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DIAGNOSTIC AND PROGNOSTIC VALUE OF SELENIUM AND SELENOPROTEIN P IN PATIENTS WITH COMORBID COURSE OF NON-ALCOHOLIC FATTY LIVER DISEASE AND ARTERIAL HYPERTENSION

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DIJAGNOSTIČKA I PROGNOSTIČKA VREDNOST SELENA I SELENOPROTEINA P KOD BOLESNIKA SA KOMORBIDNIM TOKOM NEALKOHOLNE MASNE BOLESTI JETRE I ARTERIJALNE HIPERTENZIJE

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ABSTRACT

Objective. To evaluate the diagnostic and prognostic value of Selenoprotein P and selenium in the progression of liver damage in patients with nonalcoholic fatty liver disease (NAFLD).

Methods. The study involved 120 patients: 50 with isolated NAFLD, 50 with comorbid NAFLD and hypertension, established according to the global and local guidelines. The control group included 20 relatively healthy volunteers. Liver function parameters, selenium and Selenoprotein P levels were assessed, and predictors of steatohepatitis were identified. Pearson's χ^2, Mann-Whitney test, logistic regression was used.

Results. The study found significant predominance of levels of Selenoprotein P (Sel P) and selenium in controls (71.0 (54.3; 76.1) ng/ml and 108.0 (96.9; 118.8) ng/ml respectively) compared with the NAFLD + hypertension (19.7 (8.0; 26.7) ng/ml and 43.5 (39.9; 49.1) ng/ml, p <0.001) and the NAFLD group (43.1 (41.3; 45.4) ng/ml and 67.2 (61.5; 77.4) ng/ml, respectively, p <0.001). Regression analysis determined association of Sel P and Sel levels with steatohepatitis: respectively, OR = 1,143 (95.0% CI 1,068–1,224) (p <0.001) and OR = 1,054 (95.0% CI 1,012–1,098) (p = 0.011). Other predictors of steatohepatitis were aspartateaminotransferase (OR = 1,421 (95.0% CI 1,198–1,687), p <0.001) and systolic blood pressure (OR = 1,089 (95.0% CI 1,017–1,116), p = 0.014).

Conclusion. Levels of selenium and Selenoprotein P are associated with greater liver damage in patients with NAFLD, and the concomitant increase in systemic blood pressure is an additional factor that adversely affects the course of NAFLD, increasing the intensity of liver damage in such patients.

Key words: *non-alcoholic fatty liver disease; oxidative stress; comorbidity.*

SAŽETAK

Cilj. Procena dijagnostičke i prognostičke vrednosti selenoproteina P i selena u progresiji oštećenja jetre kod pacijenata sa nealkoholnom masnom bolešću jetre (NAMBJ).

Metode. U studiji je učestvovalo 120 pacijenata: 50 sa izolovanom NAMBJ, 50 sa komorbidnom NAMBJ i hipertenzijom, utvrđenim prema svetskim i domaćim smernicama. Kontrolnu grupu činilo je 20 relativno zdravih dobrovoljaca. Procenjeni su parametri funkcije jetre, nivoi selena i selenoproteina P i identifikovani prediktori steatohepatitisa. Korišćeni su Pirsonov χ^2, Man–Vitni test, logistička regresija.

Rezultati. Studija je otkrila značajnu prevagu nivoa selenoproteina P (Sel P) i selena u kontrolama [71,0 (54,3; 76,1) ng/ml i 108,0 (96,9; 118,8) ng/ml respektivno] u poređenju sa NAMBJ + hipertenzijom [19,7 (8,0; 26,7) ng/ml i 43,5 (39,9; 49,1) ng/ml, p < 0,001] i grupa NAMBJ [43,1 (41,3; 45,4) ng/ml i 67,2 (61,5; 77,4) ng/ml, respektivno p < 0,001]. Regresionom analizom utvrđena je povezanost nivoa Sel P i Sel sa steatohepatitisom: respektivno, OR = 1,143 (95,0% CI 1,068–1,224) (p < 0,001) i OR=1,054 (95,0% CI 1,012–1,098) (p = 0,011). Drugi prediktori steatohepatitisa bili su aspartat aminotransferaza [OR = 1,421 (95,0% CI 1,198–1,687) p < 0,001] i sistolni krvni pritisak [OR = 1,089 (95,0% CI 1,017–1,116) p = 0,014].

Zaključak. Nivoi selena i selenoproteina P povezani su sa većim oštećenjem jetre kod pacijenata sa NAMBJ, a istovremeno povećanje sistemskog krvnog pritiska dodatni je faktor koji negativno utiče na tok NAMBJ, povećavajući intenzitet oštećenja jetre kod takvih pacijenata.

Ključne reči: *nealkoholna masna bolest jetre, oksidativni stres, komorbiditet*

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a cumulative concept and includes a number of pathological conditions from steatosis and non-alcoholic steatohepatitis to liver cirrhosis and hepatocellular carcinoma (1, 2, 7). It is noted that NAFLD is an independent risk factor for cardiovascular disease (9, 12). The authors add that the severity of NAFLD may be an independent predictor of the gradual development of hypertension (13). Also, a number of studies emphasize that NAFLD may be a risk factor for increased mortality from cardiovascular disease (CVD) (2, 3, 6, 12, 14). Numerous studies are currently being conducted to determine the pathophysiological links in the development of CVD on the background of NAFLD (6), so the study of aspects and mechanisms that affect the

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course of this comorbidity remains relevant (12). The greatest attention is paid to oxidative stress, atherogenic dyslipidemia, subclinical inflammation, insulin resistance, endothelial dysfunction and overexpression of cytokines (6, 12). The literature shows the presence of common risk factors and links in the pathogenesis of NAFLD and hypertension (15).

It is known that one of the factors in the development of CVD is endothelial dysfunction. In addition, changes in the hepatokin profile (fetuin-A, fibroblast growth factor 21 and Selenoprotein P) may directly affect endothelial function and lead to vascular remodeling and infiltration by inflammatory cells (15). Selenoprotein P (Sel P) is an extracellular transport glycoprotein with a mass of 42 kDa and is part of a family of 25 proteins (16, 17, 18), which provide antioxidant protection, endocrine and immune function and cellular metabolism (19). Implementation of selenium antioxidant function is by expression of Selenoproteins (20). Selenium is a part of glutathione peroxidase, which is involved in the regulation of vascular tone by maintaining a balance between the levels of superoxide anion and nitric oxide (20). This mechanism regulates apoptosis, expression of cellular adhesive molecules, eicosanoids, lipoxygenases and cyclooxygenases (20).

The aim is to determine the diagnostic and prognostic potential of selenium and Selenoprotein P in assessing the progression of NAFLD in patients with hypertension.

MATERIALS AND METHODS

By design, it was case-control retrospective study. We examined 100 patients with NAFLD and divided them into 2 groups. The study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of Kharkiv National Medical University. Informed written consent was obtained from all patients before their enrollment in this study.

The main group included 49 patients with comorbid NAFLD and hypertension (33 (67.3%) women and 16 (32.7%) men). The comparison group consisted of 51 patients with isolated NAFLD: 30 (58.8%) women and 21 (41.2%) men. The control group consisted of 20 healthy individuals, including 11 (55.0%) women and 9 (45.0%) men. According to the frequency analysis, the study groups were comparable by gender: $\chi^2 = 1,219$, $p = 0,544$.

The diagnosis of non-alcoholic fatty liver disease was established according to the Clinical Practice Guidelines for management of non-alcoholic fatty liver disease directed by the European Association for the Study of Liver Diseases and the European Association for the Study of Obesity and according to the local documents. The hypertension was diagnosed according to the Clinical Practice Guidelines for the Management of Arterial Hypertension directed by the European Society of Cardiology and the European Society of Hypertension and according to the local documents. Exclusion criteria: patients with liver diseases of viral, alcohol and other genesis (alcohol liver disease, viral hepatitis, cirrhosis etc.); hypertension 3 grade and 3 stage and secondary hypertension; coronary artery disease; type two diabetes mellitus; oncological pathology, patient's rejection of participation in study (withdrawal or unsigned informed consent).

Patients in the study groups and individuals in the control group were interviewed to determine alcohol dependence and the differential diagnosis of NAFLD with alcoholic fatty liver disease - AUDIT test (Alcohol Use Disorders Identification Test). Body mass index was calculated by the formula for the ratio of body weight in kilograms to height in meters squared: BMI = body weight, kg / (height, cm)^2. Common blood count (CBC) was performed by the optical method using current cytofluorimetry with laser semiconductors and hydrodynamic focusing. The white blood cells formula was determined microscopically using a Goryaev chamber. Levels of an alanine transaminase (ALT), aspartate transaminase (AST), total and fractional bilirubin was conducted by standard methods (kinetic method). Gamma glutamyl transferase (GGT) was measured by enzymatic colorimetric method, alkaline phosphatase (ALP) - by colorimetric method. Selenium and Selenoprotein P levels were measured by immunoassays (ELISA Kit) using ElabScience reagents (USA). Liver ultrasound examination was performed according to standard methods using the Samsung (Medison) SonoAce X8 ultrasound device.

Statistical calculation of the obtained data was performed using the application package IBM® SPSS® 25.0 (trial version) for Windows®. The distribution of quantitative and qualitative features was carried out visually graphically and using Kolmogorov-Smirnov & Lilliefors test for normality and Shapiro-Wilk's test of normality. The assessment revealed differences from the normal nature of the distribution, so the calculations were performed using non-parametric statistics.

Thus, to characterize the central tendency and variability of quantitative (continuous or interval) variables, the median (Me), lower (LQ) and upper (UQ) quarters were determined. Data were presented as Me (LQ; UQ). The significance of differences in the obtained quantitative traits in the two independent groups was determined using the Mann-Whitney U-test. Qualitative (binomial, ordinal, nominal) characteristics were presented in absolute (absolute) and relative (percentage) values. Comparisons of groups by qualitative characteristics were performed using Pearson's chi-squared test.

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Associations of the predictors with the binomial dependent variable were determined by logistic regression analysis with the calculation of β, standardized coefficients β (odds ratio; OR) and their 95.0% confidence intervals (CI). In the regression analysis, the method of simultaneous inclusion (Enter) and stepwise exclusion of Wald (Backward Wald) variables in the mathematical model were used. The threshold value of the probability level of all calculated features was taken as 0.05 (p = 0.05) with an indication of the exact value of the level of reliability "p" with three decimal places.

RESULTS

The median age of patients in the studied groups was 51.0 years (45.0; 56.0) in the main group, 52.0 years (47.0; 54.0) in the comparison group and 51.0 years (45.0; 55.5) in the control group. In the comparison of the data of the group of comorbid pathology and isolated NAFLD with controls, no statistically significant difference was found (p = 0.610 and p = 0.980, respectively), similar results were obtained when comparing the parameters of the examined groups of patients (p = 0.564).

The number of patients in the main group who had a normal body weight according to BMI was 25 (51.0%), increased - 24 (49.0%). Among patients in the comparison group: 29 (56.9%) had a normal body weight and 22 (43.1%) had an increased body weight (p = 0.343, χ^2 = 0.558).

The median BMI in all groups is shown in figure 1. The lowest was in the control group, and the highest median was determined in patients with comorbidity of NAFLD and hypertension: respectively 24.5 (23.5; 24.8) kg/m^2 and 24.9 (24.2; 25.9) kg/m^2 (p = 0.008). It should be added that patients with isolated NAFLD had a slightly lower median BMI than patients with NAFLD and hypertension, but the differences were significant (p = 0.049).

The median blood pressure of the persons involved in the study are given in Figure 2.

The median systolic blood pressure (SBP) and diastolic blood pressure (DBP) were naturally higher in patients with concomitant NAFLD and hypertension. The median SBP in patients with NAFLD was significantly higher to patients in the control group (p = 0.012), but the median levels of DBP in these groups were the same (p = 0.918) (Figure 2).

Among the main group, in which the presence of steatosis and steatohepatitis was established clinically,

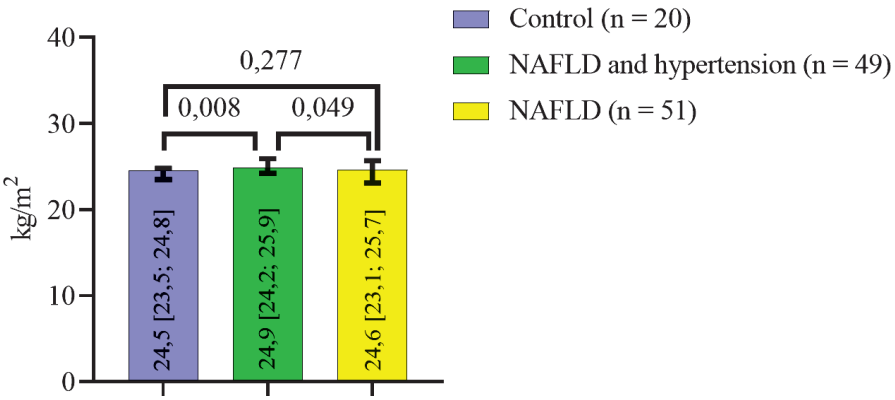

Figure 1. Comparative characteristics of patients with BMI, Me (LQ; UQ).

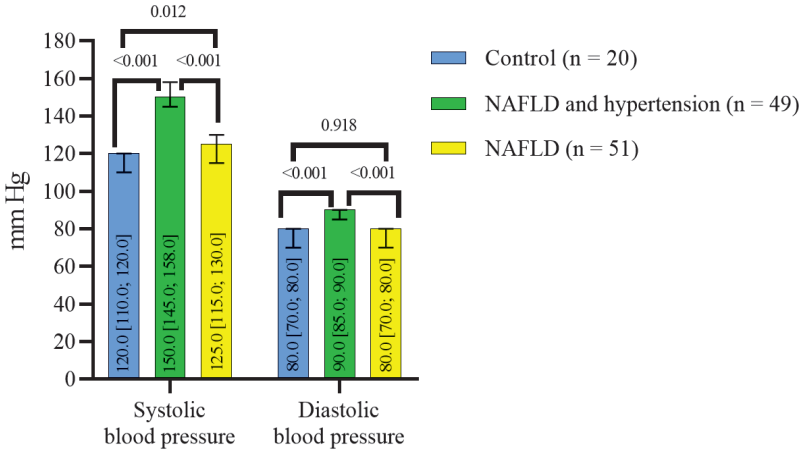

Figure 2. Blood pressure in the examined patients depending on the group, Me (LQ; UQ)

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anamnestically and laboratory-instrumentally (ALT, AST indicators, ultrasound data), 27 patients (55.1%) had steatosis, 22 patients had steatohepatitis (44.9). In the comparison group, 30 patients (58.8%) had steatosis, 21 patients (41.2%) had steatohepatitis, $\chi^2 = 0.141$, $p_{1-2} = 0.707$.

Common blood count data showed a significant increase in the median level of leukocytes in patients with isolated NAFLD compared to the control group (p = 0.002), while in the control group and NAFLD and hypertension no significant difference in this indicator was found. The levels of white blood cells (WBC), stab-and-segment neutrophils and red blood cells (RBC) did not differ significantly between study groups. The median hemoglobin (HGB) level was quantitatively higher in the control group, but no significant difference was found between the study groups (Table 1).

It should be noted that the highest level of platelets was registered in the control group and the indicator significantly prevailed over the group of comorbid (p <0.001) and isolated (p <0.001) course of NAFLD. The medians of the studied groups also varried significantly (p <0.001) with a significant predominance in patients with isolated NAFLD (Table 1).

The highest level of erythrocyte sedimentation rate (ESR) was determined in patients with comorbid NAFLD and hypertension, which significantly exceeded the ESR of the control group (p = 0.035) and the group with isolated NAFLD (p = 0.045).

Significant differences were determined by the median levels of liver tests (table 1). Thus, the level of ALT and AST significantly prevailed (p = 0.001) in patients with comorbid NAFLD and hypertension: it was almost 2 times higher than the median level of the control group and 1.3 times higher than the group with isolated NAFLD. A similar trend persisted with respect to the median levels of ALP and GGT. The highest values were recorded in patients with comorbid course, which at least 2 times significantly exceeded (p <0.001) similar values in the control group and 1.5 times - in the group of isolated NAFLD. The median AST/ALT ratio was significantly (p <0.001) the lowest in patients in the control group; in the main and comparison groups the indicators were at the same level (p = 0.167) with a slight predominance in patients of the main group. The groups were comparable in terms of total bilirubin and its fractions.

There was no significant difference in blood test results in the examined patients depending on the presence of steatosis or steatohepatitis (Table 2). It is noteworthy that the median ALT and AST levels significantly prevailed in patients with steatohepatitis compared to patients with steatosis in both the main group and the comparison group.

Table 1. Data of common blood count and biochemical blood test of patients by groups, Me (LQ; UQ).

Index	Control (n = 20)	NAFLD + hypertension (n = 49)	NAFLD (n = 51)	$p_{1–2}$	$p_{1–3}$	$p_{2–3}$
WBC, total × 10^{12}/L	5,3 (4,7; 5,6)	5,3 (4,5; 7,7)	6,4 (5,2; 7,2)	0,420	0,002	0,110
WBC, stabs, %	6,0 (6,0; 8,0)	6,0 (5,0; 7,5)	6,0 (5,0; 8,8)	0,473	0,934	0,534
WBC, segmented, %	62,5 (59,8; 66,3)	64,0 (57,0; 67,5)	63,0 (58,0; 69,0)	0,893	0,918	0,885
Lymphocytes, %	29,0 (26,0; 31,0)	29,0 (25,0; 35,5)	29,0 (24,0; 33,0)	0,723	0,924	0,401
HGB, g/L	144,0 (136,8; 154,5)	138,0 (130,0; 145,0)	142,0 (129,0; 153,0)	0,102	0,486	0,258
RBC, × 10^{12}/L	4,34 (4,20; 4,53)	4,40 (4,10; 4,60)	4,50 (4,28; 4,60)	0,788	0,327	0,210
Platelets, × 10^{9}/L	256,0 (249,3; 273,0)	202,0 (195,0; 206,5)	236,0 (230,0; 241,0)	< 0,001	< 0,001	< 0,001
ESR, mm/hour	10,5 (7,8; 13,3)	15,0 (7,0; 23,5)	10,0 (7,0; 15,0)	0,035	0,763	0,045
ALT, U/L	25,5 (24,0; 30,8)	45,0 (43,0; 47,5)	36,0 (34,0; 39,0)	< 0,001	< 0,001	< 0,001
AST, U/L	23,0 (19,3; 26,0)	53,0 (51,0; 56,0)	41,0 (40,0; 45,0)	< 0,001	< 0,001	< 0,001
AST/ALT	0,87 (0,76; 0,99)	1,16 (1,11; 1,24)	1,14 (1,08; 1,21)	< 0,001	< 0,001	0,167
ALP, U/L	129,2 (116,9; 140,6)	285,7 (217,6; 321,1)	215,5 (183,2; 246;7)	< 0,001	< 0,001	< 0,001
GGT, U/L	22,6 (16,1; 31,7)	96,2 (75,0; 108,9)	65,5 (51,5; 76,8)	< 0,001	< 0,001	< 0,001
Total bilirubin, mmol/L	13,0 (11,9; 17,8)	13,4 (12,1; 15,2)	13,2 (12,0; 16,6)	0,704	0,746	0,982
Direct bilirubin, mmol/L	4,1 (3,6; 5,5)	4,0 (3,2; 4,9)	4,1 (3,6; 5,2)	0,402	0,821	0,273
Indirect bilirubin, mmol/L	8,7 (6,8; 12,6)	9,3 (8,4; 11,4)	9,0 (7,8; 11,1)	0,403	0,587	0,462

Note: $p_{1–2}$ — significance of differences between control group and 1st group; $p_{1–3}$ — significance of differences between control group and group 2; $p_{2–3}$ — significance of differences between 1st and 2nd groups.

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Table 2. Comparison of common blood count and biochemical blood analysis of patients depending on the presence of steatosis or steatohepatitis, Me (LQ; UQ).

Index	NAFLD + hypertension (n = 49)		$p_{1–2}$	NAFLD (n = 51)		$p_{3–4}$
	Steatosis (n = 27)	Steatohepatitis (n = 22)		Steatosis (n = 30)	Steatohepatitis (n = 21)	
WBC, total, × 10^{12}/L	5.7 (4.5; 8.3)	5.3 (4.4; 6.4)	0.371	6.7 (5.4; 7.1)	5.9 (4.9; 7.8)	0.509
WBC, stabs, %	30.0 (26.0; 36.0)	28.0 (25.0; 35.0)	0.456	27.0 (24.0; 32.7)	30.0 (24.3; 33.3)	0.598
segmented, %	7.0 (5.0; 8.0)	6.0 (5.0; 7.0)	0.146	6.0 (5.0; 8.0)	8.0 (4.6; 9.6)	0.286
Lymphocytes, %	63.0 (56.0; 67.0)	64.0 (58.4; 68.3)	0.710	65.0 (60.0; 67.3)	61.0 (56.0; 69.5)	0.358
HGB, g/L	137.0 (132.0; 145.0)	139.5 (124.0; 150.0)	0.880	140.0 (126.0; 1470)	150.0 (131.5; 159.0)	0.069
RBC, × 10^{12}/L	4.38 (4.10; 4.52)	4.42 (4.0; 4.73)	0.695	4.5 (4.3; 4.6)	4.50 (4.29;4.64)	0.803
Platelets, × 10^{9}/L	202.0 (195.0; 207.0)	201.0 (194.8; 204.3)	0.770	235.5 (230.0; 240.2)	236.0 (231.0; 242.0)	0.833
ESR, mm/hour	16.0 (9.0; 24.0)	11.5 (6.8; 23.3)	0.235	10.5 (8.0; 15.3)	10.0(6.5; 14.0)	0.131
ALT, U/L	44.0 (43.0; 46.0)	47.0 (44.5; 49.0)	0.002	35.0 (33.0; 37.3)	39.0 (35.0;42.0)	0.011
AST, U/L	51.0 (49.0; 53.0)	56.5 (54.0; 57.0)	<0.001	40.0 (39.0; 41.0)	45.0 (43.0; 48.0)	<0.001
AST/ALT	1.15 (1.10; 1.21)	1.17 (1.15; 1.25)	0.129	1.14 (1.01; 1.18)	1.13 (1.08; 1.30)	0.455
ALP, U/L	286.0 (220.0; 326.7)	283.0 (211.2; 317.4)	0.469	196.8 (166.9; 237.6)	228.3 (195.7; 258.8)	0.062
GGT, U/L	93.0 (77.2; 105.6)	99.9 (72.3; 112.1)	0.410	65.3 (42.6; 73.0)	65.5 (53.6; 78.1)	0.368
Total bilirubin, mmol/L	13.1 (11.8; 14.6)	14.0 (12.2; 18.6)	0.250	13.9 (12.0; 17.3)	12.6 (11.5; 15.1)	0.154
Direct bilirubin, mmol/L	3.7 (3.3; 4.5)	4.20 (3.0; 5.1)	0.317	4.0 (3.8; 5.2)	4.10 (3.40; 4.8)	0.545
Indirect bilirubin, mmol/L	9.3 (8.3; 10.5)	9.4 (8.4; 15.6)	0.318	10.0 (8.0; 13.0)	8.3 (7.1; 10.4)	0.064

$p_{1–2}$ — significance of differences in group with NAFLD + hypertension between steatosis and steatohepatitis groups; $p_{3–4}$ — significance of differences in group with NAFLD between steatosis and steatohepatitis groups.

Table 3. Comparison of selenium and Selenoprotein P metabolism in patients depending on the group, Me (LQ; UQ).

Index	Control (n = 20)	NAFLD + hypertension (n = 49)	NAFLD (n = 51)	$p_{1–2}$	$p_{1–3}$	$p_{2–3}$
Sel P, ng/ml	71.0 (54.3; 76.1)	19.7 (8.0; 26.7)	43.1 (41.3; 45.4)	< 0.001	< 0.001	< 0.001
Selenium, µg/L	108.0 (96.9; 118.8)	43.5 (39.9; 49.1)	67.2 (61.5; 77.4)	< 0.001	< 0.001	< 0.001

$p_{1–2}$ — significance of differences between control group and 1st group; $p_{1–3}$ — significance of differences between control group and group 2; $p_{2–3}$ — significance of differences between 1st and 2nd groups.

However, the ACT / ALT ratio between the two groups tended to be significant with a predominance of the median value in patients with steatohepatitis of the main group. There was no significant difference between other indicators of liver function (Table 2).

Analysis of Selenoprotein P and selenium levels showed that significantly higher values were in the control group, compared with the main (p <0.001) and the comparison group (p <0.001). It should be added that Selenoprotein P levels were twice as high in patients with isolated NAFLD and selenium — one and a half times (Table 3).

Interestingly, it was found that the median levels of Selenoprotein P were quantitatively predominant among patients with steatohepatitis, but did not differ significantly from that in the group of patients with steatosis. At the same time, the median selenium level in patients with steatosis was quantitatively insignificantly higher in comparison with patients with steatohepatitis (Table 4). In-depth analysis of fluctuations in selenium and Selenoprotein P content as NAFLD progressed, no significant differences were found between patients with steatohepatitis and steatosis (Table 4).

Analysis of possible predictors of steatohepatitis in patients with NAFLD with and without hypertension identified a significant association of such indicators as AST (OR = 1,431 (95.0% CI 1,177-1,740), p <0.001); Selenoprotein P (OR = 1,165 (95.0% CI 1,074–1,264), p

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Table 4. Indicators of selenium and Selenoprotein P metabolism in patients with steatosis and steatohepatitis, Me (LQ; UQ).

Index	NAFLD + hypertension (n = 49)		$p_{1–2}$	NAFLD (n = 51)		$p_{3–4}$
	Steatosis (n = 27)	Steatohepatitis (n = 22)		Steatosis (n = 30)	Steatohepatitis (n = 21)	
Sel P, ng/ml	19.9 (7.3; 26.7)	19.5 (8.0; 26.8)	0.817	42.7 (40.8; 45.5)	43.2 (42.3; 45.8)	0.599
Selenium, µ/L	42.4 (34.5; 49.5)	46.0 (42.3; 49.5)	0.169	69.9 (62.4; 77.5)	66.4 (57.0; 78.1)	0.394

$p_{1–2}$ — significance of differences in group with NAFLD + hypertension between steatosis and steatohepatitis groups; $p_{3–4}$ — significance of differences in group with NAFLD between steatosis and steatohepatitis groups.

Table 5. Independent predictors of steatohepatitis (multivariate analysis by the method of simultaneous inclusion)

Predictors	Enter of variables method			Backward Wald method		
	OR	95,0 % CI	p	OR	95,0 % CI	p
ALT, U	1.064	0.898–1.260	0.473	—		
AST, U	1.431	1.177–1.740	< 0.001	1.421	1.198–1.687	< 0.001
Selenoprotein P, ng/ml	1.165	1.074–1.264	< 0.001	1.143	1.068–1.224	< 0.001
Selenium, µ/L	1.062	1.013–1.113	0.013	1.054	1.012–1.098	0.011
ALP, U/L	0.994	0.982–1.006	0.317	—		
GGT, U/L	1.017	0.986–1.049	0.292	—		
SBP, mm Hg	1.100	1.019–1.187	0.014	1.089	1.017–1.116	0.014
BMI, kg/m^2	0.933	0.611–1.423	0.746	—		

p — significance of the predictor.

<0.001); selenium (OR = 1.062 (95.0% CI 1.013–1.113), p = 0.013) and SBP (OR = 1.100 (95.0% CI 1.019–1.187), p = 0.014). However, other studied indicators of liver function or systemic inflammatory response did not show a significant association with the presence of steatohepatitis in the examined patients with NAFLD with and without hypertension (Table 5).

After analyzing all predictors, the method of their stepwise exclusion was applied to obtain the most reliable values. Thus, reliable predictors (Table 5) of the presence of steatohepatitis in patients with NAFLD were the levels of AST, Selenoprotein P, selenium and SBP. An increase in AST by 1.0 U significantly (p <0.001) was associated with an increase in the odds of steatohepatitis by 42.1%. At the same time, the increase in Selenoprotein P and selenium levels also showed a significant association with an increase in the odds of having steatohepatitis by 14.3% (p <0.001) and 5.4% (p = 0.011) respectively. It should be noted that SBP level was an independent predictor of advanced liver damage in patients with NAFLD: an increase in SBP by 1.0 mm Hg was significantly (p = 0.014) associated with an increase in the odds of steatohepatitis by 8.9% (Table 5). This confirms the negative impact of increased blood pressure on the course of NAFLD, in particular by the development of advanced stages of liver damage.

DISCUSSION

Identification of steatohepatitis biomarkers and development of prognostic and diagnostic algorithms is an affordable alternative that allows their use in clinical practice, requiring minimal invasiveness and lower economic costs. Imaging technologies, on the other hand, are highly accurate, but are often not economically available, especially when repeated measures are required. Despite the fact that NAFLD is strongly associated with metabolic syndrome and obesity, it can also develop in normal or overweight persons. Current study showed that hypertensive NAFLD patients had significantly higher body mass compared to non-hypertnesive NAFLD and the control group patients. Powell et al. (28) note that presence of metabolic violations can be considered the cause of initial fatty infiltration of hepatocytes, thus leading to NAFLD.

Another finding is related to decreased platelet levels in both hypertensive and nonhypertensive NAFLD. Compared to controls, NAFLD-only patients had significant (p<0.001) lower median platelet count, while in hypertensive NAFLD patients the median numbers were significantly (p<0.001) lower than in both other groups. However, it should be stated that no significant differences in common blood count were found depending on different liver damage grade in both hypertensive and non-hypertensive NAFLD patients. In research of Zheng et al. (30) lower mean platelet levels were observed in patients with elevated AST and ALT. Further analysis showed negative association of platelet count with liver stiffness, which correlates with data of current research. On the other hand, novel data suggest on specific role of platelets in development and promotion of systemic and especially liver inflammation. Miele et al. (29) state that intrahepatic platelet accumulation is associated with inflammation and liver damage grade especially in

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patients with steatohepatitis. Noteworthy, that this can act as possible explanation of increased risk of cardiovascular pathology in NAFLD patients due to proinflammatory platelets aggravate systemic inflammation triggering increase in different chemokines and inflammatory cells levels. Intrahepatic platelet accumulation can also explain lower levels of peripheral blood platelets in NAFLD-only and especially hypertensive NAFLD patients (29).

Presence of concomitant hypertension can be connected with overall more severe liver damage. It can be observed in results of liver tests that all parameters except bilirubin and its fractions, in hypertensive NAFLD patients were significantly higher than in NAFLD-only group. Regression analysis showed that SBP, but not presence of hypertension itself, is significant (p=0.014) predictor of steatohepatitis in NAFLD patients. In hypertension, additional damage to organs is made by increased pressure, which triggers and intensifies endothelial dysfunction, oxidative stress and apoptosis.

Considering liver damage grade, it should be stated, that a significant difference was observed only in ALT and AST levels with bigger median in patients with steatohepatitis, which can be explained by more potent and persistent inflammatory process in such patients. Preuss et al. (31) note that higher levels of liver tests, especially ALT, can serve as direct marker of initial liver damage. Peripheral levels of transaminases tend to increase because of cellular damage. Authors add that increased levels of both ALT, AST and AST/ALT ratio are also significantly associated with cardio-vascular risk. In research of Liu et al. (32) authors also state on importance of increase in liver test parameters and their association with vascular damage as initial stage of various cardio-vascular pathology. Their findings suggest a significant association of increased ASL/ALT ratio with peripheral artery disease in 1.3–1.6 times. Moreover, ASL/ALT ratio remained strong predictor of cardio-vascular damage in both crude and adjusted models. On the other hand, research by Liu et al. (33) did not find significant association of both categorical and continuous AST/ALT with brachial-ankle pulse wave velocity as primary risk factor of cardio-vascular diseases. Authors further noted on non-linear relationship of AST/ALT ratio and brachial-ankle pulse wave velocity, which was especially seen in patients less than 60 years old.

The importance of selenium as an element in the functioning of the organism is confirmed by a direct relationship between its reduction and consecutive reduction of Selenoproteins (23). However, it is difficult to establish the degree of influence of selenium levels on the development of cardio-metabolic pathology, as the data available in the literature are controversial. Some studies have shown the effectiveness of selenium supplementation in reducing the risk of cardiovascular disease (17): an increase in selenium of 20 ng/L significantly (p = 0.017) was associated with a decrease in SBP and DBP by 2.2 mm Hg and 1.5 mm Hg respectively. (24). Research of Hekmatdoost et al. (23) showed that the levels of Sel P were significantly (p = 0.002) lower in patients with NASH than in the control group and common steatohepatitis with standardization of indicators by age, sex and ALT (respectively 2.9 ± 0.4 mg/L, 5, 0 ± 0.3 mg/L and 4.2 ± 0.3 mg/L). Subsequent regression analysis performed by the authors identified an independent association only with BMI (p = 0.04).

On the other hand, data on selenium concentrations show significant controversies in different researches. Polyzos et al. (27) noted significant increase of selenium levels in patients with NAFLD compared to controls. It is worth noting that other liver diseases, such as hepatitis or alcoholic liver disease, also showed significant increase in Selenium levels compared to controls. According to researchers Polyzos S.A. et al. (11), the association of Sel P and liver damage in NAFLD is largely determined by the presence of insulin resistance in such patients. This may mean that the effect of Sel P levels on the development of NAFLD is released through the mediation of the antioxidant system against the background of insulin resistance. The study found a significantly lower median level of selenium and Selenoprotein P in patients with NAFLD and significantly lower levels of both markers in patients with comorbid NAFLD and hypertension, possibly due to depletion of the antioxidant system in patients with comorbid NAFLD.

In current research, changes in Selenoprotein P and selenium metabolism may be associated with decreased Selenoprotein P expression and impaired selenium transport to tissues under oxidative stress in patients with steatosis, followed by potent activation of the antioxidant system, against more intense liver parenchymal inflammation in patients with steatohepatitis. According to Liu et al. (25) such reduction in selenium and Selenoprotein P in patients with concomitant hypertension may be associated with additional expenditure in one of its main effects – reduction of endothelial dysfunction. This, along with modulating inflammation, inhibiting oxidative stress and protecting vascular cells, provides a protective effect of selenium on the formation and progression of cardiovascular pathology (25). Such mechanism is also discussed in detail in an in vivo and in vitro study Ren H. et al. (26), which showed that selenium inhibits homocysteine-induced endothelial dysfunction, increases the viability and migration of endothelial cells, inhibits apoptosis, prevents endothelial damage and endothelium-dependent vasodilation disorders

Current study has several possible limitations. The first is a relatively small sample of patients, which requires further increase in order to reveal additional associations

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and improve prognosis of liver damage grade. Making research a multicenter one by recruiting patients from additional hospitals could solve both sample limitation and provide additional clinical data on studied pathology (i.e. course and severity of disease). On the other hand, prospective cohort study design with repeated measures could show dynamics of Selenium and Selenoprotein P changes in such patients within the course of NAFLD and NAFLD and hypertension. It would also assist exacerbation risks assessment (i.e. increase of liver damage grade).

In conclusion, the obtained results suggest that steatosis in NAFLD patients is accompanied by a significant decrease of both selenium and Selenoprotein P, while presence of concomitant hypertension is associated with deepening of those deviations. Hypertensive NALFD patients showed significantly higher medial levels of liver function tests compared to non-hypertensive, but those differences were not observed depending on liver damage grade. The following analysis also identified systolic blood pressure to be strongly associated with steatohepatitis together with AST levels, Selenium and Selenoprotein P. Thus, it can be assumed that the key role in exaggeration of liver damage is played not only by inflammation itself, but also by imbalance of antioxidant system and systemic blood pressure, which can be used in the future to personalize diagnostic and therapeutic approaches in the patients with comorbidity of NAFLD and hypertension.

ABBREVIATIONS

ALT — alanine aminotransferase; AST — aspartate aminotransferase; BMI — body mass index; CVD — cardiovascular disease; DBP — diastolic blood pressure; ESR — erythrocyte sedimentation rate; GGT — gamma glutamine transpeptidase; HGB — hemoglobin; NAFLD — nonalcoholic fatty liver disease; RBC — red blood cells; SBP — systolic blood pressure; Sel P — Selenoprotein P.

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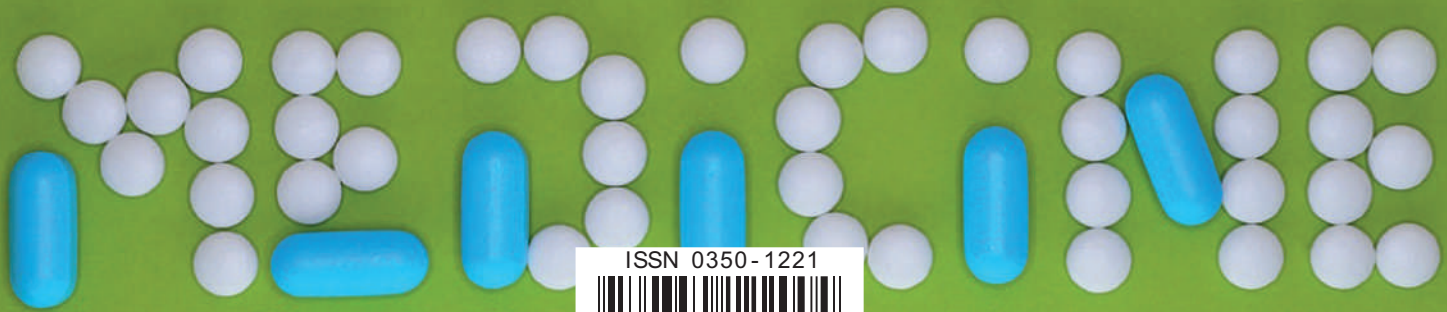

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