity and specificity of US in diagnosis of NAFLD is significantly lower in obese patients and if steatosis is less than 30%<sup>19,20</sup>. It is not possible to differentiate steatosis from steatohepatitis and fibrosis by US<sup>15,21</sup>. Therefore, liver biopsy is still the gold standard in diagnosing NAFLD.

The aim of our research was to determine the importance of laboratory and anthropometric parameters as well as MS in diagnosing NAFLD and to estimate their influence on the degree of liver steatosis as evaluated by US.

## Methods

Prospective study was carried out in the period from January 2012 to October 2014, at the Clinic of Gastroenterology and Hepatology, Clinical Center Niš. It included 86 participants, 55 of whom had fatty liver infiltration diagnosed by US. They comprised the study group. The control group consisted of 31 subjects, where the diagnosis of non-alcoholic fatty liver disease and other liver diseases were ruled out on the basis of anamnestic data, biochemical blood analyses and US examination. Inclusion criteria in the study were patients with higher transaminase values and echoso-

nographic finding of fatty liver. Exclusion criteria from research were alcohol consumption (more than 30 g/day for men, and 20 g/day for women), use of hepatotoxic drugs, the presence of metabolic or genetic liver diseases (Wilson's disease, hemochromatosis,  $\alpha$  1-antitripsin deficiency), acute and chronic virus hepatitis (hepatitis B and hepatitis C), autoimmune liver disease (primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis). The participants signed the informed consent and thus confirmed that their anamnestic, laboratory and histopathological findings could be used for the purpose of this study.

During hospitalization demographic data and a detailed anamnesis were taken from the patients regarding presence of hypertension, diabetes, existing liver diseases, use of hepatotoxic drugs and alcohol consumption.

Physical examination, anthropometric measurements, biochemical blood analyses and abdominal US were performed. Anthropometric measurements included measurement of body weight (BW), body height (BH) and waist circumference (WC). Body weight (kg) of patients was measured in light clothes, without shoes. Body height (cm) was measured using the standard measuring equipment and the scale.

Waist circumference (cm) and body mass index (BMI) were estimated in each patient according to criteria of the World Health Organization<sup>22</sup>. Waist circumference was taken by flexible meter in standing position, midway between the lower edge of the rib cage and *crista iliaca* horizontally. On the basis of the given values of BW and BH we calculated the value of BMI, as the ratio of BW in kilograms and body height in m<sup>2</sup> (kg/m<sup>2</sup>). Following these values of BMI, the patients were divided in several groups: BMI from 25 to 29.9 kg/m<sup>2</sup> – overweight or preobesity, BMI from 30 to 34.99 kg/m<sup>2</sup>, class I or mild obesity, BMI from 35 to 39.99 kg/m<sup>2</sup> class II or moderate obesity, BMI higher than 40 kg/m<sup>2</sup>, class III or severe obesity.

Arterial pressure was measured by sphygmomanometer in a seating position, after resting the patient for 10 minutes. Values of systolic (SBP) and diastolic blood pressure (DBP) were taken (mmHg). All examinees included in the study had their complete blood count (CBC) and biochemical blood analysis taken in the Central Laboratory, Clinical Centre Niš (Beckman Coulter, AU680). Biochemical blood analyses included C-reactive protein (CRP), activity of ALT and aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), values of total bilirubin (TBIL) and direct bilirubin (DBIL), urates, ferritin, transferrin saturation, ceruloplasmin, iron, fasting blood glucose (FBG), insulin, values of total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TGL), total proteins and albumins, prothrombin time, international normalized ratio (INR) and fibrinogen. Ratio AST/ALT was determined. Insulin resistance was estimated by model formula Homeostasis Model Assessment<sup>23</sup> (HOMA):

$$\text{HOMA-IR} = \frac{\text{glycaemia (mmol/L)} \times \text{insulin (mU/L)}}{22.5}$$

After detailed examination of the available medical documentation of patients, in certain cases (incomplete medical

documentation or diagnostic dilemma) additional serological analyses were performed including: serological examination of viral hepatitis B and C (HBs Ag, anti HCV At), immune complexes, immunoglobulins (IgG, IgA, IgM) and pathological antibodies (antimitochondrial, anti-smooth muscle, antinuclear antibodies) and determining the value of  $\alpha$  1-antitripsin.

The presence of MS and its components were analyzed in each patient using the American National Cholesterol Program definition (The National Cholesterol Educational Program Adult Treatment Panel – NCEP– ATPIII)<sup>24</sup>. Metabolic syndrome is present if the patient shows at least three out of the following five components: central abdominal obesity (WC > 102 cm in men, and > 88 cm in women, respectively), increased triglyceride level:  $\geq 1.7$  mmol/L, lower HDL-cholesterol level: < 1.03 mmol/L in men, and < 1.29 mmol/L in women respectively, higher blood pressure: systolic  $\geq 130$  mmHg and/or diastolic  $\geq 85$  mmHg, or already treated hypertension; increased FBG:  $\geq 5.6$  mmol/L, or existing type 2 DM.

Liver US was performed in the morning with previous regime of abstaining from food the night before the examination (instrument ACUSION Siemens model X 300 and ultrasound probe of 3.5 MHz).

We evaluated the size and structure of the liver parenchyma, echo contrast between the liver and the right kidney, degree of parenchyma echogenicity, degree of blood vessels visualization, the diaphragm, as well as the posterior segment of the right lobe, or the degree of attenuation of US waves.

The degree of liver parenchyma fatty infiltration or steatosis evaluated by US can be divided into three stages depending on the severity of US changes: grade 1 US finding, mild steatosis which is shown on US as moderately hyperechogenic parenchyma, with the visible portal vein and the diaphragm (Figure 1A); grade 2 US finding, mild steatosis when the parenchyma is more prominently hyperechogenic, so that intrahepatic blood vessels and diaphragm are less visible (Figure 1B); grade 3 US finding – the liver is highly hyperechogenic, without possibility of good visualization of the portal vein, diaphragm, posterior segment of the right lobe, that is, attenuation of US waves is present<sup>25</sup> (Figure 1 C).

This study was approved by the Ethical Committee of the Faculty of Medicine, University of Niš.

### Statistical analysis

Continuous parameters were shown by the mean values, standard deviations and medians. Attributive parameters were presented in frequencies and percentages. Normality of distribution of continuous variables was examined by Shapiro-Wilk or Kolmogorov-Smirnov test depending on the size of examinee groups. Values of continuous parameters of two independent samples were compared by Mann-Whitney and Student *t*-test of independent samples, while comparison of 3 independent samples was performed by Kruskal-Wallis test or ANOVA (depending on the normality of distribution of

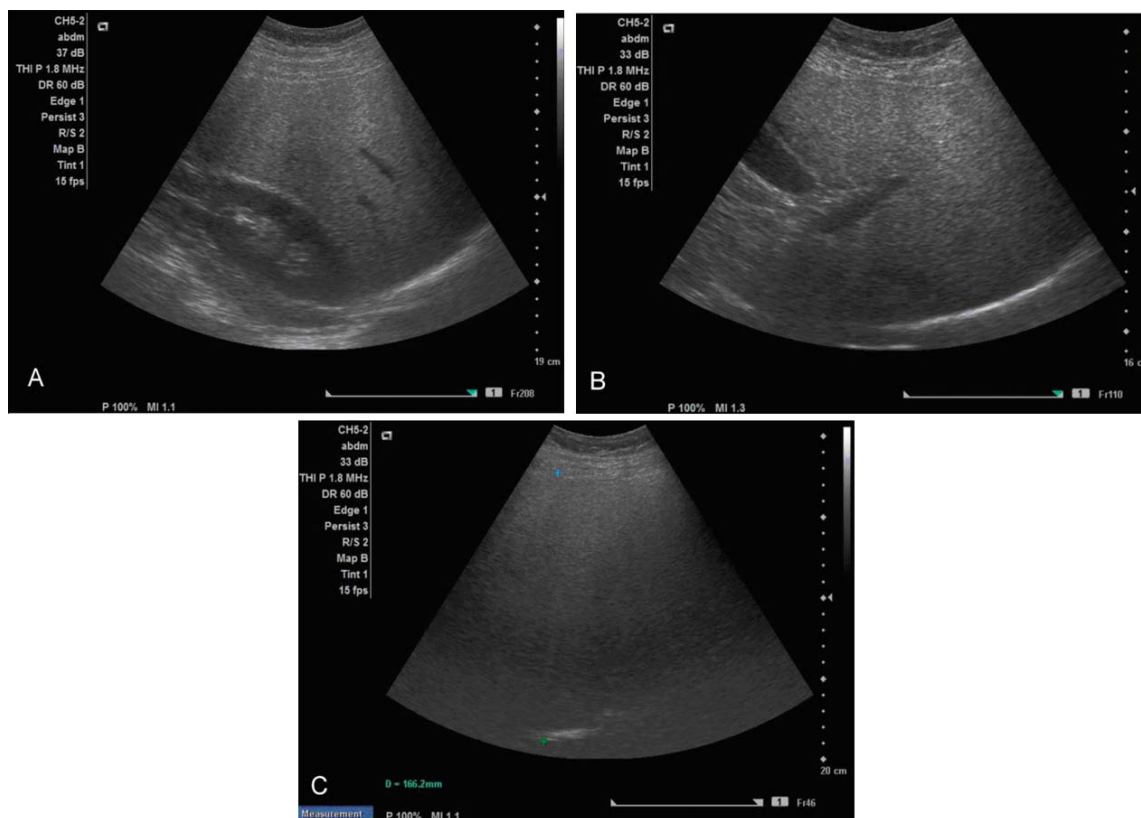


Fig. 1 – Ultrasound findings of liver steatosis: A) grade 1; B) grade 2, and C) grade 3.

variables compared), Pearson  $\chi^2$  test, and, if necessary, in case of contingency  $2 \times 2$  table, Mantel-Haenzel or Fisher's exact probability test for comparing frequency and distribution of attributive parameter modalities.

## Results

Eighty-six participants were included in the study. The NAFLD (study) group was comprised of 55 (63.95%) patients, whereas the control group included 31 (36.05%) subjects. The mean age of patients [ $x \pm SD$  (median)] in the NAFLD group was  $49.29 \pm 12.95$  (52.00) and in the control group  $47.84 \pm 10.08$  (49.00) years (Table 1). In the NAFLD group, there were 23 (41.82%) male and 32 (58.18%) female patients, and in the control group 10 (32.26%) male, and 21 (67.74%) female subjects. A statistically significant difference in age and sex of participants in the NAFLD and control groups was not found.

The BMI value was statistically significantly higher in the NAFLD group in reference to the control group ( $32.83 \pm 4.20$  vs  $22.52 \pm 2.08$  kg/m<sup>2</sup>), as well as WC ( $106.36 \pm 8.44$  cm vs  $78.87 \pm 7.18$  cm) ( $p < 0.001$ ) (Table 1).

In the NAFLD patients, statistically significantly higher values of LDL-cholesterol were found, as well as those of TBIL ( $p < 0.05$ ), total cholesterol ( $p < 0.01$ ), TGL, urates, CRP, ferritin, fibrinogene, FBG, insulin and HOMA-IR ( $p < 0.001$ ), while the value of HDL-cholesterol was higher in the control group ( $p < 0.05$ ). Values of INR and platelets did not statistically differ in the examined groups. In the NAFLD

group, the values of AST, ALT, GGT ( $p < 0.001$ ) and ALP ( $p < 0.01$ ) were significantly higher (Table 1).

In the NAFLD group, there were statistically significantly more patients with hypertension (72.73% vs 12.90%,  $p < 0.001$ ) (Table 2), with values of systolic and diastolic blood pressure significantly higher when compared to the control group ( $p < 0.001$ ) (Table 1). Type 2 DM was present in 26 (47.27%) patients of the NAFLD group, while there were no diabetes patients in the control group ( $p < 0.001$ ) (Table 2).

Most patients in the NAFLD group, 23 (41.82%) of them, had an US finding of fatty liver grade 3, and there were 20 (36.36%) patients with grade 2 finding and 12 (21.82%) with grade 1 finding.

Kruskal-Wallis test and ANOVA analysis showed statistically significant differences between the groups with different US grade for insulin, LDL-cholesterol, WC, BMI ( $p < 0.05$ ), as well as HOMA-IR and BW ( $p < 0.01$ ) (Table 3).

Based on Student's *t*-test and Mann-Whitney test, by comparing the groups with different US grades separately, the patients from the NAFLD group with grade 2 US finding had BMI values significantly higher in reference to those from the group with grade 1 US finding,  $33.26 \pm 4.16$  (33.29) vs  $29.85 \pm 3.11$  (33.26) kg/m<sup>2</sup> ( $p < 0.05$ ). Also, the patients with grade 3 US finding, in comparison with grade 1, had statistically significantly higher mean values of WC ( $109.26 \pm 7.76$  vs  $100.75 \pm 6.15$  cm) and BMI ( $34.01 \pm 4.12$  vs  $29.85 \pm 3.11$  kg/m<sup>2</sup>) ( $p < 0.01$ ). Body weight mean value was the highest in the patients with grade 3 US finding, statistically significantly higher than in the patients with grade 2 ( $p < 0.05$ ) and 1 ( $p < 0.001$ ) (Table 3) findings.

**Table 1**  
**Demographic, anthropometric and biochemical parameters in the patients with non-alcoholic fatty liver disease (NAFLD) and control group subjects**

Parameter	NAFLD group (n = 55)		Control group (n = 319)	
	$\bar{x} \pm SD$	Md	$\bar{x} \pm SD$	Md
Sex, n (%)				
male	23 (41.82)		10 (32.26)	
female	32 (58.18)		21 (67.74)	
Age (yrs)	49.29 $\pm$ 12.95	(52.00)	47.84 $\pm$ 10.08	(49.00)
WC (cm)	106.36 $\pm$ 8.44 <sup>‡</sup>	(105.00)	78.87 $\pm$ 7.18	(79.00)
BW (kg)	92.22 $\pm$ 14.83 <sup>‡</sup>	(92.00)	65.00 $\pm$ 8.90	(62.00)
BH (m)	1.68 $\pm$ 0.12	(1.65)	1.70 $\pm$ 0.09	(1.68)
BMI (kg/m <sup>2</sup> )	32.83 $\pm$ 4.20 <sup>‡</sup>	(32.87)	22.52 $\pm$ 2.08	(22.77)
SBP (mmHg)	137.18 $\pm$ 18.60 <sup>‡</sup>	(140.00)	116.45 $\pm$ 13.86	(120.00)
DBP (mmHg)	84.73 $\pm$ 10.69 <sup>‡</sup>	(90.00)	72.90 $\pm$ 9.98	(70.00)
Urates ( $\mu$ mol/L)	373.01 $\pm$ 94.55 <sup>‡</sup>	(375.10)	239.98 $\pm$ 56.57	(237.40)
TBIL ( $\mu$ /L)	12.78 $\pm$ 5.66 <sup>*</sup>	(12.20)	10.17 $\pm$ 2.91	(9.90)
DBIL ( $\mu$ /L)	2.23 $\pm$ 1.08	(2.00)	1.81 $\pm$ 0.54	(1.60)
Albumins (g/L)	45.10 $\pm$ 3.09	(45.20)	44.05 $\pm$ 3.84	(44.90)
Total cholesterol (mmol/L)	5.86 $\pm$ 1.06 <sup>†</sup>	(5.82)	5.16 $\pm$ 1.08	(5.12)
HDL (mmol/L)	1.15 $\pm$ 0.25	(1.13)	1.28 $\pm$ 0.28 <sup>*</sup>	(1.34)
LDL (mmol/L)	3.76 $\pm$ 0.92 <sup>*</sup>	(3.80)	3.26 $\pm$ 0.87	(3.30)
TGL (mmol/L)	2.28 $\pm$ 1.12 <sup>‡</sup>	(1.91)	1.20 $\pm$ 0.37	(1.08)
CRP (mg/L)	6.20 $\pm$ 10.38 <sup>‡</sup>	(3.40)	1.69 $\pm$ 1.63	(1.10)
Ferritin ( $\mu$ /L)	145.38 $\pm$ 113.45 <sup>‡</sup>	(113.90)	45.65 $\pm$ 28.17	(39.30)
INR	1.07 $\pm$ 0.11	(1.06)	1.08 $\pm$ 0.08	(1.06)
Fibrinogen (g/L)	4.47 $\pm$ 1.02 <sup>‡</sup>	(4.27)	3.48 $\pm$ 0.86	(3.55)
Platelets ( $\times 10^9$ /L)	242.69 $\pm$ 66.60	(235.00)	253.90 $\pm$ 50.48	(261.00)
AST (U/L)	40.17 $\pm$ 21.82 <sup>‡</sup>	(34.80)	20.84 $\pm$ 4.25	(20.10)
ALT (U/L)	59.56 $\pm$ 43.94 <sup>‡</sup>	(52.50)	16.85 $\pm$ 6.23	(16.10)
ALP (U/L)	71.85 $\pm$ 28.67 <sup>†</sup>	(63.10)	55.50 $\pm$ 16.56	(49.80)
GGT (U/L)	61.62 $\pm$ 67.10 <sup>‡</sup>	(38.40)	19.45 $\pm$ 14.97	(14.70)
FBG (mmol/L)	6.56 $\pm$ 2.38 <sup>‡</sup>	(6.00)	5.00 $\pm$ 0.74	(5.00)
Insulin ( $\mu$ U/L)	39.16 $\pm$ 28.88 <sup>‡</sup>	(4.27)	12.41 $\pm$ 4.37	(13.00)
HOMA-IR	13.67 $\pm$ 18.88 <sup>‡</sup>	(7.47)	2.77 $\pm$ 1.07	(2.88)

\* $p < 0.05$ . <sup>†</sup> $p < 0.01$ . <sup>‡</sup> $p < 0.001$ ;  $\bar{x}$  – mean value; SD – standard deviation; Md – median; WC – waist circumference; BMI – body mass index; BH – body height; BW – body weight; SBP – systolic blood pressure; DBP – diastolic blood pressure; TBIL – total bilirubin; DBIL – direct bilirubin; LDL – low density lipoprotein cholesterol; HDL – high density lipoprotein cholesterol; TGL – triglycerides; CRP – C reactive protein; INR – international normalized ratio; AST – aspartate aminotransferase; ALT – alanine aminotransferase; ALP – alkaline phosphatase; GGT – gamma-glutamyl transpeptidase; FBG – fasting blood glucose; HOMA-IR – homeostasis model assessment.

**Table 2**  
**Prevalence of diabetes mellitus (DM) and hypertension in the non-alcoholic fatty liver disease (NAFLD) and control group subjects**

Parameter	NAFLD group (n = 55)	Control group (n = 31)
	n (%)	n (%)
Hypertension	40 (72.73) <sup>‡</sup>	4 (12.90)
DM type 2	26 (47.27) <sup>‡</sup>	0 (0.00)

<sup>‡</sup> $p < 0.001$ .

**Table 3**  
**Demographic, anthropometric and biochemical parameters of non-alcoholic fatty liver disease (NAFLD) patients in relation to ultrasound (US) grades**

Parameter	NAFLD US grade 1 (n = 12)		NAFLD US grade 2 (n = 12)		NAFLD US grade 3 (n = 12)	
	$\bar{x} \pm SD$	Md	$\bar{x} \pm SD$	Md	$\bar{x} \pm SD$	Md
Age (years)	48.00 ± 15.18	(53.00)	50.80 ± 12.74	(54.00)	48.65 ± 12.36	(52.00)
FBG (mmol/L)	5.49 ± 0.93	(5.35)	7.10 ± 2.97 <sup>a*</sup>	(6.00)	6.64 ± 2.24 <sup>a*</sup>	(6.10)
Insulin (mu/L)*	22.92 ± 8.16	(22.00)	48.03 ± 33.13 <sup>a†</sup>	(35.40)	39.92 ± 29.15 <sup>a*</sup>	(30.00)
HOMA-IR <sup>†</sup>	5.61 ± 2.12	(5.16)	18.11 ± 21.99 <sup>a†</sup>	(8.50)	14.01 ± 20.03 <sup>a*</sup>	(7.92)
Total cholesterol (mmol/L)	6.27 ± 0.94	(5.83)	5.99 ± 0.98	(6.17)	5.54 ± 1.13	(5.28)
HDL (mmol/L)	1.25 ± 0.24	(1.18)	1.12 ± 0.22	(1.08)	1.12 ± 0.27	(1.12)
LDL (mmol/L)*	4.15 ± 0.83 <sup>c*</sup>	(4.05)	3.92 ± 0.90	(3.95)	3.41 ± 0.9	(3.50)
TGL (mmol/L)	2.24 ± 1.25	(1.83)	2.32 ± 0.94	(1.87)	2.27 ± 1.23	(1.96)
AST (U/L)	38.51 ± 23.19	(33.20)	43.32 ± 26.89	(35.00)	38.30 ± 16.14	(34.80)
ALT (U/L)	50.04 ± 21.91	(46.45)	67.36 ± 61.87	(54.55)	57.74 ± 32.93	(55.30)
ALP (U/L)	72.26 ± 26.44	(65.55)	71.88 ± 28.34	(61.80)	71.61 ± 31.21	(65.90)
GGT (U/L)	84.10 ± 113.11	(39.70)	50.68 ± 50.56	(35.10)	59.41 ± 45.37	(41.70)
CRP (mg/L)	5.69 ± 4.90	(4.15)	7.11 ± 14.95	(3.65)	5.68 ± 7.65	(3.20)
WC (cm)*	100.75 ± 6.15	(101.00)	106.40 ± 9.00	(103.50)	109.26 ± 7.76 <sup>a†</sup>	(107.00)
BW (kg) <sup>†</sup>	82.10 ± 9.22	(79.10)	89.77 ± 13.59	(91.50)	99.63 ± 14.8 <sup>a†b*</sup>	(100.00)
BMI (kg/m <sup>2</sup> )*	29.85 ± 3.11	(29.27)	33.26 ± 4.16 <sup>a*</sup>	(33.29)	34.01 ± 4.12 <sup>a†</sup>	(34.22)
SBP (mmHg)	132.92 ± 13.22	(140.00)	132.50 ± 15.60	(130.00)	143.48 ± 21.92	(140.00)
DBP (mmHg)	82.08 ± 10.33	(80.00)	83.00 ± 9.51	(80.00)	87.61 ± 11.57	(90.00)
AST/ALT	0.78 ± 0.26	(0.69)	0.80 ± 0.34	(0.74)	0.78 ± 0.39	(0.63)

\* $p < 0.05$ , <sup>†</sup> $p < 0.01$ , <sup>‡</sup> $p < 0.001$ ; <sup>a</sup> vs US gr 1, <sup>b</sup> vs US gr 2, <sup>c</sup> vs US gr 3;  $\bar{x}$  – mean value; SD – standard deviation; Md – median; WC – waist circumference; BMI – body mass index; BW – body weight; SBP – systolic blood pressure; DBP – diastolic blood pressure; LDL – low density lipoprotein cholesterol, HDL – high density lipoprotein cholesterol, TGL – triglyceride; CRP-C – reactive protein; AST – aspartate aminotransferase; ALT – alanine aminotransferase, ALP – alkaline phosphatase; GGT – gamma- glutamyl transpeptidase; FBG – fasting blood glucose; HOMA-IR – homeostasis model assessment.

In patients with grade 1 US finding, insulin and HOMA-IR values were significantly higher than in the patients with grade 2 ( $p < 0.01$ ), and grade 3 ( $p < 0.05$ ) US findings. The values of FBG were statistically higher in patients with grades 2 and 3 in comparison to those with grade 1 US finding ( $p < 0.05$ ).

We did not find statistically significant differences in transeminase, ALP, GGT, total cholesterol, TGL, CRP, fibrinogen and ferritin values in examinees with different US grades.

On the basis of contingency table  $3 \times 2$ , the presence of hypertension and type 2 DM in the NAFLD group was statistically significantly different in reference to US grade ( $p < 0.05$ ).

By separate comparison, hypertension occurred more frequently in US findings grades 2 and 3 in comparison with US finding grade 1 ( $p < 0.05$ ). Also, type 2 DM which was

mostly represented in the group with US findings grade 3, which was statistically more frequent in reference to grade 1 US findings ( $p < 0.05$ ) (Table 4).

In the NAFLD group, there were 17 (30.91%) overweight (BMI from 25 to 29.9 kg/m<sup>2</sup>) and 38 (69.09%) obese patients (BMI  $\geq 30.0$  kg/m<sup>2</sup>). The largest number of patients in the obesity group, 22 (40.00%) of them, had first degree obesity (BMI from 30 to 34.99 kg/m<sup>2</sup>). It was confirmed that obesity as a category of nutritional status (BMI  $\geq 30.0$  kg/m<sup>2</sup>) was statistically more common in patients with grades 2 and 3 US finding in reference to grade 1 US finding ( $p < 0.05$ ), and the same significance was achieved on the basis of contingency table  $3 \times 2$ . Mild, moderate and severe obesity as a unique category of obesity are statistically significantly more frequent in patients with grades 2 and 3 US finding in reference to grade 1 US finding ( $p < 0.05$ ) (Table 5).

There were 48 (87.27%) patients in the study group

**Table 4**  
**Prevalence of diabetes mellitus (DM) and hypertension in patients with non/alcoholic fatty liver disease (NAFLD) in reference to ultrasound (US) grades**

Parameter	NAFLD US grade 1, n = 12		NAFLD US grade 2, n = 20		NAFLD US grade 3, n = 23	
	n	%	n	%	n	%
Hypertension*	5	41.56%	16	80.00% <sup>a*</sup>	19	82.61% <sup>a*</sup>
DM type 2*	2	16.67%	10	50.00%	14	60.87% <sup>a*</sup>

\* $p < 0.05$ ; a vs ultrasound grade 1.



**Table 5**  
**Correlation between body mass index (BMI) and ultrasound (US) grade of fatty liver**

Parameter	NAFLD US grade 1 (n = 12)	NAFLD US grade 2 (n = 20)	NAFLD US grade 3 (n = 23)
BMI, n (%)			
< 18.5 kg/m <sup>2</sup>	0 (0.00)	0 (0.00)	0 (0.00)
from 18.5 to 24.9 kg/m <sup>2</sup>	0 (0.00)	0 (0.00)	0 (0.00)
from 25 to 29.9 kg/m <sup>2</sup>	8 (66.67)	4 (20.00)	5 (21.74)
≥ 30.0 kg/m <sup>2</sup> *	4 (33.33)	16 (80% a <sup>ax</sup> )	18 (78.26 <sup>ax</sup> )
Obesity, n (%)			
No obesity	8 (66.67)	4 (20.00)	5 (21.74)
BMI from 30 to 34.99 kg/m <sup>2</sup>	3 (25.00)	10 (50.00% <sup>ax</sup> )	9 (39.13 <sup>ax</sup> )
BMI from 35 to 39.99 kg/m <sup>2</sup>	1 (8.33)	5 (25.00)	7 (30.43)
BMI higher than 40 kg/m <sup>2</sup>	0 (0.00)	1 (5.00)	2 (8.70)

\*  $p < 0.05$ ; <sup>a</sup> – vs ultrasound grade I, <sup>x</sup> – patients with BMI 30 kg/m<sup>2</sup> compared as unique category.

with MS, therefore with 3 or more components of MS. The equal number of the NAFLD group patients, *ie* 16 (29.09%) of them, had 3, 4 and 5 MS components, respectively.

The most frequent MS component in the NAFLD group was hypertension or increased SBP or DBP, which was present in 50 (90.91%) patients. Other components of metabolic syndrome were represented as follows: central obesity (WC) – 87.27%, higher FBG or already existing type 2 DM – 69.9%, higher triglyceride level – 65.45% and lower HDL-

cholesterol – 60.00%. The presence of hypertension, as a metabolic component of NAFLD, was statistically more frequent than the presence of metabolic components of higher triglyceride levels, the presence of DM or higher FBG ( $p < 0.01$ ), as well as lower levels of HDL-cholesterol ( $p < 0.001$ ) (Table 6).

Metabolic syndrome was statistically more frequent in the patients with grades 2 and 3 US findings ( $p < 0.01$ ) in relation to grade 1 US finding (Table 7).

**Table 6**  
**Frequency of metabolic syndrome (MS) components in the non-alcoholic fatty liver disease (NAFLD) group**

MS component	n (%)
Waist circumference (cm)	48 (87.27)
Lower HDL (mmol/L)	33 (60.00)
Higher TGL (mmol/L)	36 (65.45)
Hypertension/ SBP and/or DBP (mmHg)	50 (90.91 <sup>bc†,ax</sup> )
FBG ≥ 5.6 mmol/L / type 2 DM	38 (69.09)

†  $p < 0.01$ , ‡  $p < 0.001$ ; <sup>a</sup> vs HDL, <sup>b</sup> vs TGL, <sup>c</sup> vs FBG ≥ 5.6 mmol/L / type 2 DM; FBG – fasting blood glucose; HDL – high density lipoprotein cholesterol, TGL – triglyceride; SBP – systolic blood pressure; DBP – diastolic blood pressure; DM – diabetes mellitus.

**Table 7**  
**Frequency of metabolic syndrome (MS) components in the non-alcoholic fatty liver disease (NAFLD) group in relation to ultrasound (US) grade**

Parameter	NAFLD (US grade 1, n = 12)	NAFLD (US grade 2, n = 20)	NAFLD (US grade 3, n = 23)
MS, n (%)	6 (50.00)	20 (100.00 <sup>a†</sup> )	22 (95.65 <sup>a†</sup> )
Waist circumference, n (%)	9 (75.00)	18 (90.00)	21 (91.30)
Lower HDL, n (%)	3 (25.00)	15 (75.00 <sup>a†</sup> )	15 (65.22 <sup>a*</sup> )
Higher TGL, n (%)	7 (58.33)	13 (65.00)	16 (69.57)
Hypertension, n (%)	10 (83.33)	18 (90.00)	22 (95.65)
FBG ≥ 5.6 mmol/L / type 2 DM, n (%)	5 (41.67)	15 (75.00)	18 (78.26 <sup>a*</sup> )
MS component, n (%)			
0	0 (0.00)	0 (0.00)	0 (0.00)
1	1 (8.33)	0 (0.00)	0 (0.00)
2	5 (41.67)	0 (0.00)	1 (4.35)
3	3 (25.00)	6 (30.00)	7 (30.43)
4	1 (8.33)	9 (45.00 <sup>ax</sup> )*	6 (26.09 <sup>ax</sup> )
5	2 (16.67)	5 (25.00)	9 (39.13)

\*  $p < 0.05$ ; †  $p < 0.01$  a vs US grade 1, x – patients with 4 and 5 MS components compared as unique categories; FBG – fasting blood glucose; HDL – high density lipoprotein cholesterol; TGL – triglyceride; SBP-systolic blood pressure; DBP – diastolic blood pressure; DM – diabetes mellitus.

The frequency of lower values of HDL-cholesterol as metabolic component in relation to the patients with grade 1 US finding was statistically significantly higher in the patients with grade 2 US finding ( $p < 0.01$ ), as well as grade 3 ( $p < 0.05$ ). Already existing type 2 DM or higher FBG as metabolic component was most frequent in the patients with grade 3 US finding, and as such statistically more frequent than grade 1 US finding ( $p < 0.05$ ) (Table 7).

The number of patients with 4 or 5 metabolic components was statistically significantly higher in the patients with grades 2 and 3 US findings in relation to the patients with grade 1 ( $p < 0.05$ ) (Table 7).

## Discussion

Non-alcoholic fatty liver disease is the most common chronic liver disease nowadays and it is the most common reason of high aminotransferase levels in hepatology wards<sup>26</sup>.

The presence of multiple metabolic disorders such as DM, obesity, dyslipidemia and hypertension carries a high risk of disease progression and development of non-alcoholic steatohepatitis and fibrosis in NAFLD patients<sup>27,28</sup>. It is important to recognize the patients with NAFLD so as to enable timely action on joined risk factors and prevent development of more severe diseases<sup>29</sup>. The prevalence of NAFLD is on the increase, which is the consequence of obesity pandemic. Liver biopsy is a gold standard for diagnosing the disease, but it is not widely used due to ethical reasons, since we are dealing with patients mainly without clinical symptoms, with frequently normal transaminase values. Considering a good correlation between fatty liver ultrasound finding and the pathohistological one, the ultrasound is recommended to be the first diagnostic method. In this study we adhered to the criteria of Needleman et al.<sup>25</sup> who, after comparing pathohistological and ultrasound findings of fatty liver, confirmed the precision of ultrasound findings at 88% in diagnosing and studying non-alcoholic fatty liver.

In this study, we compared BMI, the presence of MS, certain components of MS and laboratory parameters of the control and study groups with the aim to estimate the impact of these parameters on fatty liver development, as well as the association between these parameters and the degree of steatosis estimated by ultrasound. The patients in the study group had statistically higher values of BMI, WC, BW, FBG, insulin, HOMA-IR, AST, ALT, ALP, GGT, total cholesterol, LDL-cholesterol, TGL, CRP, ferritin, urates and fibrinogen in reference to the control group. The majority of patients in the study group had the ultrasound finding of grade 3 fatty liver. By comparing the examined parameters of certain grades of ultrasound findings, ANOVA analysis showed that BMI, WC, higher fasting blood glucose, insulin and HOMA-IR were statistically significantly related to the degree of ultrasound grade.

In our study, the majority of NAFLD patients (69,09%) were obese, with BMI higher than 30 kg/m<sup>2</sup> and the mean values of BMI and WC statistically were significantly higher in relation to the control group.

Earlier studies estimate that liver steatosis develops in 57–74% of obese people, in 90% of people with third degree

obesity, and that more than a third of asymptomatic people with severe obesity have histological characteristics of NASH<sup>2,30</sup>. Abdominal obesity correlates with the prevalence of NAFLD, or NASH respectively, and is closely related to insulin resistance, a major pathogenetic factor for developing NASH<sup>31</sup>.

In the study of Leite et al.<sup>32</sup> central obesity is an independent risk factor for NAFLD development. Most examinees in NAFLD group in the study of Williams et al.<sup>33</sup> were obese with mean values of BMI statistically higher in relation to the control group (not-NAFLD). Similar results can be found in the study of Kirovski et al.<sup>34</sup> that confirms statistically higher mean values of BMI and WC in examinees from the NAFLD group in relation to the control group.

In the study by Cheah et al.<sup>35</sup>, the prevalence of central obesity, DM, hypertension, higher fasting blood glucose and triglycerides was statistically much higher in the NAFLD group. This study showed that NAFLD develops 1.2 times more often in patients with larger waist circumference<sup>35</sup>.

In the study by Abangah et al.<sup>36</sup> most examinees had an ultrasound finding of grade 2 fatty liver, while BMI and TG statistically significantly correlated with the degree of steatosis. Our study also confirms statistically significant correlations between BMI and higher ultrasound grade.

The presence of type 2 DM considerably increases the risk of developing NAFLD and progression into more serious forms of the disease, non-alcoholic steatohepatitis and different degrees of fibrosis<sup>37,38</sup>. The prevalence of NAFLD in patients with diabetes goes up to 79%<sup>32,33,39,40</sup>. Having observing a group of patients with type 2 DM, Dvorak et al.<sup>40</sup> confirmed the NAFLD prevalence of 79%. Patients with NAFLD have a higher body weight, waist circumference, BMI, ALT and triglycerides in relation to non-NAFLD examinees.

The prevalence of NAFLD increases with higher fasting blood glucose, from 27% in patients with normal values, 43% in patients with higher fasting blood glucose, to 62% in patients with type 2 DM<sup>41</sup>.

In prospective studies type 2 DM is an independent risk factor of NAFLD progression, fibrosis development, HCC and mortality<sup>42</sup>.

The prevalence of diabetes in our NAFLD examining group reached 47,27%, which is similar to results of previous studies. Mean values of fasting blood glucose were statistically higher in the NAFLD group than in the control one. The prevalence of hypertension in our study group was also statistically higher than in the control group (72.73% vs 12.90%,  $p < 0.001$ ), which was similarly to the results of other studies<sup>34,35</sup>. At the same time, hypertension and diabetes were statistically more frequent in the examinees with grades 2 and 3 US findings in relation to grade 1.

Chitturi et al.<sup>43</sup> determined that 87% patients with NAFLD had characteristics of MS (94% central obesity, 82% dyslipidemia and 50% glucose intolerance), with practically 98% of patients having IR, which was more frequent and serious in patients with NASH than in patients with chronic hepatitis C. In the study of Caballeria et al.<sup>44</sup>,



MS and IR are independent risk factors of NAFLD development.

A larger number of MS characteristics in a person combines with multiply higher risk of developing NAFLD, whereas the presence of only one characteristic carries 3.6 times higher risk<sup>45</sup>. The presence of MS is a predisposing factor for disease progression and development of more acute forms of NAFLD<sup>46,47</sup>.

In our study, the prevalence of MS in NAFLD group was 87.27%. All the examinees showed one or more metabolic risk factors, whereas 29.09% showed at least three metabolic risk factors which is the minimum for diagnosing MS. In earlier studies, more than 90% of patients with NAFLD had one or more components of metabolic syndrome, whereas 33% of them had the complete diagnosis<sup>48,49</sup>.

Examinees with NAFLD had statistically significantly higher prevalence of hypertension, central obesity, higher fasting blood glucose, which is similar to other studies<sup>34, 50</sup>. The study demonstrated that there was the association between the MS components and the US degree of fatty liver infiltration. Among the patients with US finding of fatty liver grades 2 and 3, there were statistically more patients with four or five MS components, similar to studies where the results imply that the presence of a larger number of metabolic disorders carries a higher risk of developing more severe forms of the disease<sup>27, 28, 51</sup>.

Insulin resistance is closely connected with NAFLD, both with development of steatosis and progression of the disease to steatohepatitis, cirrhosis and liver carcinoma<sup>52</sup>. Mean values of insulin and insulin resistance expressed by HOMA-IR were statistically much higher in the study group than in the control group, which was similar to the results obtained by de Salgado et al.<sup>53</sup>. The patients in the study group with US findings grades 2 and 3 had statistically higher values of insulin and HOMA-IR than the patients with grade 1 US finding, which was expected since the patients with US findings grades 2 and 3 had higher prevalence of DM and fasting blood glucose, thus confirming the influence of insulin, insulin resistance and the presence of type 2 DM on the degree of the disease severity.

In a study by Ghamar-Chehreh et al.<sup>54</sup>, univariate analysis showed a statistically significant association between insulin, HOMA-IR, higher fasting blood glucose, transaminases, triglycerides, body weight and the grade of fatty liver.

It is known that a large number of NAFLD patients have normal transaminase values, so their sensitivity in diagnosing the disease is low.

Higher values of AST and GGT correlate with the degree of liver steatosis, but are less sensitive in relation to ALT<sup>55</sup>. In a study by Leite et al.<sup>32</sup> the examinees from the NAFLD group had higher serum levels of ALT than those from the control group. In a multivariate analysis, a high serum triglycerides level and a high-normal ALT level were independently associated with hepatic steatosis, together with either the presence of obesity or increased waist circumference. Razavizade et al.<sup>56</sup> determined a correlation between the serum levels of ALT and the degree of steatosis estimated by US.

In our study, the mean values of AST, ALT, GGT and ALP were statistically significantly higher in the study group in relation to the control group, however, no statistical significance was found with respect to the degree of steatosis estimated by ultrasound, which indirectly implies that we cannot estimate the degree of disease progression on the basis of aminotransferase levels.

Dyslipidemia is a risk factor for developing NAFLD and progression of the disease<sup>32, 35, 36, 40, 57</sup>. Our study results were similar, *ie* the patients with NAFLD had statistically much higher values of total cholesterol, LDL cholesterol and TGL in relation to the control group.

The values of HDL cholesterol were statistically significantly lower in the study group, which is similar to the results of Kirovski et al.<sup>34</sup>. No statistically significant correlations were found between the degree of liver steatosis estimated by US and dyslipidemia.

However, there are several limitations of our study to be considered. Firstly, patients did not have liver biopsy done, which is a gold standard for establishing NAFLD diagnosis. Secondly, ultrasound has certain disadvantages. The sensitivity and specificity of US in NAFLD diagnosis rapidly decrease in obese patients. In our study, the largest number of patients had BMI  $\geq 30$  kg/m<sup>2</sup>, and we should consider the low sensitivity of ultrasound for steatosis less than 30%. It is not possible to differentiate steatosis from steatohepatitis and fibrosis by ultrasound, and the majority of our examinees had multiple risk factors for disease progression, such as DM, MS, hypertension, dyslipidemia, obesity. On the other hand, liver biopsy is an invasive method, frequently demanding patient's hospitalization and sedation, certain costs, and is followed by possible complications. Biopsy sample represents 50 000 part of the liver parenchyma tissue, which during the progression of the disease is not equally affected by pathological changes, which influences the variability of the sample itself, and consequently the validity of pathohistological findings, as well<sup>58</sup>.

All this poses a challenge both for the clinician to estimate which patients with US finding of fatty liver should be considered candidates to undergo liver biopsy and for patients themselves to easily undergo this intervention, as they commonly do not present with significant disease symptoms and often have normal transaminase values.

The results of a recent meta analysis show that ultrasound is a precise, reliable method for diagnosing more than 20–30% fatty infiltrated liver parenchyma, in comparison to histology, with sensitivity of 84.8% and specificity of 93.6%<sup>59</sup>.

## Conclusion

The results of our study show that a large percentage of patients with high risk factors (DM, MS, dyslipidemia, hypertension) have NAFLD. A strong association between certain elements of metabolic syndrome and the presence of NAFLD was demonstrated, particularly between obesity and hypertension.

We also confirmed the importance of insulin resistance estimated by HOMA-IR in the development of the disease,

as well as its influence on development of more severe grades of the disease estimated by ultrasound. Despite statistically significantly higher values of liver enzymes in NAFLD group, a correlation with ultrasound has not been established. A high prevalence of obesity and metabolic syndrome in higher US grades was found, *ie* the association with progressive forms of the disease was confirmed.

Ultrasound is cheap and available, compared with other diagnostic methods, which makes it a technique of choice for screening patients on NAFLD presence, particularly in conditions of obesity pandemic. The severity of hepatic steatosis estimated by ultrasound in the presence of metabolic syndrome is a better non-invasive method of disease monitoring in relation to liver enzymes.

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