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Original Article

Efficacy of a non-invasive model in predicting the cardiovascular morbidity and histological severity in non-alcoholic fatty liver disease

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ABSTRACT

Background: Insulin resistance (IR) in cases of non-alcoholic fatty liver disease (NAFLD) is connected to remarkable liver cell inflammation and cardiovascular complications. Given the prevalence of NAFLD and its association with potential sequels, there is a strong need for an accurate non-invasive tool to monitor the progression of NAFLD.

Methods: 272 patients with NAFLD and cardio-metabolic risk factors were tested for HOMA-IR, mean platelet volume (MPV), neutrophil-lymphocyte ratio (NLR), uric acid, ferritin, lipid profile, liver stiffness measurement (LSM), controlled attenuation parameter (CAP) by fibroscan and carotid intima media thickness (CIMT). Liver biopsy was performed to validate the results.100 healthy controls were selected. A score was constructed and applied to a validation group (n = 61).

Results: Logistic regression revealed that significant fibrosis and cardiovascular risk in NAFLD were independently associated with AST/ALT ratio (p = 0.000), GGT (p = 0.000), CIMT (p = 0.001), uric acid (p = 0.000), VLDL (p = 0.000), HOMA-IR (p = 0.000), ferritin (p = 0.000) CAP (p = 0.000), LSM (p0.000). A non-invasive model was formulated by which a value > 15 was accurate in identification of advanced fibrosis and cardiovascular risk with a sensitivity of 97.3%, specificity 97%.

Conclusion: The score correlated well with the results of liver biopsy and can be repeated with great flexibility to assess severity of NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is predicted to become the next rising metabolic epidemic with an estimated prevalence approaching 30% [1]. It is characterized by an elevated hepatic fat content of more than 5% as evidenced by imaging or histological examination in the absence of hereditary or secondary causes of fat accumulation in the liver including the use of steatogenic drugs [1,2].

Risk factors for the development of NAFLD are aging, male sex, diet high in saturated fat, rs738409 G allele in PNPLA3 (Patatin-like phospholipase domain-containing 3) encoding I148 M [2,3].Metabolic and endocrine disorders associated with NAFLD are mainly obesity, dyslipidemia, diabetes and polycystic ovary [4].

Non-alcoholic steatohepatitis (NASH) is distinguished by progressive hepatocellular ballooning with or without fibrosis [4]. NASH may progress to fibrosis or cirrhosis if therapeutic interventions or life style modifications are not followed, thus NAFLD is regarded as an important cause of cryptogenic cirrhosis [5].

Insulin resistance (IR), metabolic syndrome and NAFLD are linked; NAFLD is the hepatic element of IR and metabolic syndrome [6]. Biomarkers associated with hepatic steatosis and IR include alanine aminotransferase (ALT) > 29 U/l, gamma-glutamyl transferase (GGT) > 25 U/L, hyperuricemia due to its involvement in endothelial dysfunction and increased arterial stiffness, hyperferritinemia >300 μg/dl due to its association with the increased risk of diabetes and the metabolic syndrome [7–10].

Endothelial dysfunction is an important triggering factor of atherosclerosis and carotid intima media thickness (CIMT) is a

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significant radiological sign of subclinical atherosclerosis and may predict the development of vascular adverse events [11].The continual inflammatory state of NAFLD may enhance the occurrence of subclinical carotid atherosclerosis, designating NAFLD as an independent risk factor for cardiovascular disease [12,13].

Liver biopsy is important in evaluating histological severity in NAFLD; however it is uncomfortable for the patient, there are possible errors in sampling, especially when a small cylinder of tissue is obtained and impractical as a repeated follow up technique.

The current steatosis indices provide a high suspicion of the presence of NAFLD without throwing any light on the potential behavior or severity of the disease, so the aim of the study was to derive a score based on easily obtained biochemical parameters and liver stiffness attenuation parameters by transient elastography (Fibroscan) capable of distinguishing between individuals with and without advanced fibrosis or cirrhosis, and of minimizing the need to resort to liver biopsy prior to the commencement of therapeutic interventions.

2. Materials and methods

2.1. Patients selection

A prospective study that evaluated 630 patients for the eligibility to be included the study from May 2014 until March 2018 in the Hepatology and Tropical medicine outpatient clinics, Zagazig and Tanta University hospitals, Egypt. 230 patients (36.5%) were excluded due to infection with HCV (n = 89), HBV (n = 13), use of steatogenic drugs (n = 67), alcohol abuse (n = 3), refusal to participate (n = 58).

400 patients with documented clinical features of cardiometabolic syndrome were included if a waist circumference more than 88 cm in females and 102 cm in males, arterial blood pressure ≥130/85 mmHg, antihypertensive drug intake, and body mass index (BMI) ≥ 25 [14], fatty liver by abdominal ultrasound with elevated transaminases. 128/400 were excluded because they refused to go through with the liver biopsy, finally 272 completed the study. The indication for liver biopsy were restricted to patients with features of metabolic syndrome with raised transaminases or GGT, increased values of liver stiffness by fibroscan >7 kPa, Controlled Attenuation Parameter (CAP) value > 212 dB/m and to exclude other causes of liver diseases.

Exclusion criteria were pregnancy, infection with HCV or HBV; use of steatogenic drugs or drugs which induce insulin resistance (B-blockers, steroids, immunosuppressives, thiazide diuretics, and antipsychotics such as clozapine and risperidone); patients with type 2 diabetes on insulin therapy; excess alcohol consumption (>20 g/day for males and females, approximately 60 ml of whisky = 200 ml of wine = 480 ml of beer) [15].

An informed consent was obtained from all participants before enrollment in the study which was approved by the ethical committee of Zagazig university hospital at the 1st of March 2014.

All procedures were performed in accordance with the ethical standards of the Zagazig University, faculty of medicine research committee and with the 1975 Declaration of Helsinki and its later amendments.

2.2. Laboratory analysis

-After a 12-h overnight fast, complete blood count was performed to detect the mean platelet volume and the neutrophil-lymphocyte ratio by Sysmex KX 21 N hematology auto analyzer (Sysmex Corporation, Kobe, Japan) from ethylenediamine tetra-acetic acid blood samples. ALT, AST, and GGT were estimated using the Cobas Integra 400 chemistry auto analyzer; AST/ALT ratio was calculated for every patient.

-Serum insulin was measured quantitatively by electrochemiluminescence immunoassay with a cutoff value 8.64μIU/ml. Insulin resistance was calculated by HOMA-IR using the following formula: fasting glucose (mg/dl) X fasting insulin (μU/ml)/405, with a value greater than 2 indicative of insulin resistance [16]. Diabetes was diagnosed if fasting plasma glucose level was>100 mg/dl, 2-h plasma glucose >140 mg/dl with HbA1c > 6.5% [17].

2.2.1. Biomarkers associated with insulin resistance and hepatic steatosis

-Serum uric acid was estimated by Cobas Integra 400 chemistry autoanalyzer with a cut off value 6.3 mg/dl that is optimal for identifying the risk of metabolic syndrome [18]. Serum ferritin was determined quantitatively by ECLIA using Cobas e 411 immunoassay (n = 30–300 ng/ml) [19].

-Serum Triglycerides, total cholesterol and high density lipoprotein –C (HDL-C) levels were evaluated using the spectrophotometric method. Low density lipoprotein-C (LDL-C) was calculated using the Friedewald formula: LDL-C (mg/dl) = Total cholesterol − HDL-C − (triglycerides/5) [20]. VLDL cholesterol was estimated by dividing the triglycerides in mg/dl by 5 which is valid if the triglyceride values are less than 450 mg/dl [21].Total cholesterol/HDL and LDL/HDL ratios were calculated.

2.3. Abdominal ultrasonography

A non-invasive and efficient diagnostic tool for hepatic steatosis especially when more than 33% of the liver is affected. US has a sensitivity of 94% and a specificity of 84% [22].

It was performed by two radiologists who were blinded for clinical data; inter-observer variability was quantified according to kappa values.

The ultrasonographic steatosis score (USS) classifies steatosis as Grade 0 (no steatosis: defined as normal liver echotexture), grade1 (mild steatosis: slight, diffuse increase in the fine parenchymal echoes with normal visualization of portal vein and diaphragm), grade 2 (moderate steatosis: moderate and diffuse increase in fine echoes with slight impairment of visualization of portal vein and diaphragm, grade 3 (severe steatosis: defined as fine echoes with poor or no visualization of the portal vein, diaphragm, and posterior portion of the right lobe) [23].

2.4. Liver stiffness measurement (LSM)

LSM was performed by two experienced physicians blinded to the clinical data of the patients using Fibroscan®. Liver stiffness values 2.5–7, 7–9.5, 9.5–12.5 and > 12.5 kPa accordingly denote fibrosis stages F 0-1; F2; F3 and F4 or cirrhosis [24,25]. Inter-observer variability was quantified according to kappa values.

2.5. Controlled Attenuation parameter (CAP)

CAP detects the extent of ultrasound attenuation by hepatic fat. CAP was measured with the M probe 3.5 MHz if skin-liver capsule distance is between 25 and 65 mm or with the XL probe at a distance from 66 up to 75 mm. CAP is detected at the same time as LSM, so they were correlated simultaneously. CAP values are expressed in decibel/meter (dB/m), it ranges from 100 to 400 dB/m. CAP values were interpreted as follows:

- Steatosis grade S0 cutoff is 212, with a range of 212–265 dB/m
- S1 (5-33% steatosis): cutoff value is 266, with a range of 266–303 dB/m

- S2 (34-66%): cutoff value is 304, with a range of 304–320 dB/m
- S3 (>66%): cutoff value is 321 dB/m, with a range of 321–400 dB/m [26].

2.6. Carotid artery intima-media thickness (CIMT)

The common carotid arteries were scanned bilaterally by two experienced radiologists blinded to the clinical data using B-mode duplex ultrasound with a 7.5 MHz linear probe (Siemens G60®) ultrasound system, inter-observer variability was quantified according to kappa values.

CIMT was measured from the intima lumen interface to the media adventitia interface, a value exceeding 0.9 mm is considered abnormal. Three measures were taken on either side; the mean CIMT was defined as the mean of the right and left CIMT [27].

2.7. Liver biopsy

Liver biopsies were stained with hematoxylin&eosin and Masson's trichrome stain. The slides were examined by two experienced histopathologists. Nonalcoholic fatty liver disease activity score (NAS) was calculated. A score of ≥5 with steatosis and hepatocyte ballooning is diagnostic of NASH, however NASH is considered with a lower score value and hepatocyte ballooning [28]. Hepatic steatosis was graded as follows: S0 = steatosis <5%, S1 = steatosis 5%–33%, S2 = steatosis 34%–66% and S3 = steatosis >66%. Liver fibrosis staging was assessed and added to NAS score to make the NAFLD activity score or (NASH CRN scoring system) increasing the total score to 12; Significant fibrosis is defined as stage 3, cirrhosis as stage 4 (Table1supplementary) [29].

2.8. Control group

100 healthy subjects of matched age and sex were recruited from the outpatient clinics; they were not DM or hypertensive with average body built (BMI mean value 23.4 ± 0.9 K/m^2, with normal transaminases, normal liver by abdominal ultrasound with normal values of CAP and fibroscan and after exclusion of other causes of liver diseases.

2.9. Validation group

It was composed of 83 patients with fatty liver disease and cardio-metabolic features of metabolic syndrome who fulfilled the same inclusion and exclusion criteria of the study group to confirm reproducibility of the scoring system.22 patients rejected performing liver biopsy and were excluded, so finally 61 patients were enrolled.they were recruited from the outpatient clinics.

2.10. Statistical analysis

All data were statistically analyzed using SPSS 20 for windows (SPSS Inc., Chicago, USA).The sample size was calculated from the equation = (Z-score)^2*SD*(1-SD)/(margin of error)^2 so, (1.96)^2*0.5*(0.5)/(0.05)^2 = a sample size of at least 384 people would be necessary. Quantitative data were expressed as the mean ± SD.

Qualitative data were expressed as absolute frequencies (number)& (percentage). F test was used to compare between more than two groups of normally distributed variables. Spearman's correlation coefficient (r) for ordinal variables and Pearson correlation for continuous variables. $P < 0.05$ was considered to be statistically significant.

Inter-observer variability between radiologists or pathologists was quantified using the kappa statistic, which measures agreement while accounting for chance, observer agreement was categorized by kappa values as poor (<0.20), fair (0.20–0.39), moderate (0.40–0.59), good (0.60–0.79), or excellent (>0.80) [30].

The competing variables tested were age, sex, BMI, waist circumference, GGT, AST/ALT ratio, Alpha fetoprotein, MPV, NLR, HOMA-IR, uric acid, ferritin, triglycerides (TGs), LDL, HDL, VLDL, LSM, CAP and CIMT. Multivariate linear regressive analysis to select variables independently associated with CAP and LSM.

Logistic regression analysis was performed to select variables independently associated with significant fibrosis and cirrhosis in NAFLD. Significant fibrosis is defined as LSM of 9.5–12.5 kPa and by liver biopsy as NAS fibrosis stage = 3.Cirrhosis if LSM >12.5 kPa, NAS fibrosis stage 4. The analysis was adjusted for age and sex, logistic regression controlled the confounding variables by calculating the adjusted odds ratio.

The scoring model obtained from logistic regression provides β coefficient values, with their corresponding adjusted odds ratios (OD). Points were assigned to each parameter significantly associated with significant fibrosis or cirrhosis based on the strength of its OD; the smallest OD was given 1 point and the other parameters were given points according to the strength of their ODs when divided by the smallest OD, the resulting number was approximated to the nearest whole number for example, a parameter (b) had OD 3.8 and the parameter (a) had the smallest OD 1.3, so the product of division will be 2.92 that will be approximated and so parameter (b) will be assigned 3 points.

The summed points formed the score of the model which was classified into risk categories as follow: low risk (<15%), intermediate (15%–49%), high (50%–79%), and very high (>80%) [31].

A receiver operating characteristic (ROC) analysis was carried out to detect the cut off values which were selected at the highest sensitivity and specificity for each parameter of the model, then the performance of the cut-off value was evaluated by Youden's J Statistical equation: sensitivity + specificity-1; value near 1 indicates good performance [32].

3. Results

The demographic, laboratory and radiologic characteristics of the studied patients (n = 272) and control group (n = 100) are shown in Table 1. BMI, WC, metabolic biomarkers, CAP by fibroscan and CIMT were significantly higher in the patient group compared to the control group.

According to CAP cut off values for steatosis grading; patients were stratified into 4 subgroups (Table 2): S0 (n = 71), S1 (n = 21), S2 (n = 77), S3 (n = 103). Carotid intima media thickness was significantly higher in S2 and S3 when compared to S0, S1 with good agreement between the readings of the radiologists (kappa value 0.72, p = 0.001) (Table 2, Fig. 1).

The results of liver biopsy for the 272 patients are shown in Table 2 and the agreement between the readings of pathologists was excellent (kappa value 0.82, p = 0.001). 93 patients showed NAFL without inflammation or ballooning, 107 patients showed NASH with ballooning and finally 72 patients were diagnosed with NASH cirrhosis.

CAP measurements were compared to the results of liver biopsy and their corresponding LSM reading by fibroscan; higher values of CAP denoting higher hepatic fat infiltration were associated with higher liver stiffness and advanced fibrosis staging (Fig. 2, Table 2), agreement between the readings of physicians who performed fibroscan was excellent (kappa value 0.87, p = 0.001).

Severity of hepatic steatosis by CAP was positively correlated with BMI (r = 0.589, p = 0.001), AST/ALT (r = 0.379, p = 0.004), GGT

Table 1
Baseline demographic, laboratory and radiological findings of the study, control and validation subgroups.

N	100	272	61	–
Sex (M/F)	70/30	190/82	39/22	0.78
Age	38.2 ± 1.8	35.5 ± 4.7	40 ± 4.5	0.6
BMI (K/m^2)	23.4 ± 0.9	30.7 ± 1.7	31.2 ± 1.2	0.03
WC (cm)	80 ± 4.2	113.5 ± 8.9	116.5 ± 7.5	0.001
AST (IU/L)	28.7 ± 4.6	76.1 ± 18.2	60.2 ± 10	0.001
ALT (IU/L)	30.3 ± 2.2	83.9 ± 19.8	65.8 ± 15	0.0012
AST/ALT	0.87 ± 0.13	0.91 ± 0.28	0.93 ± 0.13	0.18
GGT (IU/L)	22.2 ± 3.6	64.9 ± 7.9	57 ± 9	0.01
MPV (fl)	8.2 ± 0.5	11.6 ± 0.6	11.3 ± 0.7	0.02
NLR	1.6 ± 0.1	2.97 ± 0.37	2.67 ± 0.26	0.01
FBS (mg/dl)	78.5 ± 4.3	118 ± 7.7	120.8 ± 12	0.034
Insulin (μIU/ml)	6.1 ± 0.7	10.4 ± 0.5	9.6 ± 1.1	0.004
HbA1c %	4.4 ± 0.37	6.9 ± 0.6	7.2 ± 0.56	0.032
HOMA-IR	1.4 ± 0.2	2.9 ± 0.6	2.8 ± 0.7	0.001
TGs (mg/dl)	113.7 ± 14.3	280 ± 13.4	309 ± 20.4	0.001
VLDL (mg/dl)	22.3 ± 4.3	56.6 ± 3.9	42.5 ± 5.6	0.04
Ferritin (ng/dl)	196 ± 18.7	383 ± 40.2	402 ± 20.2	0.001
Uric acid (mg/ml)	4.6 ± 0.4	7.9 ± 0.7	7.4 ± 0.9	0.02
LDL mg/dl	88.5 ± 8.2	188.3 ± 13.6	201 ± 9.5	0.034
HDL mg/dl	48.3 ± 6.2	33.7 ± 4.2	34.2 ± 6.6	0.01
Total cholesterol mg/dl	163.6 ± 15.5	273.6 ± 10.4	310.5 ± 11.2	0.003
TC/HDL ratio	3.3 ± 0.8	7.2 ± 1.9	8.5 ± 0.8	0.001
LDL/HDL ratio	1.8 ± 0.9	5.8 ± 1.7	5.5 ± 0.9	0.001
Fibroscan (kPa)	3.6 ± 1.2	11.9 ± 1.01	12.5 ± 2.8	0.001
CAP (dB/m)	175.4 ± 24.3	279.2 ± 38.2	282 ± 21	0.001
CIMT (mm)	0.73 ± 0.12	1.28 ± 0.19	1.32 ± 0.2	0.01

P < 0.05 is considered significant.

(r = 0.397, p = 0.007), MPV (r = 0.268, p = 0.04), NLR (r = 0.370, p = 0.001) uric acid (r = 0.756,p = 0.000), ferritin (r = 0.664,p = 0.000), TGs (r = 0.370,p = 0.000), VLDL (r = 0.688,p = 0.000), HOMA-IR (r = 0.794,p = 0.000), CIMT (r = 0.680,p = 0.000), LSM by fibroscan (r = 0.691, p = 0.000) and fibrosis stage by liver biopsy (r = 0.702, p = 0.000).

Logistic regressive analysis revealed that presence of significant fibrosis in NAFLD was independently associated with AST/ALT ratio (p = 0.001), GGT (p = 0.001), BMI (p = 0.001), HOMA (p = 0.000), ferritin (0.000), VLDL (p = 0.000), CAP (p = 0.000), and CIMT (p = 0.000).

According to the ROC curve performed (Table 3) to determine cut-off values of the high risk variables that could independently predict significant fibrosis in NAFLD with their corresponding Youden's J value; values near 1 indicated good performance of the cut off value.

A non-invasive model to predict significant fibrosis risk in NAFLD was composed of the cut off value for each variable (Table 4). The mean value of the patients score (n = 272) was 10.5 ± 8.1. There was a highly significant positive correlation between the score and the stage of fibrosis (r = 0.866, p = 0.000).A score> 15 was accurate to identify advanced fibrosis in NAFLD via ROC curve analysis with a sensitivity of 97.3% specificity 97%, AUC 0.913, Youden's J value = 0.94, p = 0.000.

Comparative evaluation of the proposed model and the other fatty liver indices is shown in Table 5. The proposed non-invasive model corresponded well with the results of liver biopsy. On the other hand the NAFLD fibrosis score was not sufficient to discriminate between F1 and F2.

The steatosis and fatty liver indices revealed non-significant associations with results of liver biopsy (p = 0.24, 0.08 respectively), making liver biopsy essential. The steatosis index showed the positive probability of NAFLD but was not informative regarding its severity. FIB-4 did not succeed in discrimination between stages of liver fibrosis in NAFLD (p = 0.34).

A third group was enrolled to validate the model (n = 61), their laboratory, metabolic and laboratory characteristics are shown in Table 1. Liver biopsy revealed F0 (n = 12), F1 (n = 13), F2 (n = 7), F3 (n = 13), F4 (n = 16), and their corresponding scores were (2.4 ± 0.45, 6.7 ± 2.1, 11.7 ± 1.9, 16.8 ± 1.3 respectively); higher fibrosis stages were associated with higher score values (p = 0.001).

Table 2
Laboratory values of the study patients when stratified by the CAP cutoff values which define grades of hepatic steatosis.

Variable	S0 (<5%)	S1 (5-33%)	S2 (34-66%)	S3 (>66%)	*p*
N	71	21	77	103	
Age	35.7 ± 3.1	32.3 ± 6.2	34.3 ± 4.1	35.9 ± 2.8	0.1
BMI k/m^2	27.9 ± 1.7	28.3 ± 1.5	29.1 ± 1.3	30.2 ± 1.6	0.02
WC cm	110.2 ± 3.7	112 ± 3.9	115.4 ± 9.7	122.6 ± 8.2	0.032
AST IU/L	38.8 ± 18	58 ± 19	54.7 ± 26.3	87.5 ± 17.7	0.01
ALT IU/L	48 ± 19	65 ± 23	70.4 ± 30	78.2 ± 11.2	0.02
AST/ALT	0.8 ± 0.12	0.89 ± 0.2	0.84 ± 0.17	1.03 ± 0.13	0.03
GGT IU/L	41 ± 10	46 ± 17	60.7 ± 14	73.4 ± 8.4	0.01
MPV fl	8.7 ± 0.8	9.9 ± 0.8	11.1 ± 0.9	12.3 ± 0.6	0.02
NLR	1.8 ± 0.4	2.1 ± 0.5	2.8 ± 0.5	3.1 ± 0.4	0.003
HOMA-IR	1.8 ± 0.5	2.2 ± 0.8	2.9 ± 0.7	3.2 ± 0.5	0.01
VLDL-C	28.8 ± 3.2	39 ± 10	54.2 ± 9.2	62.6 ± 7.5	0.001
TGs mg/dl	147.4 ± 20	164 ± 17	310 ± 16.5	400 ± 13	0.02
LDL mg/dl	112.7 ± 10.7	135 ± 15	158.9 ± 16.4	192.2 ± 11.6	0.03
HDL mg/dl	38.5 ± 4.2	41 ± 6	33.3 ± 5.2	32.7 ± 3.9	0.056
Total cholesterol	220.3 ± 17.3	252 ± 28	305.8 ± 22.3	365.6 ± 16.7	0.1
TC/HDL ratio	5.4 ± 1.9	6.96 ± 0.8	8.1 ± 0.9	9.12 ± 0.7	0.01
LDL/HDL ratio	2.72 ± 0.61	3.19 ± 0.9	5.82 ± 0.78	6.06 ± 0.93	0.01
Ferritin ng/dl	213 ± 56.5	310 ± 49	380.7 ± 81.4	417.6 ± 48.2	0.01
Uric acid mg/dl	5.8 ± 1.1	6.9 ± 0.9	7.7 ± 1.5	8.9 ± 0.9	0.02
LSM (kPa)	5.6 ± 0.8	8 ± 0.7	9.9 ± 0.9	12.4 ± 4.6	0.001
CAP (dB/m)	177.5 ± 14.1	278 ± 11	308 ± 4.6	359 ± 12.3	0.001
CIMT	0.88 ± 0.12	0.98 ± 0.18	1.13 ± 0.2	1.3 ± 0.14	0.03
Liver biopsy					
F0 (n = 66)	62	4	0	0	0.001
F1 (n = 26)	9	16	1	0	0.002
F2 (n = 26)	0	1	23	2	0.003
F3 (n = 82)	0	0	53	29	0.001
F4 (n = 72)	0	0	0	72	0.0001

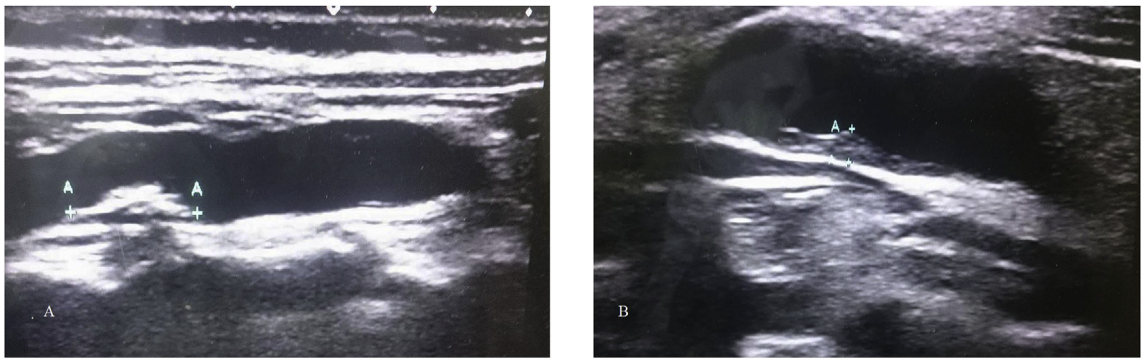

Fig. 1. Carotid ultrasound in a patient with NAFLD revealed athermanous plaque **(A)** and increased carotid intima-media thickness **(B).**

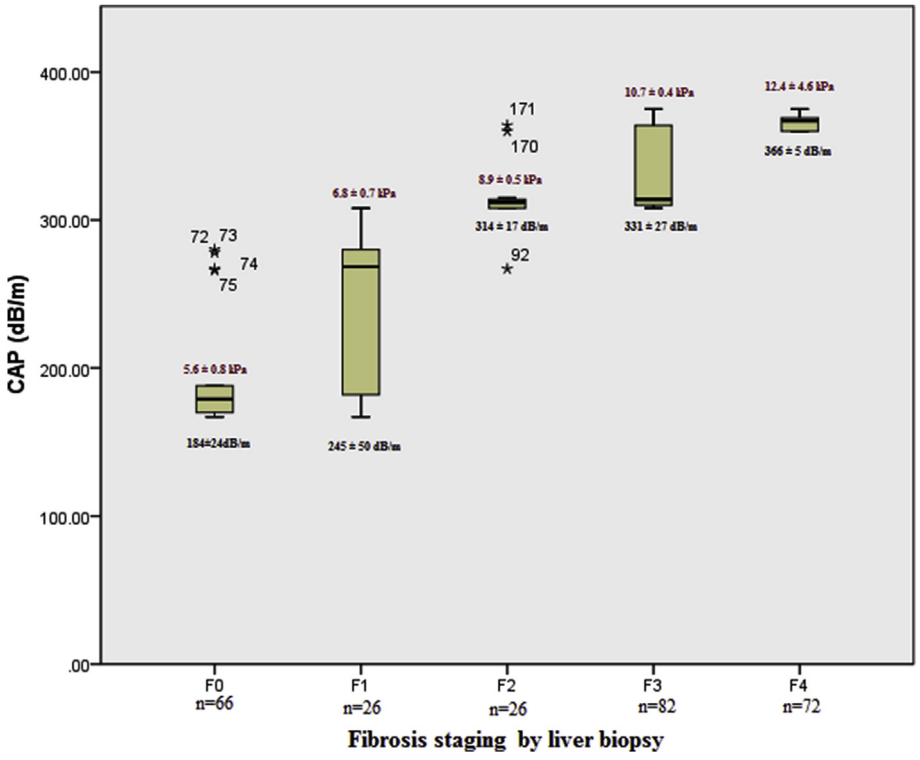

Fig. 2. Comparability of CAP measurement with the results of liver biopsy and their corresponding LSM by fibroscan.

Table 3
Cut-off values (by ROC curve) of the high risk variables independently associated with significant fibrosis.

	Cut off	Youden's J value	Sensitivity%	Specificity%	AUC	CI 95%	P
AST/ALT ratio	1.2	0.77	89	88	0.809	0.77–0.85	0.001
BMI	30	0.79	92	87	0.816	0.77–0.86	0.001
GGT	52	0.74	89	85.2	0.811	0.78–0.89	0.001
CIMT	1.2	0.89	95	93.7	0.845	0.81–0.88	0.0001
Ferritin	321	0.86	95.8	90	0.809	0.77–0.85	0.001
VLDL	46	0.87	96	91	0.825	0.78–0.87	0.001
HOMA-IR	2.11	0.79	93	86	0.843	0.80–0.88	0.0001
CAP	301.5	0.92	94.1	98.1	0.860	0.993–0.99	0.0001
LSM	9.75	0.96	97.8	98	0.880	0.94–0.97	0.0001

CAP: controlled association parameter**; LSM**: Liver stiffness measurement.

Table 4
Non-invasive model for identification of significant fibrosis and cardiovascular risk in NAFLD.

independent predictor	Exponential B	Odds ratio	Score
1-AST/ALT >1.2	0.416	1.37	1
2-BMI > 30	0.396	1.32	1
3-GGT > 52 IU/l	0.469	1.41	1
4-HOMA-IR > 2.11	1.32	3.86	3
5-VLDL > 46 mg/ml	1.21	3.72	3
6-Ferritin > 321 ng/ml	0.713	2.74	2
7-CIMT > 1.2 mm	0.692	2.49	2
8-LSM > 9.75 kPa	0.792	2.9	2
9-CAP> 301.5 dB/m	1.445	4.92	4
Total score			19
Low			≤3
Intermediate			4–9
High			10–15
Very high			16–19

LSM: Liver stiffness measurement; **CAP**: Controlled assessment parameter.

4. Discussion

Liver histology in NAFLD cannot be repeatedly assessed by liver biopsy to monitor the disease progression or the impact of therapeutic interventions.

The drawbacks of liver biopsy are the sampling errors due to heterogeneous disease distribution and the potential histopathological difference of fatty deposition in different hepatic areas [33] in contrast to CAP and LSM which simultaneously give a comprehensive overview of fatty infiltration and severity of fibrosis in NAFLD [33].

Insulin resistance is directly proportional to the severity of NAFLD and is linked to the progression into fibrosis or even cirrhosis [34].Measurement of carotid intima-media thickness (CIMT) is a noninvasive method used to identify subclinical cardiovascular risk. Patients with NAFLD have an increased CIMT and a higher frequency of carotid plaques than healthy controls, also the severity of liver histopathological changes is directly proportional to CIMT and inflammatory markers of atherosclerosis [35].

Controlled attenuation parameter could be useful for the diagnosis and follow-up of NAFLD patients, CAP has been shown to have a significant correlation with actual liver fat content in NAFLD [36,37]. In the current study, CAP correlated with BMI, liver transaminases and liver stiffness as measured by fibroscan.

Several serum biomarker based indices for NAFLD have been postulated; the NAFLD liver fat score which includes AST/ALT ratio, metabolic syndrome, presence of diabetes and fasting insulin; the score with a cut-off −0.640 predicts increased hepatic fat content with AUC of 0.87 but it does not have the ability to discriminate non-significant fibrosis from advanced fibrosis or cirrhosis [38].The hepatic steatosis index which includes AST/ALT ratio, presence of diabetes and BMI, at a cut off >36 score can predict the presence of hepatic steatosis with AUC of 0.812, values less than 36 are inconclusive and require a liver biopsy, also information about the NAFLD severity is lacking [39].

The fatty liver index includes BMI, waist circumference, triglycerides and GGT; values above 60 provide a suspicion of fatty liver with AUC 0.84 yet cannot discriminate stages of hepatic fibrosis [40]. Fibrosis-4 score (FIB-4), utilizes platelets, ALT, AST and age. FIB-4 scores >3.25 have a 97% specificity and a positive predictive value of 65% for advanced fibrosis with an AUC of 0.85 (95% CI 0.82−0.89).However, it may be a misleading tool in case of normal platelet count leading to a falsely low FIB4 if NAFLD with significant fibrosis is associated with normal transaminases [41–44].

These indices provide a high suspicion of the presence of NAFLD without assessing the risk for advancing fibrosis and with limited information regarding the potential behavior of the disease.

In the current study, significant fibrosis in NAFLD was predicted by a non-invasive model composed of nine easily applied parameters including BMI, AST/ALT, GGT, ferritin, HOMA-IR, VLDL, CIMT, and the fibroscan parameters; liver stiffness measurement and controlled attenuation parameter.

A Score >15 was efficient in identifying significant fibrosis in NAFLD and the inclusion of CIMT as a parameter of the model allowed prediction of subclinical atherosclerosis. LSM values noting significant fibrosis or cirrhosis are lower when compared to corresponding stages in other liver diseases as HCV or HBV induced cirrhosis.

The proposed model in the current study has the advantage of real life quantitative evaluation of fibro fatty liver tissue through addition of CAP and LSM. The inclusion of CIMT in the model provides information about the cardiovascular risk. CAP and LSM by fibroscan are significantly cheaper and more readily available than the sophisticated and expensive fat quantification methods by magnetic resonance spectroscopy. A large number of patients were included and consented to have a liver biopsy which is the gold standard that validated our results. The score can be repeated with great flexibility to assess progression or regression of NAFLD.

The limitations of the current study were it being a two center study, alcoholic fatty liver disease patients were not enrolled. We recommend that NAFLD patients with significant fibrosis or cirrhosis should be strictly managed and followed up by the model and should be a point for future research.

To our knowledge; the proposed model is the first one which assesses the severity of advancing fibrosis and cardiovascular risk in NAFLD. The score was confirmed on a large number of patients and further multicenter studies are warranted before adopting this model in general practice especially in the coming era of developing new medication for NAFLD.

Table 5
Comparative evaluation of the proposed model and the other fatty liver indices.

	F0 (n = 66)	F1 (n = 26)	F2 (n = 26)	F3 (n = 82)	F4 (n = 72)	P
CAP	184 ± 24	245 ± 50	314 ± 17	331 ± 27	366 ± 5	0.001
Fibroscan	5.6 ± 0.8	6.8 ± 0.7	8.9 ± 0.5	10.7 ± 0.4	12.4 ± 4.6	0.001
proposed model	2.4 ± 0.45	4.1 ± 1.1	9.34 ± 1.2	13.8 ± 1.65	16.1 ± 1.1	0.001
NAFLD fibrosis score	−2.47 ± 0.4 **(F0-2)**	−1.3 ± 0.6 (**indeterminate**)	−0.97 ± 0.5 (**indeterminate**)	0.86 ± 0.4(**F3-4**)	0.74 ± 0.6(**F3-4**)	0.001
Steatosis Index	35 ± 1.4 (**inconclusive**)	38.3 ± 1.3 (+ **ve** NAFLD)	39.2 ± 1.5 (+ **ve** NAFLD)	38.9 ± 1.2 (+ **ve** NAFLD)	39.8 ± 1.8 (+ **ve** NAFLD)	0.24
Fatty Liver Index	79.7 ± 2.9	85.8 ± 7.6	89.8 ± 5.9	92.8 ± 8.9	90.4 ± 5.3	0.08
FIB-4	1.31 ± 0.4	1.47 ± 0.34	1.77 ± 0.53	1.42 ± 0.47	1.67 ± 0.23	0.34

Inconclusive: there is a need for liver biopsy. + ve NAFLD: the positive probability of NAFLD is high but not informative about the severity.

Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2019.05.032.

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