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ANALYSIS OF PATHOGENETIC RELATIONS BETWEEN CLINICAL AND METABOLIC PARAMETERS IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE ON THE BACKGROUND OF HYPERTENSION

Abstract. The article presents the factor analysis of pathogenetic relationships between metabolic parameters, EL levels and clinical parameters in patients with liver steatosis on the background of hypertension. The study have found that the most significant factor load on the severity of liver steatosis is reproduced by the concentration of insulin, triglycerides, HDL, and proatherogenic hyperdyslipidemia is closely associated with diet and alcohol intake.

Key words: NAFLD, hypertension, endothelial lipase, dyslipidemia

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver function test results in both adults and children [1]. NAFLD in fact covers a histological spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), advanced fibrosis, and cirrhosis [2]. Simple steatosis without fibrosis or inflammation has a benign clinical course in most but not in all cases without excess mortality [3]. The possible role of NAFLD as an adjunctive risk factor for the development of CV diseases has been debated for a long time, and only recent evidence has demonstrated an existing relationship between these conditions [4]. Insulin resistance is frequently detected in patients with NAFLD, as it is in those without obesity and diabetes [5] An increasing number of patients have been described with normal body mass index (BMI), although these individuals may have central adiposity and occult insulin resistance [6] NAFLD is frequently associated with the components of metabolic syndrome, such as type 2 diabetes mellitus (T2DM), obesity, hypertension, and dyslipidemia [7]. Several studies have shown that the adoption of a healthy lifestyle, weight loss, and pro-active management of individual components of metabolic syndrome can help to prevent, retard or reverse NAFLD-related liver damage [8].

Endothelial lipase (EL) is a strong determinant of structural and functional properties of high-density lipoprotein (HDL) [9]. EL - a new marker of cardiovascular risk, which is closely associated with dyslipidemia and insulin resistance, and almost has not been studied in the presence of NAFLD [10].

Independently, NAFLD increases the risk of premature cardiovascular disease and associated mortality, so investigation and monitoring of the liver metabolic function and early detection of EL accumulation having great importance.

The purpose of the study was to conduct a factor analysis of pathogenetic relationships between metabolic parameters, EL levels and clinical parameters in patients with liver steatosis on the background of hypertension.

Materials and methods 80 patients have been examined on the basis department of internal medicine №1 of Kharkiv National Medical University and National Institute of Therapy named by L.T. Malaya of National Academy of Medical Sciences of Ukraine.

The study has been conducted according to the requirements European Convention for the Protection of Vertebrate Animals (Strasbourg, 08.03.1986), Directive of the Council of the European Economic Society for the Protection of Vertebrate Animals (Strasbourg, 24.11.1986) of the Law of Ukraine "About medications", 1996, articles 7,8,12, according to ICH GCP (2008), GLP (2002), according to the requirements and norms, the standard provisions on ethics of the Ministry of Health of Ukraine No. 690 of 23.09.2009. The research was approved by the Ethics Commission of the Kharkiv National Medical University (Protocol No. 7 of 13.09.2016) and conducted in accordance with the principles of the Helsinki Declaration.

The patients have been divided into three groups according to the severity of liver steatosis. The first group consisted of 16 patients with hypertension without laboratory or instrumental signs of liver steatosis (hypertension group). Patients who, in addition to hypertension, had signs of steatosis during ultrasound and normal level of transaminases (ALT, AST), formed a group with moderate liver steatosis (MLS, n = 20). Patients with hypertension who, in addition to the echoscopic features of hepatic steatosis had increased level of transaminases, were assigned to the group with severe liver steatosis (group SLS, n = 24). The control group consisted of 20 practically healthy individuals. The patients' ages ranged from 45 to 60 years, with an average age of 52.12 $\pm$ 5.24 years. Among them 28 were female (46.66%) and 32 were male (53.33%)

The NAFLD diagnosis was established according to the Protocol № 826 of the Ministry of Health of Ukraine of 6.11.2014, based on the criteria of the American Association for the Study of Liver Diseases [11] and European Recommendations for the Diagnosis and Treatment of NAFLD [12], to be exact, in the presence of ultrasound criteria of liver steatosis and severity of metabolic disorders.

The diagnosis of hypertension was established according to the Protocol No. 384 of the Ministry of

Health of Ukraine dated 24.05.2012, determining the stage and degree of hypertension is according to the clinical guidelines for arterial hypertension (2017) of the European Society for Hypertension (ESH) and the European Society of Cardiology (ESC) [13].

To clarify the existing steatosis and confirm its severity, we have calculated and investigated the surrogate coefficients recommended by EASL-EASD-EASO for calculation in this contingent of patients. So for identification of liver steatosis and its severity we have used liver fat index (NAFLD liver fat score), which includes such indicators as the presence of metabolic syndrome and T2DM, serum insulin level, AST and the ratio AST/ALT and is calculated by the formula [14]:

NAFLD liver fat score= - 2.89+1.18×metabolic syndrome (yes=1/no=0)+ 0.45×type 2 diabetes (yes=2/no=0)+0.15× fasting serum Insulin (mU/L)+ 0.04 × fasting serum AST(U/L) – 0.94 × AST/ALT.

The FIB-4 index has been used to identify liver fibrosis, which includes indicators such as AST, ALT, platelet count, and is calculated by the formula [15]:

FIB4 = Age (years) ×AST (IU/L)/platelet count ($\times 10^9$/L)×√ALT (IU/L)

The body mass index (BMI) has been accessed for all patients. Measurement of blood pressure (BP) has been performed according to the standard auscultatory method by N.S. Korotkov (office measurement) using a sphygmomanometer No. 31304500 (Erka, Chemnitz, Germany).

For the diagnosis of overweight and obesity we have used BMI, which was determined by the Kettle formula where body weight (in kg) is divided by height (in meters) squared. The results have been interpreted according to WHO recommendations. To diagnose the obesity phenotype, a visual assessment of the localization of fat deposits has been carried out - in the upper, in the lower half of the body, the measurement of WC (in cm), the ratio of WC/HC.

The examination plan also included: complete blood count, urine test, determination of serum glucose, studies of total protein, total bilirubin and its fractions, aminotransferase activity, alkaline phosphatase, thymol test, determination of total cholesterol, triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoprotein (VLDL) and atherogenic coefficient. In order to analyze the carbohydrate metabolism, glucose and fasting insulin levels have been determined. The HOMA index is calculated by the formula: HOMA-IR = (fasting blood insulin (U / ml) x fasting blood glucose (mmol/l)) / 22.5. HOMA-IR values ≥ 2.77 were regarded as presence of insulin resistance.

Serum endothelial lipase (EL) concentration was determined by enzyme-linked immunosorbent assay using Aviscera Bioscience INC reagent kit (USA) using a Labline 90 enzyme immunoassay analyser.

For excluding the alcoholic genesis of NAFLD all patients have been interviewed to determine alcohol units. This test has international standardization and allows detecting alcohol abuse by the formula:

Alcohol units = amount (liters) × alcoholic strength (%) × 0.789

Alcohol abuse was eliminated by less than 14 units per week regardless of gender [16].

In order to monitor the implementation of dietary recommendations, we have used a questionnaire designed by the original questionnaire, which asked patients about the consumption of 15 basic foods that are not recommended for overweight, carbohydrate metabolism disorders and liver steatosis. They are aerated waters, fast food, potatoes, baking, including bread, fried meat, sugar, "yellow" fruits, honey, spices, canned food, semi-finished products, cereals (semolina, millet porridge, corn, white rice, muesli), chocolate, milk products with more than 2.5% fat, smoked foods.

Suggested answers included options for the frequency of use of the products: every day; several times a week; several times a month; several times a year; never and they had a gradation of points from 4 to 0, respectively. Result was scored as: 0-15 points - factor 0: dietary recommendations were followed almost without breakdowns; 15-30 - Coefficient1: Dietary recommendations were followed with rare disruptions; 30-45 - Coefficient 2: Dietary recommendations were followed with frequent disruptions; 45-60 - Coefficient 3: Dietary recommendations are practically not followed.

The statistical processing of the survey data has been performed using Microsoft Exel and Statistica 7.0 using standard methods of virion statistics. To analyse the association between the features, Pearson parametric and non-parametric Spearman parameter correlation was performed. The relationship was considered statistically significant at $p < 0.05$. In order to establish patterns of grouping of features, cluster and factor analyses have been performed. To identify the dependence of different features, linear and nonlinear (logistic regression) regression analysis (standard and stepwise) have been reproduced with model creation.

Results and discussion. Results of studies are presented in table 1.

There was a significant difference in groups with surrogate coefficient of hepatic steatosis (NAFLD), which also takes into account insulin sensitivity, which significantly (SS effect 27.39; MS effect 13.69; $F = 6.73$; $p = 0.002$) increased from group to groups. The most sensitive and specific for the determination of hepatic fibrosis, the FIB-4 surrogate coefficient increased significantly from group to group. However, diagnostically significant levels were only available in patients with severe steatosis, suggesting disorders that correspond to F1-F2 fibrosis on the METAVIR scale. The severity of liver steatosis is directly associated with overweight and abdominal fat. All hypertension patients had a proatherogenic lipidogram profile and increased EL concentration, but the severity of lipid disorders was exacerbated as steatosis increases and was associated with an increase in insulin resistance. The level of endothelial lipase in patients with steatosis on the background of hypertension was significantly higher than in patients with hypertension without steatosis and significantly higher than the value in the

control group. In addition, an increase in endothelial lipase was the highest in patients with severe steatosis and was associated with metabolic disorders. So in general EL level can be attributed to independent markers of atherosclerotic process and cardiovascular risk.

Table 1.

ANTHROMETRIC, LABORATORY AND SURROGATE RATIOS INDICATING THE SEVERITY OF LIVER STEATOSIS

Parameter	Control, n=20		Hypertension group, n=16		MLS group, n=20		SLS group, n=24		Significance of difference, P
	0		1		2		3		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
AST/ALT, U	0.73	0.34	0,95	0,26	1,19	0,38	1,75	0,77	12, 23, 13
ALT, U/L	18,15	7,26	22,44	6,29	24,65	7,19	62,54	40,78	23, 13
AST, U/L	11,45	3,78	24,56	7,84	22,90	10,18	38,71	20,70	23, 13
AP, mmol/l	1334,90	464,11	1422,50	302,46	1457,13	384,29	1834,06	690,13	13, 23
NAFLD liver fat score	-1,93	0,65	-0,308	1,14	2,308	2,43	4,48	3,21	For all groups < 0,001
Fib-4	0,43	0,16	1,07	0,36	1,14	0,72	1,36	0,63	With control - all groups< 0.0001 13, 23
BMI, kg/m2	21,44	1,57	25,91	3,42	30,00	2,79	29,04	5,44	01, 02, 03, 12
WC, cm	75,50	6,83	79,31	8,58	98,08	10,53	104,10	8,67	02, 03, 12, 13, 23
WC/height, U	0,44	0,03	0,47	0,04	0,57	0,05	0,60	0,04	01, 02, 03, 12, 13, 23
SBP, mm Hg	116,00	4,17	161,56	17,77	163,89	17,54	169,17	22,20	01, 02, 03
DBP, mm Hg	73,50	5,16	101,56	7,47	102,78	8,26	101,46	9,94	01, 02, 03
Cholesterol, mmol/l	3,85	0,77	5,25	1,47	5,74	0,85	5,80	1,42	01, 02, 03
Triglycerides, mmol/l	0,92	0,16	1,13	0,38	1,70	0,83 0,33	1,96	0,67 0,37	12, 13, 23
HDL, mmol/l	1,77	0,28	1,47	0,42	1,42	0,30	1,20	0,27	13, 23
LDL, mmol/l	2,36	0,46	3,45	1,41	3,34	0,85	3,75	1,25	
VLDL, mmol/l	0,38	0,05	0,56	0,16	0,70	0,40	0,92	0,31	13, 23
Endothelial lipase, ng / ml	8,23	2,47	10,54	2,69	13,21	3,59	13,71	3,71	01, 02, 03, 12, 13
Diet	2,36	0,81	2,57	0,53	2,64	1,15	2,08	0,86	
Alcohol units	4,26	2,27	4,29	1,82	6,39	2,99	6,62	2,98	02, 03, 12, 13
Fasting glucose , mmol/l	4,36	0,72	5,01	0,60	6,32	1,75	5,73	0,91	12, 13
Fasting insulin, mU/l	7, 91	3,71	17,77	6,86	24,51	9,49	33,28	13,82	12, 13, 23
HOMA-IR	1,55	0,85	3,61	1,80	7,02	4,76	8,35	5,25	12, 13
HbA1C, %	-	-	5,40	0,63	6,64	1,76	5,79	0,49	12, 23, 13

A factor analysis of the model has been carried out, which contained the studied parameters. Analysis of variants with orthogonal rotation of factors, minimizing the number identified 2 main factors of model distribution (Table. 2). Factor 1 included the grouping of factors with the most significant factor loadings of the following parameters: severity of liver steatosis, concentration of insulin, triglycerides, HDL (negative contribution), VLDL. Significant factor 2 load is associated with cholesterol, LDL, diet (negative contribution) and alcohol consumption (negative contribution).

Table 2

FACTOR LOAD OPTION FOR DISTRIBUTION BY MAJOR COMPONENTS BY VARIMAX NORMALIZED

Parameter	Factor - 1	Factor– 2
Insulin 1	0,73	0,01
HbA1C	0,24	0,08
EL 1	0,38	0,38
Cholesterol	0,24	0,84
Triglycerides	0,79	0,14
HDL	-0,63	-0,01
LDL	0,15	0,81
VLDL	0,69	0,06
SAD 1	0,36	-0,02
DAD 1	0,26	-0,12
BMI 1	0,19	0,07
WC/ height	-0,53	-0,19
Alcohol units	0,38	-0,62
Diet	0,15	-0,78
NAFLD liver fat score	0,77	0,02
Expl.Var	3,32	2,55
Prp.Totl	0,23	0,18

Using the quartimax method, which minimizes the number of factors needed to explain the variable, it was concluded that blood glucose and triglyceride measurements should at least be obtained to predict NAFLD severity. To predict proatherogenic hyperlipidemia, one must be aware of diet and alcohol intake (Table 3).

Table 3

FACTOR LOAD OPTION OF QUARTIMAX NORMALIZED DISTRIBUTION BY MAJOR COMPONENTS

Parameters	Factor – 1	Factor – 2
WC/ height	0,56	0,07
Insulin 1	0,72	0,01
HbA1C	0,27	-0,06
EL 1	0,39	-0,36
Cholesterol	0,26	-0,83
TG	0,74	-0,14
HDL	-0,61	-0,01
LDL	0,18	-0,79
VLDL	0,65	-0,06
SAD 1	0,35	0,03
DAD 1	0,25	0,12
BMI 1	0,33	-0,02
Alcohol units	0,37	0,63
Diet	0,11	0,77
NAFLD liver fat score	0,78	0,01
Expl.Var	3,57	2,53
Prp.Totl	0,24	0,17

The ratio of factor loadings in factor 1 versus factor 2 is presented in figure 1, showing that blood pressure, BMI and glycated hemoglobin are located in the middle, so they have the least variability, even though they form the model from a clinical point of view (criteria of selection and severity). The location of the EL also has its place in the middle of the system, although it has a certain distance relative to the named central components. Also significant is the fact that the expressiveness of the factor load of EL in both factors is the same - 0.381, which explains the significant dependence of the parameter on the ratio of other components of the system.

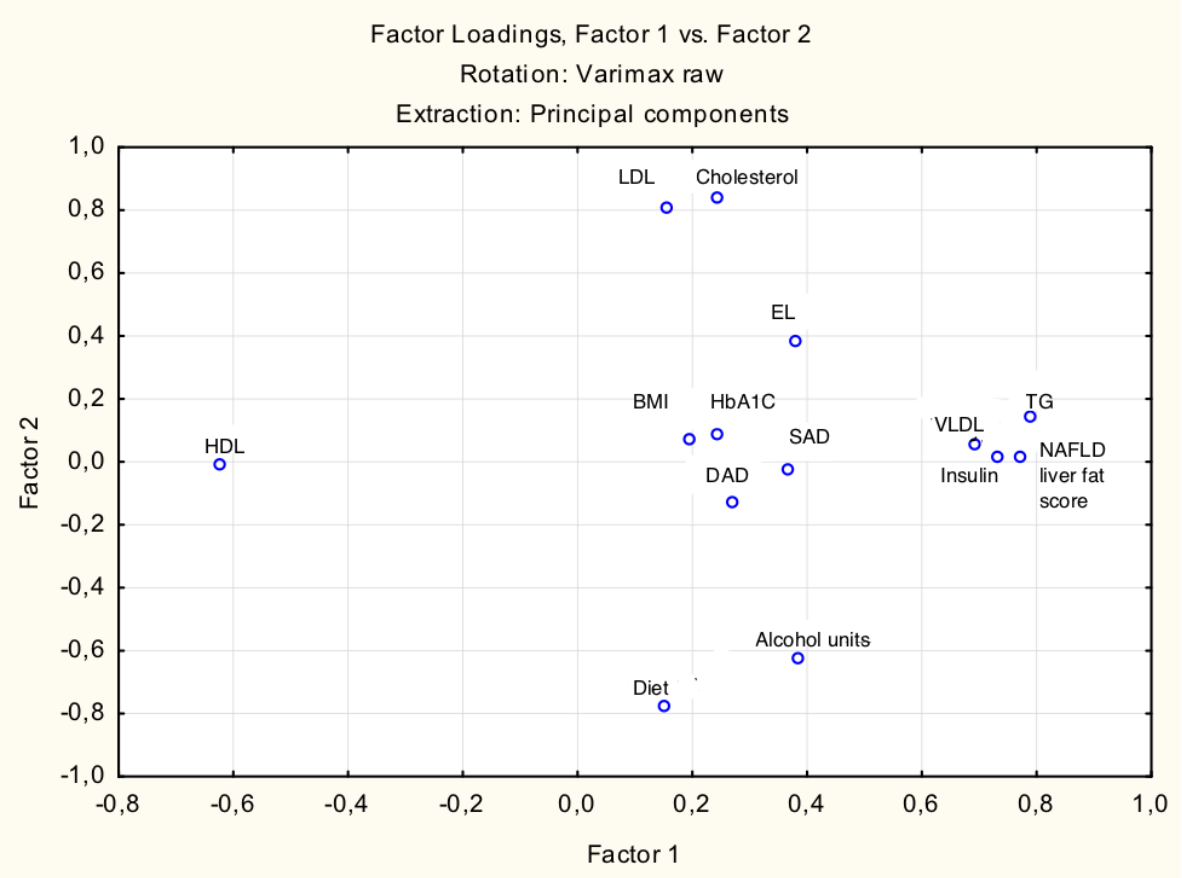

Fig 1. Factor load ratio of model components in factor 1 and factor 2.

Thus, the system itself formed by clinical parameters is variable due to the unstable lipid-carbohydrate ratio between the parameters. Indeed, after selecting the most significant covariable factors, cholesterol levels (-0.98) and LDL (-0.88) remain for factor 1, insulin levels (-0.88) and steatosis (- 0.94), which is presented in table 4. The cumulative percentage of variability for factor 1 is 20.73% (eigenvalue of factor is 2.90), for factor 2 it is 34.96% (eigenvalue of factor is 1.99).

Table 4

THE MOST SIGNIFICANT FACTORS BY THE FACTOR LOAD

Parameter	Factor - 1	Factor– 2
Insulin 1	-0,281143	-0,884764
HbA1C	-0,009640	-0,208619
EL 1	-0,310367	-0,195030
Cholesterol	-0,981871	0,126769
TG	-0,383178	-0,324554
HDL	0,105576	0,300576
LDL	-0,883342	0,171048
VLDL	-0,275324	-0,248849
SAD 1	0,034232	-0,053297
DAD 1	0,062614	-0,037207
BMI 1	-0,094491	-0,145286

Alcohol units	0,183776	-0,360396
Diet	0,395081	-0,192957
NAFLD liver fat score	-0,309226	-0,941152
Expl.Var	2,453105	2,245520
Prp.Totl	0,175222	0,160394

Conclusions

1. In patients with NAFLD on the background of hypertension additional association between EL and glycemic control and hypertension (MR = 0.47; F (5.44) = 2.56; P <0.05) forms, so endothelial lipase can be considered as an additional predictor of cardiovascular risk.

2. In the expressiveness of factor load EL depends equally on other metabolic parameters, such as dyslipidemia, insulin resistance and severity of steatosis.

3. The most significant factor load on the severity of liver steatosis is reproduced by the concentration of insulin, triglycerides, HDL (negative contribution). And pro-atherogenic hyperdyslipidemia is tightly linked to diet and alcohol consumption.

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