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RESEARCH ARTICLE

Assessment of hepatic steatosis algorithms in non-alcoholic fatty liver disease

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Abstract:
Background: In order to optimize the identification of persons with non-alcoholic fatty liver disease (NAFLD), several algorithms for hepatic steatosis were developed. These available algorithms, as well as an algorithm, derived using biochemical and anthropometric data of our participants, are compared in a cross-sectional pilot study.
Material and methods: We included 77 participants with abdominal obesity: 43 with NAFLD and 33 without NAFLD. Body mass index (BMI), waist circumference (WC) and hip circumference (HC), systolic and diastolic blood pressure were assessed. Fibrinogen, high sensitive C-reactive protein (hsCRP), aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), uric acid, ferritin, glucose, insulin, homocysteine, lipid status parameters, apolipoprotein A-I, apolipoprotein B and Lp(a)-lipoprotein were measured. Fatty liver was assessed by ultrasound with the presence or absence of hepatic steatosis. Discovering the most significant factor in the presence of NAFLD is assessed through logistic regression modeling. The predictor variables were chosen according to an algorithm derived from conducted factor analysis and other available algorithms for hepatic steatosis.
Results: Participants with NAFLD had significantly higher BMI (34.38 ± 9.73 vs 28.05 ± 4.79 kg/m^2, p =0.001), WC (108.05 ± 11.47 vs 96.15 ± 14.27 cm, p =0.001), HC (114.93 ± 11.01 vs 108.21 ± 9.82 cm, p =0.050), systolic (128.98 ± 8.67 vs 122.42 ± 10.62 mmHg, p =0.010) and diastolic blood pressure (83.64 ± 5.94 vs 78.33 ± 7.57 mmHg, p =0.001), AST (23.93 ± 6.91 vs 21.70 ± 5.21 U/L, p =0.014), ALT (30.50 ± 13.70 vs 23.00 ± 11.75 U/L, p =0.007), hsCRP (4.34 ± 5.56 vs 2.98 ± 2.34mg/l, p =0.004) and uric acid (358.02 ± 83.29 vs 296.78 ± 84.54μmol/l, p =0.001), in comparison non NAFLD. Logistic regression model with algorithm derived from factor analysis showed the best performance. From other available algorithms, only fatty liver index (FLI) and hepatic steatosis index (HSI) had statistically significant discriminatory power.
Conclusions: Elevation of WC, HC, BMI, DBP, SBP, Fbg, hsCRP, glucose, and uric acid, incorporated in our hepatic steatosis prediction model, had the best predictive power among all assessed algorithms. HIPPOKRATIA 2018, 22(1): 10-16.

Keywords: Non-alcoholic fatty liver disease, NAFLD, algorithms, body mass index, abdominal obesity

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Introduction

Non-alcoholic fatty liver disease (NAFLD) has become popular in the recent years because of its high prevalence rate of more than 30 % in the general population and up to 70 % in the high-risk groups, such as morbidly obese individuals and patients with type 2 diabetes mellitus (T2DM)[1].

NAFLD represents a broad spectrum of disease from isolated steatosis (NAFL: non-alcoholic fatty liver) up to a more severe form, known as non-alcoholic steatohepatitis (NASH) which is associated with lobular inflammation, ballooning, and progression to fibrosis and cirrhosis[2]. According to the American Association for the Study of Liver Disease (AASLD) NAFLD is evidence of hepatic steatosis in more than 5 % of hepatocytes; either by imaging or histology in the absence of other causes of secondary hepatic fat accumulation, such as significant alcohol consumption, steatogenic medication, viral hepatitis or hereditary disorders[3].

NAFLD is strongly associated with progressive hepatic disease, but also with cardiometabolic disorders. It may accelerate the development of T2DM and cardiovascular disease[4]. Therefore, it is vital to identify patients with hepatic steatosis for early risk intervention.

Although liver biopsy is considered to be the gold standard for diagnosis of NAFLD, it is invasive and therefore not applicable as a screening method in the routine clinical practice. Proton magnetic resonance spectroscopy (^{1}H-MRS) is considered the most accurate non-invasive method for measuring liver fat content[5]. However, it is only available in highly specialized institutions due to its high cost. Routine ultrasound is also being used to diagnose NAFLD, but it has its limitations. Ultrasound is subjective, and operator dependent, which has a low sensitivity for the detection of mild steatosis and there may be technical issues in morbidly obese patients. Therefore, there has been considerable interest in blood markers alone or in combination with clinical parameters which might help in identifying patients with NAFLD[2]. Several steatosis algorithms have been reported in the literature since 2005, and their diagnostic performances for prediction of hepatic steatosis are reported in Table 1[6-10].

The current study aimed to compare the different existing steatosis algorithms as well as an algorithm that was made according to biochemical and anthropometric data of participants from our study group.

Material and methods

Subjects

The cross-sectional pilot study was conducted from July 2016 until July 2017. We evaluated 77 subjects of both sexes (42 female and 35 male). Inclusion criterion for the participants was abdominal obesity [waist circumference (WC) ≥102 cm in males and ≥88 cm in females according to the National Cholesterol Educational Program Adult Treatment Panel III (NCEP ATP III) criteria for abdominal obesity][11]. Participants were both genders, between 18 and 55 years old, with females in their reproductive period. Patients with cardiovascular diseases other than hypertension, renal diseases, infective, hepatic, malignant, and autoimmune diseases were excluded from this study. We also excluded smokers, subjects in acute stress situations or with infections [high sensitive C-reactive protein (hsCRP) >10 mg/l], as well as those with recent (i.e. within three months) weight and physical activity changes. Subjects which used drugs that could affect biomarker levels of endothelial dysfunction, lipid metabolism, glucose metabolism, and menstrual cycle were also excluded. Patients that consumed alcohol more than 20g/day were excluded[3]. Participants with positive surface antigen of the hepatitis B (HB) virus (HBsAg), HB antibodies (anti-HBs) or hepatitis C antibodies (anti-HCV) were also excluded.

Fatty liver was assessed as presence or absence of hepatic steatosis and the grading of hepatic steatosis obtained by ultrasound, identified by a professional operator using the National Health and Nutrition Examination Survey (NHANES) III: Hepatic Steatosis, Ultrasound Images Assessment, Procedures Manual, November 2010[12]. According to that protocol, participants with hepatic steatosis were graded into three degrees of steatosis: mild, moderate, and severe. The study was conducted according to the Declaration of Helsinki and approved by the Ethical Committee of the Clinical Centre of Vojvodina (00-55/336-21/05/2012). Written consent was obtained from every participant.

Study protocol

All participants attended the Department for Nutrition and Food Safety, Center of Hygiene and Human Ecology Institute of Public Health of Vojvodina for anthropometric measurements and clinical examination. Data was collected for each patient regarding the average daily alcohol consumption via a seven-day alcohol consumption survey. Twenty four hours before blood sampling, participants were asked to refrain from strenuous physical activity and consumption of alcoholic and caffeinated beverages. Venous blood was drawn from the antecubital vein after a 12h overnight fast. Analyses were performed immediately after sampling. Ultrasound examination was done the same day using GE healthcare LOGIQ7 (GE Yokogawa Medical Systems Ltd, Tokyo, Japan) and convex probe of 4 Hz.

Anthropometric and clinical measurements

Body height (BH) in centimeters was determined using Martins anthropometer with 0.1 cm precision. Body weight (BW) in kilograms was counted using a decimal scale with movable weights and precision 0.1 kg. Body mass index (BMI) was calculated as BW/BH^2 (kg/m^2). WC was measured using a non-elastic tape band with 0.1 cm precision. WC was measured in standing position in the horizontal plane midway between the lowest rib and the iliac crest. Hip circumference (HC) was measured

Table 1: Algorithms available in literature for the diagnosis of hepatic steatosis.

Algorithm	Parameters	Population	Cut-off	Sensitivity	Specificity	AUROC	Ref
FLI[6]	BMI, TG, WC, GGT	n =496 (SLD, no SLD)	<30 ≥60	87% 61%	64% 86%	0,84	US
HSI[7]	AST/ALT, BMI, DM	n =10,724	<30 >36	93% 46%	40% 92%	0.81	US
LAP[8]	WC, TG, gender	n =588 (SLD, no SLD)	- -	- -	- -	0.79	US
TyG[9]	TG, glucose	n =50	4.59	87%	69%	0.856	LB
VAI[10]	WC, BMI, TG, HDL	n =324	2.45	79%	92%	0.92	LB

Ref: reference used for diagnosis of steatosis, n: number, US: ultrasound, LB: liver biopsy, BMI: body mass index, TG: triglycerides, WC: waist circumference, GGT: gamma glutamyl transferase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, DM: diabetes mellitus, SLD: suspected liver disease, FLI: fatty liver index, HSI: hepatic steatosis index, LAP: lipid accumulation product, VAI: visceral adiposity index, TyG: triglyceride and glucose index, AUROC: area under ROC curve.

using a non-elastic tape band with 0.1 cm precision in standing position around the widest portion of the buttocks, with the tape parallel to the floor[13]. The blood pressure was measured according to standard protocol (Riva Rocci method, mercury sphygmomanometer), in sitting position after ten minutes of rest. For the calculation of the average daily alcohol consumption, >20 g/day of ethanol, the following formula was used:

D (g) = F x Vol (ml) x 0.8 g/ml

where D is grams of ethanol, F is the fraction of ethanol (%v/v), Vol is the volume of drink in milliliters, and 0.8 g/ml is the specific gravity if ethanol.

Analytical procedures

Standard biochemical methods were used to assess blood glucose levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), uric acid, immunoturbidimetry for determination of hsCRP, and ferritin on ADVIA® 1800 Chemistry (Siemens Healthcare Diagnostics, Marburg, Germany). Parameters of lipid status: total cholesterol (TC), triglyceride (TG), and high-density cholesterol (HDL-C) were determined using standard biochemical methods while apolipoprotein A-I (apo A-I), apolipoprotein B (apo B), and Lp(a)-lipoprotein [Lp(a)] using an immunoturbidimetry method (ARCHITECT ci4100, Abbott Laboratories, USA). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation, and in cases TG ≥4.5 mmol/l was determined using the direct method on ARCHITECT ci4100. Coagulametric Clauss method was used to assess fibrinogen on BCxp (Siemens Healthcare Diagnostics, Marburg, Germany). Insulin was measured using an electrochemiluminescent immunoassay (ECLIA) on Elecsys 2010 (COBAS - Roche Diagnostics, USA). HBsAg and anti-HCV antibodies were obtained using enzyme-linked fluorescence assay (EFLA) using mini Vidas (bioMerieux, Marcy-l'Etoile, France). Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as fasting insulin (μIU/ml) x fasting glucose (mmol/l) / 22.5 and homeostatic model assessment for beta cell function (HOMA-%B) as 20 × fasting insulin (μIU/ml)/fasting glucose (mmol/ml) - 3.5[14,15].

Fatty liver index (FLI) was calculated as: [(e0.953 × loge (TG) + 0.139 × BMI + 0.718 × loge (GGT) + 0.053 × (WC) - 15.745) / (1 + e0.953 × loge (TG) + 0.139 × BMI + 0.718 × loge (GGT) + 0.053 × (WC) - 15.745)] × 100[6]. Hepatic steatosis index (HSI) was calculated as 8 × ALT / AST + BMI (+2 if T2DM was present; +2 if female sex)[7]. Lipid accumulation product (LAP) was calculated for males as: (WC [cm] - 65) × (TG [mmol/L]), and for females as: (WC [cm] - 58) × (TG [mmol/L])[8]. Triglyceride and glucose index (TyG) was calculated as: Ln (TG (mg/dl) x glucose (mg/dl)/2)[9] and visceral adiposity index (VAI) for males as: (WC/ 39.68 + (1.88 × BMI) x (TG/1.03) x (1.31/HDL-C), and for females as: (WC/ 36.58 + (1.89 × BMI) x (TG/0.81) x (1.52/HDL-C)[16].

Statistical analyses

Summary statistics are presented as mean and standard deviation for metric variables and as frequencies for categorical variables. Kolmogorov-Smirnov normality test was used to test if a variable follows a normal distribution. Independent T-test and Mann-Whitney U-test were used to compare differences between two independent groups, depending on the distribution of the dependent variables. Spearman correlation coefficient was used to test the association between two ranked variables. Factor analysis was used to extract four-factor structure from biochemical and anthropometrical parameters obtained in the sample. Discovering factors that have a significant influence on the presence of NAFLD is assessed through logistic regression modeling. A total of six logistic regression models were built. In the first model, the independent variable was chosen according to the selected extracted factor. In all other models, independent variables were available diagnostic algorithms for hepatic steatosis. The regression analyses results are presented as coefficients (B), standard errors (S.E.), tests for significance (Wald, df, p), and odds ratios. The odds ratio above 1 indicates a positive association between independent and dependent variables, i.e. increase of the likelihood of dependent variable. The ratio below 1 standard, describes negative associations, i.e., that predictor decreases the likelihood of dependent variable. A significant level of 5 % is considered in each test (IBM SPSS Statistics for Windows, version 20.0, IBM Corp., Armonk, NY, USA).

Results

In this study, we included 77 participants (35 male and 42 female), 44 with NAFLD (23 male and 21 female), and 33 (12 male and 21 female) without NAFLD. Anthropometric and biochemical measurements are shown in Table 2. Subjects with NAFLD had significantly higher BMI, WC, HC, and systolic (SBP) and diastolic blood pressure (DBP). Levels of AST, ALT, uric acid, and hsCRP were significantly higher in participants with NAFLD. There were significantly higher mean values of algorithms for hepatic steatosis FLI, HSI, and LAP in participants with NAFLD.

Correlation analysis between algorithms for hepatic steatosis and degree of NAFLD and HOMA-IR is shown in Table 3. There is a positive statistically significant correlation between the degree of NAFLD determined by ultrasound and FLI, TyG, LAP, and HSI. There were no significant correlations between the degree of NAFLD and VAI. Finally, given the close pathogenic link between steatosis and insulin resistance, we investigated if steatosis scores independently correlated with HOMA-IR. There is a statistically positive correlation between HOMA-IR and all the algorithms of hepatic steatosis except HSI.

According to biochemical and anthropometrical parameters determined in our subjects using factor analysis, four-factor structure was extracted explaining 56.713 % total variance. All factor loadings show values above 0.3 on the main factor; the KMO values of 0.6 indicate dis-

Table 2: Demographic and metabolic characteristics of the 77 participants with abdominal obesity: 43 with non-alcoholic fatty liver disease (NAFLD) and 33 without NAFLD.

Parameter	Subjects without NAFLD n= 33		Subjects with NAFLD n=44		p
	mean	SD	mean	SD	
Age (years)	39.36	6.40	42.34	6.57	0.057[1]
BMI (kg/m2)	28.05	4.79	34.38	9.73	**0.001[1]**
WC (cm)	96.15	14.27	108.05	11.47	**0.001[1]**
HC (cm)	108.21	9.829	114.93	11.010	**0.050[1]**
SBP (mmHg)	122.42	10.62	128.98	8.67	**0.010[1]**
DBT (mmHg)	78.33	7.57	83.64	5.94	**0.001[1]**
hsCRP (mg/l)	1.98	2.34	4.34	5.56	**0.004[1]**
Fbg (g/l)	3.10	0.81	3.32	0.77	0.341[1]
Neu/Ly	1.45	0.43	1.61	0.72	0.71[1]
Uric acid (μmol/l)	296.76	74.06	358.02	83.29	**0.001[2]**
AST (U/L)	21.70	5.21	23.93	6.91	**0.014[1]**
ALT (U/L)	23.00	11.75	30.50	13.70	**0.007[1]**
GGT (U/L)	26.58	21.22	30.77	18.00	0.138[1]
Ferritin (μg/l)	94.97	104.56	129.06	125.08	0.310[1]
Glucose (mmol/l)	5.37	1.12	5.54	0.70	0.23[1]
Insulin (mIU/l)	13.98	7.02	16.18	9.33	0.404[1]
HOMA-IR	3.41	1.92	4.06	2.66	0.33[1]
HOMA-%B	161.60	76.01	166.80	93.57	0.76[1]
TC (mmol/l)	5.49	1.09	5.56	1.08	0.805[1]
TG (mmol/l)	1.78	1.60	1.86	0.91	0.102[1]
HDL-C (mmol/l)	1.28	0.29	1.20	0.29	0.232[1]
LDL-C (mmol/l)	3.43	1.02	3.51	0.93	0.712[2]
Lp(a) (g/l)	0.18	0.28	0.25	0.30	0.095[1]
Apo AI (g/l)	1.38	0.16	1.39	0.21	0.98[1]
Apo B (g/l)	1.08	0.29	1.11	0.24	0.28[1]
Hcy (μmol/l)	10.50	2.65	10.75	2.88	0.75[1]
FLI	51.75	33.6	76.80	21.6	**0.001[1]**
TyG	8.71	0.6	8.91	0.5	0.088[2]
LAP	70.54	80.6	86.17	43.7	**0.008[1]**
HSI	37.58	6.6	45.77	10.9	**0.001[1]**
VAI	2.72	3.10	2.87	2.32	0.237[1]

NAFLD: non-alcoholic fatty liver disease, SD: standard deviation, BMI: body mass index, WC: waist circumference, HC: hip circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma glutamyl transferase, hsCRP: high sensitivity C-reactive protein, Fbg: fibrinogen, TC: total cholesterol, TG: triglyceride, HDL-C: HDL-cholesterol, LDL-C: LDL-cholesterol, Lp(a): Lp(a)-lipoprotein, Neu/Ly: neutrophil/lymphocyte ratio, Apo A-I: apolipoprotein A-I, Apo B: apolipoprotein B, Hcy: homocysteine, HOMA: homeostasis model assessment, FLI: fatty liver index, HSI: hepatic steatosis index, LAP: lipid accumulation product, VAI: visceral adiposity index, TyG: triglyceride and glucose index, [1]: Mann–Whitney U-test, [2]: independent T-test.

Table 3: Correlation analysis between algorithms for hepatic steatosis and degree of non-alcoholic fatty liver disease (NAFLD) and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR).

		FLI	TyG	LAP	HSI	VAI
Degree of NAFLD	CC	0.532	0.301	0.412	0.371	0.167
	p	**0.001**	**0.008**	**0.001**	**0.001**	0.149
HOMA-IR	CC	0.442	0.454	0.332	0.189	0.274
	p	**0.001**	**0.001**	**0.003**	0.100	**0.016**

NAFLD: non-alcoholic fatty liver disease, SD: standard deviation, CC: correlation coefficient, FLI: fatty liver index, HSI: hepatic steatosis index, LAP: lipid accumulation product, VAI: visceral adiposity index, TyG: triglyceride and glucose index, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance.

tinct and reliable factors. The Barlett test was significant (χ^2 =1145.382, df =210, p <0.001) indicating that correlations between items are significantly different from zero. Table 4 shows pattern matrix and indicates the following factor structure: A - obesity (WC, TC, BMI, DBP, SBP, Fbg, hsCRP, glucose, uric acid); B - atherogenic factor (LDL-C, TC, APOB/A-I, Ne/Ly); C - liver damage (ALT, GGT, ferritin, AST), and D - insulin resistance (TG, HDL-C, HOMA-%B, HOMA-IR).

Table 4: Factor analysis - pattern matrix for the following factor structure: A - obesity; B - atherogenic factor; C - liver damage and D - insulin resistance.

Factor	A	B	C	D
HC (cm)	**.807**			
WC (cm)	**.761**			
BMI (kg/m2)	**.675**			
DBT (mmHg)	**.651**			
SBP (mmHg)	**.535**			
Fbg (g/l)	**.543**			
hsCRP (mg/l)	**.538**			
Glucose (mmol/l)	**.380**			
Uric acid (μmol/l)	**.463**			
LDL-C (mmol/l)		**-.853**		
TC (mmol/l)		**-.842**		
Apo B/A-I		**-.732**		
Neu/Ly		**.566**		
ALT (U/L)			**-.794**	
GGT (U/L)			**-.660**	
AST (U/L)			**-.631**	
Ferritin			**-.620**	
TG (mmol/l)				**-.768**
HDL-C (mmol/l)				**.698**
HOMA -%B				**-.676**
HOMA-IR				**-.580**

WC: waist circumference, HC: hip circumference, BMI: body mass index, DBP: diastolic blood pressure, SBP: systolic blood pressure, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma glutamyl transferase, hsCRP: high sensitivity C-reactive protein, Fbg: fibrinogen, TG: triglyceride, HDL-C: HDL-cholesterol, Neu/Ly: neutrophil/lymphocyte ratio, Apo B/A-I: apolipoprotein B/A-I, Hcy: homocysteine, HOMA: homeostasis model assessment.

In order to determine the best predictor of the presence of NAFLD, six logistic regression models have been developed. Each model has a different predictor variable. The first model predicts the presence of NAFLD using variable derived from FACTOR A as the predictor variable. The other models use hepatic steatosis algorithms: FLI, HSI, TyG, LAP, and VAI as independent variables, respectively. The results are given in Table 5. The estimated coefficient for variable derived from FACTOR A, in the first model, is 1.455 and the variable has a statistically significant influence on the presence of NAFLD. Increasing the value of FACTOR A increases the chance for presence of NAFLD for more than four times. This model has an overall classification rate of 70.1 %. The second model predicts the presence of NAFLD using FLI as an independent variable with an overall classification rate of 66.2 % and the estimated regression coefficient of 0.031. The results show that increasing the values of FLI will significantly increase the probability of having NAFLD. The third model uses HSI as an independent variable for prediction of NAFLD presence with an overall classification rate of 55.8 % and a regression coefficient of 0.107. In all other built models, the predictors did not show statistically significant influence on the prediction of NAFLD presence.

Discussion

Considering the hepatic and metabolic consequences of fat accumulation in the liver[1-3], there is an essential medical need for a simple, accurate and cost-effective biomarker of liver fat content. There have been several proposed diagnostic scores as surrogate markers to diagnose hepatic steatosis: FLI, HSI, LAP, VAI, and TyG index[6-10].

We evaluated five available algorithms for estimation of hepatic steatosis (FLI, HSI, LAP, VAI, and TyG). According to biochemical and anthropometrical parameters determined in our subject group using factor analysis, four factors have been extracted, only algorithm derived from FACTOR A - obesity (WC, HC, BMI, DBP, SBP, Fbg, hsCRP, glucose, and uric acid) is significantly positively associated with NAFLD with the best overall classification rate 70.1 %. In our study group, all parameters incorporated in factor A were significantly higher in sub-

Table 5: Logistic regression (FACTOR A derived from factor analysis and available algorithms for hepatic steatosis prediction).

		B	S.E.	Wald	df	Sig.	Exp (B)
Model 1	FACTOR A	1.455	0.433	11.292	1	**0.001**	4.283
	Constant	0.376	0.271	1.918	1	0.166	1.456
Model 2	FLI	0.031	0.009	11.491	1	**0.001**	1.032
	Constant	-1.764	0.663	7.081	1	0.008	0.171
Model 3	HSI	0.107	0.043	6.137	1	**0.013**	1.112
	Constant	-3.984	1.713	5.411	1	0.020	0.019
Model 4	TyG	0.652	0.436	2.240	1	0.135	1.920
	Constant	-5.459	3.840	2.022	1	0.155	0.004
Model 5	LAP	0.005	0.004	1.124	1	0.289	1.005
	Constant	-0.080	0.409	.039	1	0.844	0.923
Model 6	VAI	0.022	0.089	0.063	1	0.802	1.023
	Constant	0.225	0.338	0.444	1	0.505	1.253

FLI: fatty liver index, HSI: hepatic steatosis index, LAP: lipid accumulation product, VAI: visceral adiposity index, TyG: triglyceride and glucose index, B: coefficient, S.E.: standard error, Wald: Wald, df: degree of freedom, Sig: significance, Exp (B): exponential B coefficient.

jects with NAFLD. Uric acid was especially interesting factor because it was significantly higher in participants with NAFLD and grouped itself in FACTOR A. The association of high uric acid levels and NAFLD is still not clear, but there are several mechanisms that may contribute to NAFLD pathogenesis mainly through interaction with insulin resistance, induction of intracellular and mitochondrial stress and activation of the NLRP3 inflammasome[17]. Oxidative stress inhibits enzyme of Krebs cycle aconitase and leads to accumulation of citrate and stimulation of ATP citrate lyase resulting in increased fat synthesis and inhibition of enoyl CoA hydratase which leads to impaired beta-oxidation of fatty acids and inhibition of AMPK-activated protein kinase[18].

The second best predictor of NAFLD in our study group was FLI algorithm. These results were in line with the findings of previous studies[19]. Four parameters were incorporated into FLI: two anthropometric (BMI and WC) and two biochemical (GGT and TG)[17] that are part of the metabolic syndrome phenotype and its prevalence increases in obese individuals[20]. In some studies, GGT was considered as an independent predictor of NAFLD considering that its prevalence increases in NAFLD. On the other hand among the markers of dyslipidemia TG is strongly associated with NAFLD[21].

In our study, there were no significant differences between levels of GGT and TG in participants with and without NAFLD. This may explain why FLI did not have better discriminatory power in comparison with our algorithm. HSI was the third best predictor of NAFLD in our study group. Overall classification rate was moderate 55.8 %. HSI had relatively lower predictive power according to other studies, too[7-10].

Other models (VAI, LAP, TyG) did not have statistically predictive power but they all significantly correlated with insulin resistance as assessed by HOMA-IR. Adipose tissue dysfunction (increased lipolysis, imbalanced adipocytokine production) which is indirectly expressed by VAI could directly participate in both liver steatosis and induction of inflammation and an inverse correlation with insulin sensitivity. It was anticipated that VAI would be associated with liver fat and that demonstrated in histologically defined steatosis in genotype 1 infected patients with HCV[22] but not in patients with NAFLD[23]. TyG was effective in identifying the risk of insulin resistance assessed by HOMA-IR in Chinese population[24] and glucose clamp studies in Mexican population[25]. It is proposed that TyG is not a good measure of peripheral insulin resistance but rather hepatic insulin resistance since it correlates well with hepatic fat content[26]. In our study, there was a significant correlation with TyG index and degree of NAFLD determined by ultrasound as well as in Chinese study[9]. Only three parameters were incorporated in LAP: gender, WC, and TG. WC is a surrogate measure of visceral fat which is thought to play a key role in insulin resistance and lipotoxicity. TG are commonly elevated in the presence of insulin resistance and strongly associated with hepatic triacylglycerol content. LAP may be valuable in recognizing patients with insulin resistance along with ectopic lipid deposition as well as liver fat accumulation. In our study, there was a significant correlation with LAP index and degree of NAFLD determined by ultrasound as well as in Italian study[8].

This study has several limitations. It is a cross-sectional pilot study with a small number of patients. Presence and degree of NAFLD were determined using ultrasound which has limited sensitivity, specificity, and is operator-dependent.

In conclusion, the elevation of all anthropometrical and biochemical parameters which are incorporated in the first model for prediction of hepatic steatosis (WC, HC, BMI, DBP, SBP, Fbg, hsCRP, glucose, and uric acid) showed the best discriminatory power in the prediction of hepatic steatosis determined by ultrasound.

From the algorithms derived, the results of international studies, FLI and HSI had the best discriminatory power in predicting hepatic steatosis. Other algorithms did not have statistically significant discriminatory power but correlated with the degree of insulin resistance assessed by HOMA-IR which is thought to be a predisposition for the development of NAFLD. Further trials with a larger number of participants are needed to construct an adequate algorithm for prediction of hepatic steatosis.

Conflict of interest

The authors state that they have no financial interests and conflict of interests to disclose regarding any of the products or methods used in this study. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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