MEDICINE

CLUSTER ANALYSIS OF THE PATHOGENETIC RELATIONSHIPS OF METABOLIC PARAMETERS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE ON THE BACKGROUND OF HYPERTENSION

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ABSTRACT

The aim of the study was to conduct a cluster analysis of pathogenetic relationships between metabolic parameters, endothelial lipase levels, the severity of steatosis, and clinical parameters in patients with non-alcoholic fatty liver disease with hypertension. To analyze pathogenetic relationships, a cluster analysis was performed with the distribution of parameters into 4 clusters using the Ward's method. The most dense metabolic link by cluster analysis endothelial lipase forms with NAFLD liver fat score (2.639 cu), HbA1C (2.084 cu), total cholesterol (2.272 cu), and alcohol units (2.797 cu).

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Non-alcoholic fatty liver disease (NAFLD) is the most common cause of impaired liver function in adults and children [1]. NAFLD covers the histological spectrum from simple steatosis to non-alcoholic steatohepatitis (NASH), progressive fibrosis and cirrhosis [2]. Simple steatosis without fibrosis or inflammation in most cases has a benign clinical course, but often leads to an increase in mortality [3]. The possible role of NAFLD as a risk factor for the development of cardiovascular diseases has been discussed for a long time, and only recent data have demonstrated the existing relationship between these conditions [4]. Insulin resistance is often detected in patients with NAFLD, as in patients without obesity and diabetes [5]. NAFLD is often associated with components of the metabolic syndrome, such as type 2 diabetes mellitus (T2DM), obesity, hypertension, and dyslipidemia [7]. However, an increasing number of patients with a normal body mass index (BMI) have been described, with central obesity and latent insulin resistance. [6] Several studies have shown that adopting a healthy lifestyle, reducing weight, and proactively correcting individual components of the metabolic syndrome can help prevent, slow down, or reverse liver damage associated with NAFLD [8].

Endothelial lipase (EL) is a strong determinant of the structural and functional properties of high density lipoproteins (HDL) [9]. EL is a new marker of cardiovascular risk, which is closely associated with dyslipidemia and insulin resistance and has hardly been studied in the presence of NAFLD [10].

Regardless of this, NAFLD increases the risk of premature cardiovascular disease and related mortality, therefore, research and monitoring of the metabolic function of the liver and early detection of accumulation of EL, as well as the relationships between them, are of great importance.

The purpose of the study was to conduct a cluster 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 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 + 5.24 years. Among them 28 were female (46.66%) and 32 were male (53.33%).

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 [11]:

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 [12]:

FIB4 = Age (years) ×AST (IU/L)/platelet count (×109/L)×√ALT (IU/L)

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 [13].

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.

The statistical processing of the survey data has been performed using Microsoft Exel and Statistica 7.0 using standard methods of virion statistics.

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

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	
1	2	3	4	5	6	7	8	9	10
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

Continuation of table 1

1	2	3	4	5	6	7	8	9	10
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
EL, 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

For the analysis of pathogenetic relationships, a cluster analysis was performed. The graph of parameter merging using Ward's method showed that it is advisable to distribute data into 3-4 clusters (Fig. 1).

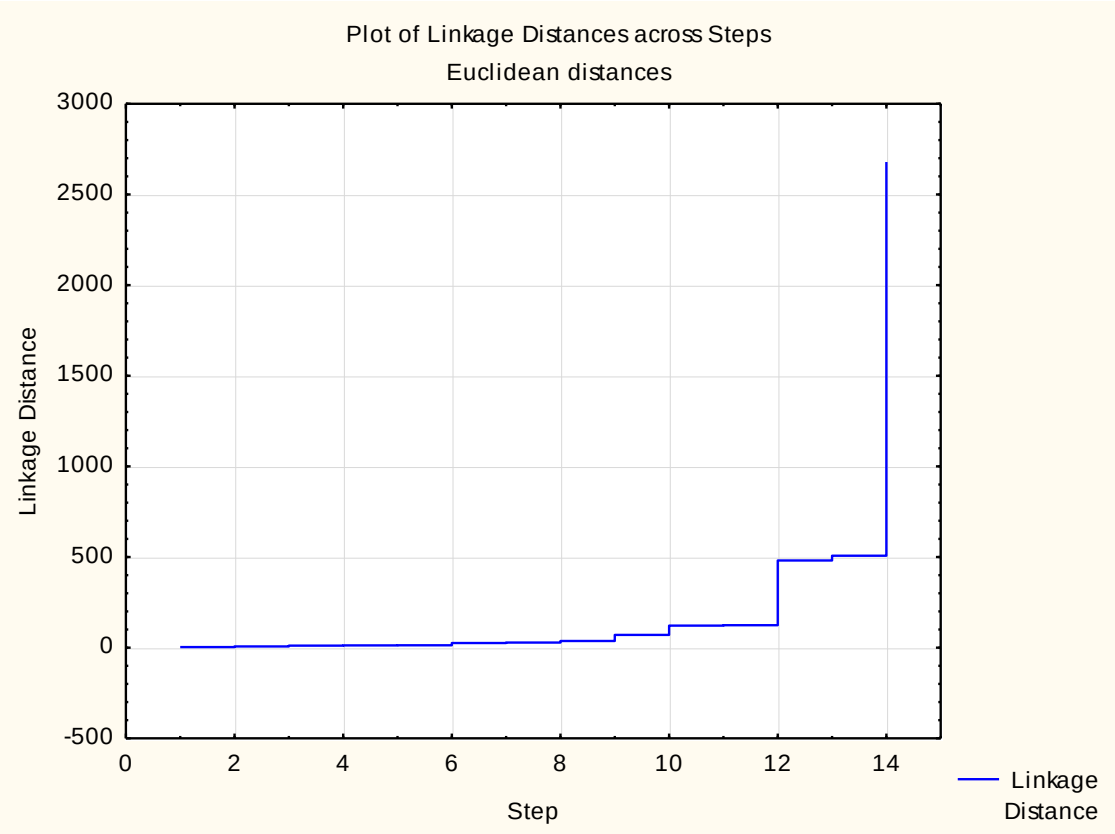

Fig. 1. Cluster aggregation of data parameters

The first and second cluster illustrates the existence of offline hypertension and the close relationship of hyperinsulinism with BMI.

The third cluster covered liver steatosis associated with alcohol consumption, compensation for carbohydrate metabolism (by HbA1C) and the level of total cholesterol and EL.

The fourth cluster demonstrates the connection of lipid profile parameters with the patient's nutritional preferences (Table 2).

Table 2. Clustering of model components containing parameters of lipid-carbohydrate metabolism with hypertension and liver steatosis

Cluster 1		**Cluster 2**		**Cluster 3**		**Cluster 4**	
Parameter	**Mean**	**Parameter**	**Mean**	**Parameter**	**Mean**	**Parameter**	**Mean**
SBP	33,04	Fasting insulin	8,520	HbA1C	2,084	Triglycerides	0,840
DBP	33,04	BMI	8,520	EL	5,386	HDL	1,231
				Cholesterol	2,272	LDL	1,864
				Alcohol units	2,797	VLDL	1,470
				NAFLD liver fat score	2,639	Diet	1,306
						WC/height	1,472

Grouping the results of the examination of patients allowed us to identify 4 main clusters. Cluster 1 - overweight patients with abdominal fat distribution and moderate hypertension, moderate hyperinsulinism with prediabetic levels of HbA1C, increased levels of total cholesterol, LDL and borderline HDL, moderate steatosis (table 3).

Table 3. Basic statistical analysis of clinical and laboratory parameters Cluster 1

Parameters	**Valid N**	**Mean**	**Minimum**	**Maximum**	**Variance**	**Std.Dev.**	**Coef.Var.**
WC/height	13	0,5391	0,3593	0,6221	0,00544	0,073755	13,68212
Fasting insulin	13	25,0715	15,7000	46,0500	93,55990	9,672637	38,58015
HbA1C	13	6,1092	4,3300	10,3000	2,36916	1,539207	25,19477
EL	13	12,0465	7,0665	17,4200	9,18047	3,029928	25,15198
Cholesterol	13	5,9615	4,0200	7,7900	1,40600	1,185748	19,88996
Triglycerides	13	1,4846	0,7900	4,1600	0,73911	0,859715	57,90828
HDL	13	1,4046	0,6900	2,0000	0,13968	0,373734	26,60755
LDL	13	3,7492	2,2800	5,3500	0,86937	0,932402	24,86917
VLDL	13	0,6277	0,1900	1,8700	0,16587	0,407270	64,88377
SBP	13	142,6923	130,0000	150,0000	52,56410	7,250111	5,08094
DBP	13	94,6154	80,0000	100,0000	56,08974	7,489309	7,91553
BMI	13	27,5394	16,8525	32,4500	17,56233	4,190744	15,21728
Alcohol units	13	6,4231	2,0000	9,2000	5,46692	2,338145	36,40226
Diet	13	2,7692	1,0000	4,0000	0,85897	0,926809	33,46809
NAFLD liver fat score	13	2,1543	-0,8161	6,1263	3,98455	1,996134	92,65719

Cluster 2 included patients with grade 1 obesity with severe abdominal fat distribution and severe hypertension, a pre-diabetic level of HBA1C, an increase in total cholesterol, triglycerides, LDL and a distinct decrease in HDL and severe liver steatosis (Table 4).

Table 4. Basic statistical analysis of clinical and laboratory indicators of representatives of Cluster 2

Parameters	Valid N	Mean	Minimum	Maximum	Variance	Std.Dev.	Coef.Var.
WC/height	11	0,5934	0,5058	0,7317	0,0038	0,06164	10,38688
Fasting insulin	11	53,0082	35,3300	73,3500	172,3410	13,12787	24,76574
HbA1C	11	6,0109	4,8600	7,4400	0,9214	0,95992	15,96965
EL	11	13,6655	7,7600	19,7200	21,8332	4,67261	34,19283
Cholesterol	11	6,4600	4,1500	8,5500	1,4985	1,22414	18,94954
Triglycerides	11	2,5227	0,9700	5,6900	1,8036	1,34298	53,23534
HDL	11	1,0718	0,7600	1,5600	0,0545	0,23340	21,77624
LDL	11	4,2218	1,9400	6,4000	1,8807	1,37138	32,48310
VLDL	11	1,0982	0,3700	2,5600	0,4095	0,63995	58,27362
SBP	11	175,9091	160,0000	180,0000	54,0909	7,35465	4,18094
DBP	11	105,0000	100,0000	120,0000	45,0000	6,70820	6,38877
BMI	11	30,0625	22,9481	48,3343	51,2784	7,16089	23,82002
Alcohol units	11	6,1909	1,5000	10,0000	8,6669	2,94396	47,55298
Diet	11	2,9091	1,0000	4,0000	1,0909	1,04447	35,90352
NAFLD liver fat score	11	6,8779	0,9228	10,9828	11,5463	3,39799	49,40435

Cluster 3 was composed of overweight patients with abdominal fat distribution, a slight increase in insulin at normal HbA1C, a slight increase in total cholesterol and LDL, normal triglycerides and marginal HDL (Table 5).

Table 5. Basic statistical analysis of clinical and laboratory indicators of representatives of Cluster 3

Parameters	Valid N	Mean	Minimum	Maximum	Variance	Std.Dev.	Coef.Var.
WC/height	23	0,5448	0,4606	0,6875	0,00439	0,066239	12,1574
Fasting insulin	23	18,1643	4,8600	37,1000	85,67632	9,256150	50,9578
HbA1C	23	5,6678	4,3700	7,2800	0,61653	0,785192	13,8535
EL	23	11,2209	5,3300	18,9900	10,31109	3,211089	28,6171
Cholesterol	23	5,3009	2,8400	6,9900	1,65284	1,285627	24,2531
Triglycerides	23	1,3804	0,7500	2,6900	0,29569	0,543770	39,3912
HDL	22	1,3318	0,8000	2,3000	0,11832	0,343977	25,8276
LDL	22	3,2109	1,0000	5,1600	1,38308	1,176042	36,6265
VLDL	23	0,6991	0,3400	1,5000	0,10728	0,327538	46,8493
SBP	23	168,2609	150,0000	180,0000	53,65613	7,325034	4,3534
DBP	23	103,0435	100,0000	120,0000	31,22530	5,587960	5,4229
BMI	23	28,1174	22,6003	37,1255	13,17259	3,629407	12,9080
Alcohol units	23	5,3000	1,5000	11,5000	8,36909	2,892938	54,5837
Diet	23	3,0000	1,0000	4,0000	0,90909	0,953463	31,7821
NAFLD liver fat score	23	0,8359	-2,7277	4,9742	4,40681	2,099239	251,1388

Cluster 4 is the least numerically representative, but the most unexpected. It included patients with severe abdominal obesity, diabetic levels of HbA1C, moderate hyperinsulinism, with an increase in total cholesterol, TG, LDL and a decrease in HDL (Table 6).

Table 6. Basic statistical analysis of clinical and laboratory indicators of representatives of Cluster 4

Parameters	Valid N	Mean	Minimum	Maximum	Variance	Std.Dev.	Coef.Var.
WC/height	5	0,5782	0,4821	0,6180	0,0032	0,05692	9,84298
Fasting insulin	5	21,9720	16,1700	27,5400	26,5516	5,15283	23,45179
HbA1C	5	6,6280	5,4200	9,9600	4,8687	2,20652	34,86912
EL	5	13,9380	9,2300	18,2800	14,5143	3,80977	27,33367
Cholesterol	5	5,6600	4,6000	6,7600	0,7388	0,85951	15,18561
Triglycerides	5	2,2500	1,2300	3,2900	0,8322	0,91222	40,54322
HDL	5	1,1440	0,7800	1,6100	0,1012	0,31817	27,81177
LDL	5	3,5060	2,4400	4,5000	0,6001	0,77468	22,09585
VLDL	5	1,0000	0,5000	1,4800	0,1811	0,42562	42,56172
SBP	5	203,0000	190,0000	240,0000	445,0000	21,09502	10,39164
DBP	5	114,0000	100,0000	130,0000	130,0000	11,40175	10,00154
BMI	5	30,1405	24,8016	33,9100	12,8248	3,58118	11,88161
Alcohol units	5	6,8800	4,5000	11,5000	10,6970	3,27063	47,53818
Diet	5	2,6000	2,0000	3,0000	0,3000	0,54772	21,06625
NAFLD liver fat score	5	1,7530	0,2018	2,9370	1,5633	1,25032	71,32395

For better visualization, we have reduced the average values of the parameters for the clusters into a common table (Table 7), which allows us to compare trends.

Table 7. The average values of clinical and laboratory indicators with the distribution of clusters

Parameters	**Cluster 1**	**Cluster 2**	**Cluster 3**	**Cluster 4**
WC/height	0,54	0,59	0,54	0,58
Fasting insulin	25,07	53,01	18,16	21,97
HbA1C	6,11	6,01	5,67	6,33
EL	12,05	13,67	11,22	13,94
Cholesterol	5,96	6,46	5,30	5,66
Triglycerides	1,48	2,52	1,38	2,25
HDL	1,40	1,07	1,33	1,14
LDL	3,75	4,22	3,21	3,51
VLDL	0,63	1,10	0,70	1,00
SBP	142,69	175,91	168,26	203,00
DBP	94,62	105,00	103,04	114,00
BMI	27,54	30,06	28,12	30,14
Alcohol units	6,42	6,19	5,30	6,88
Diet	2,77	2,91	3,00	2,60
NAFLD liver fat score	2,15	6,88	0,84	1,75

The lowest variability of characteristics in the first cluster is inherent in indicators of blood pressure, BMI and insulin concentration, and the largest - in the severity of liver steatosis.

The lowest variability in the second cluster is inherent in blood pressure and anthropometric parameters, as well as indicators of carbohydrate metabolism. The variability of the severity of steatosis is half that of the previous group.

Cluster 3 from cluster 1 is distinguished by lower numbers of blood pressure, less severe liver steatosis and less alcohol abuse. The indicated group is determined by the relative stability of lipid

profile parameters, the stability of carbohydrate metabolism compensation, but the high variability of NAFLD. Thus, it is understood that the formation of steatosis is not latent even under conditions of a moderate shift in metabolic parameters.

Cluster 4 from cluster 2 is distinguished by pronounced hypertension, low insulin values, less compensation for carbohydrate metabolism, but also less severity of liver steatosis. In addition, alcohol abuse is the highest in this group, and the lowest adherence to dietary recommendations. The fact that dyslipidemia is isolated is also obvious, which is confirmed by the data of a large population study under the auspices of NHANES, which included more than 23 thousand Americans, in patients with hepatic pathology with high levels of transaminases lipid profiles with low LDL and high HDL can be recorded, which may be caused by a defect in the synthesis of lipoproteins or a violation of the synthetic function of the liver and a marker of latent hepatopathies [14].

Conclusions. 1. Clustering of patient examination results demonstrates a reliable distribution of groups according to the severity of liver steatosis.

2. In case of non-compliance with dietary recommendations and the use of alcohol even within acceptable limits, the progression of liver steatosis occurs even against the background of minimal metabolic disturbances

3. The presence of genetically determined hyperlipidemia in combination with insulin resistance is an unfavorable background in the implementation of the clinical manifestations of metabolic disorders.

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