

April 2021

New Screening non- invasive tool for Nonalcoholic Steatohepatitis in High-Risk Individuals

Yahya Mohamed Elhamdi

M.B.B.Ch, Faculty of Medicine – Zagazig University, larynyahya@gmail.com

Alaa Ahmed Farag

Assistant Professor of Internal Medicine, Faculty of Medicine, Zagazig University, alaa.farag@hotmail.com

Ayman Magd Eldin Mohammed Sadek

Lecturer of Internal Medicine, Faculty of Medicine, Zagazig University, ayman.sadek@hotmail.com

Sameh Saber Bayoumi

Assistant Professor of Radiology, Faculty of Medicine, Zagazig University, sameh.bayoumi@gmail.com

Follow this and additional works at: <https://digitalcommons.aaru.edu.jo/zumj>

Recommended Citation

Elhamdi, Yahya Mohamed; Farag, Alaa Ahmed; Sadek, Ayman Magd Eldin Mohammed; and Bayoumi, Sameh Saber (2021) "New Screening non- invasive tool for Nonalcoholic Steatohepatitis in High-Risk Individuals," *Zagazig University Medical Journal*: Vol. 27 : Iss. 3 , Article 19.

Available at: <https://digitalcommons.aaru.edu.jo/zumj/vol27/iss3/19>

This Original Article is brought to you for free and open access by Arab Journals Platform. It has been accepted for inclusion in Zagazig University Medical Journal by an authorized editor. The journal is hosted on [Digital Commons](#), an Elsevier platform. For more information, please contact rakan@aar.edu.jo, marah@aar.edu.jo, u.murad@aar.edu.jo.



Manuscript ID ZUMJ-2006-1883

DOI 10.21608/zumj.2020.33313.1883

ORIGINAL ARTICLE

New Screening Non- Invasive Tool for Nonalcoholic Steatohepatitis in High-Risk Individuals

Alaa A. Farag⁽¹⁾, Yahya Mohamed Elhamdi⁽²⁾, Sameh Saber Bayoumi⁽³⁾, Ayman M. E. M. Sadek⁽¹⁾

(1) Internal Medicine Department, Faculty of Medicine - Zagazig University

(2) Internal Medicine Department, Tripoli University – Libya

(3) Radiology Department, Faculty of Medicine - Zagazig University



Corresponding author:

Yahya Mohamed Elhamdi
M.B.B.Ch, Internal Medicine Department,
Faculty of Medicine,
Tripoli University - Libya
larynyahya@gmail.com

Submit Date 2020-06-24

Revise Date 2021-03-04

Accept Date 2020-11-18

ABSTRACT

Background: Nonalcoholic fatty liver disease (NAFLD) is a chronic liver disease which includes simple steatosis and nonalcoholic steatohepatitis (NASH). The current study aimed to develop a non-invasive tool to predict NASH in patients with NAFLD. Patients and Methods: A cross-sectional study was carried on (80) patients presenting to the Hepatology /Internal Medicine Outpatient Clinic, Zagazig University Hospitals suffering from dyspeptic symptoms, fatigue and unexplained elevation of aminotransferases from July 2019 to January 2020. All patients were subjected to liver function tests, lipid profile, fasting blood sugar, serological markers, abdominal ultrasonographic examination and Fibroscan evaluation. The new model score included the following clinical and laboratory parameters [body mass index (BMI), US of liver, Liver stiffness, low-density lipoproteins (LDL), high-density lipoprotein (HDL), triglyceride (TG), Alanine transferase (ALT), Aspartame transaminase (AST), AST/ALT ratio, Platelet, mean platelet volume (MPV), Ferritin and Fasting Blood Glucose] **Results:** current results showed a sensitivity of the new score in the detection of NASH (97.9%) versus 91.7% and 89.6% for NAFLD score and BARD score respectively, but GULAB score was associated with a 100% sensitivity, at a cutoff level of 1.5. The area under the curve for the new score was is 0.789 versus 0.921, 0.925, and 0.996 for NAFLD, BARD, and GULAB scores respectively. **Conclusion:** The new model score considered simple, non-invasive and low-cost tool and can be used as a good negative test to exclude NASH in the screening of high risk patients and markedly reduce the need for liver biopsies in NAFLD patients.

Keywords: Nonalcoholic steatohepatitis, Steatosis, Fibrosis

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is rapidly becoming one of the most common causes of chronic liver disease worldwide and is now a major cause of liver-related morbidity and mortality [1].

NASH is a pathophysiological stage activated by the continuous deposition of excess liver triglycerides (steatosis) due to increased dietary fat intake or by de novo lipogenesis. It is also characterized by insulin resistance (IR), inflammation and oxidative

stress that eventually lead to fibrosis, cirrhosis, and in some cases, liver cancer [2].

However, NASH is more progressive and includes features of steatosis with hepatocyte injury, lobular inflammation, and fibrosis. Evidence suggested a possible increase in the risk of liver cirrhosis and hepatocellular carcinoma [3].

Conventionally, NASH is a histologic diagnosis based on liver biopsy (LB) when all other causes of liver damage have been discarded in which hepatocyte ballooning, inflammation, and fibrosis is demonstrated.

Alternatively, non-invasive strategies that include liver stiffness measurement (LSM) using transient elastography (TE) and other surrogate scores such as European Liver Fibrosis (ELF), Fibrosis-4 (FIB-4), and Non-alcoholic fatty liver disease Fibrosis Score (NFS) are used to spare the patient of histology examination. However, some non-invasive diagnostic tools may be unfeasible for screening patients at early stages of disease among the general population who are overweight and obesity, which may also hinder the study of the natural history of NASH [4].

The current study aimed to develop a non-invasive tool to predict NASH in patients with NAFLD.

PATIENTS AND METHODS

A cross-sectional study was carried on (80) Egyptian patients presenting to the Hepatology Outpatient Clinic, Zagazig University Hospitals suffering from dyspeptic symptoms, fatigue and unexplained elevation of aminotransferases, during the period from July 2019 to January 2020. Fig. (1), showed the flow chart of the patients' selection process.

A written formal consent to participate in the study was signed by the patients, the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University (ZU-IRB # 4418). The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Sample size: assuming that number of steatohepatic patient is 120 and ppv of biochemical and imaging is 80% ,sample was calculated to be 80 cases using EPI info 7 program with test power 80% ,CI 95%

Inclusion criteria: Patients who are suggested to have fatty liver by ultrasound. Age: > 18 years. Gender: male or female

Exclusion criteria: Alcoholic fatty liver disease (alcohol intake exceeding 40 g/d in males and 20 g/d in females, over the past 5 years). Concomitant chronic hepatitis B and C. Autoimmune hepatitis or primary biliary cholangitis. Drug induced hepatitis or toxic hepatitis. Genetic and metabolic liver

diseases. Liver and biliary malignancy other than HCC.

The participants were allocated into two groups according to presence of NASH as follow:

- 1- **Group I (48 patients): This group included patients with risk of NASH.**
- 2- **Group II (32 patients): This group included patients without risk of NASH.**

Flow-chart for the screening of NAFLD

The EASL-EASD-EASO Guidelines recommended to screen the presence of NAFLD through a first step based on the use of ultrasound or serum biomarkers to evaluate the presence of liver steatosis. When steatosis is identified, they suggest using non-invasive serum biomarkers to look for liver fibrosis. Patients at low risk for advanced fibrosis are followed-up every 2 years by repeating liver enzymes and fibrosis scores, whereas those at intermediate or high risk need to be referred to specialists to exclude other chronic liver diseases, to better assess disease severity and possibly to initiate specific therapy [5].

Definition of risk for NASH:

Risk for NASH was defined as those who presented at least one of the following biochemical and metabolic parameters: fasting glucose ≥ 100 mg/dL, TG ≥ 150 mg/dL, AST > 54 IU/L and ALT > 42 IU/L [6].

All patients were subjected to:

Full history taking with special attention to : Presence of any symptoms that may accompany with fatty liver disease (e.g.: fatigue and right hypochondrial pain). Presence of dyslipidemia signs (elevated serum cholesterol and TG levels). Presence of diabetes.

Diabetes mellitus was diagnosed when fasting glucose was ≥ 126 mg/dl or the patient was treated with anti-diabetic drugs, or had impaired fasting glucose (IFG)—when the fasting glucose level ranged between 100 and 125 mg/dL [7]. History of alcohol intake. Symptoms suggesting liver cirrhosis and/or liver cell failure (e.g.: history of jaundice, ascites, hepatic encephalopathy.....etc.).

Clinical examination: General examination with attention to BMI: Height and weight were measured while the patients had light

clothes and no shoes, and the BMI measured was according to the equation:

$$\text{BMI} = \text{Weight (kg)} / \text{Height}^2 \text{ (m)}.$$

Obesity was diagnosed when BMI was $\geq 30 \text{ kg/m}^2$, and overweight when BMI was ≥ 25 and $< 30 \text{ kg/m}^2$. [7].

Local abdominal examination with especial attention to: Presence of hepatomegaly or splenomegaly. Any stigmata of liver cirrhosis and/or liver cell failure (e.g.: jaundice, ascites, spider nevi ...etc.).

Laboratory investigations including: Liver profile [Serum levels of Aspartate transferase enzyme (AST), Alanine transferase enzyme (ALT) Albumin, Gamma glutamyl transferase (γ GT)]. Coagulation profile Prothrombin time (PT) and (INR), to exclude liver cell failure (LCF). Fasting blood sugar (FBS). Complete blood count (CBC). Fasting blood sugar (FBS). Lipid profile including: Triglyceride, total cholesterol & low- density lipoprotein (LDL), high density lipoprotein (HDL). Serological markers to exclude other causes of chronic liver diseases, autoimmune hepatitis and metabolic liver disease. Viral markers (HBs Ag, HBc IgG, HCV Ab). Autoantibodies (Anti smooth muscle antibody (ANA) and Anti mitochondrial antibody (ASMA). Serum Ferritin.

Abdominal ultrasonography: The equipment used was Medisone Sonoace 9900 Duplex ultrasonography equipment with a curved convex 3.5-5 MHz transducer that has a real time B mode imaging system with pulsed wave and Color Doppler facilities was used. For presence of ultra-sonographic features of fatty liver, which include increased echogenicity of liver by ultrasound examination which is the characteristic ultrasonographic finding that identified hepatic steatosis. The increased echogenicity compared to the spleen and kidney. A loss definition of definition of the hemi-diaphragm and decreased detail of the intrahepatic architecture (particularly the portal veins and the hepatic vein trunk) are supportive findings [8].

Fibroscan technique:

Transient elastography (TE) (FibroScan; Philips IU22) is a new tool, designed for the non- invasive study of liver

stiffness. TE uses an ultrasound transducer probe, mounted on the axis of a vibrator.

Fibroscan (EchoSens) examination was done as follows:

Patients lied in the dorsal decubitus position with their right arm in maximal abduction. The tip of the probe transducer was covered with coupling gel. The tip of the probe transducer was placed on the skin between the ribs at the level of the right lobe of the liver. A vibration of mild amplitude and low frequency was transmitted from the vibrator to the tissue by the transducer itself. A pulse-echo acquisition was performed at this time to follow the propagation of the shear wave and measure its velocity, which was directly related to the liver stiffness, the harder the tissue, the faster the propagation of the shear wave [9].

Liver stiffness measurement was expressed in kilopascals (KPa). Ten successful acquisitions were performed on each patient. The median value was expressed as final result of the liver stiffness. The success rate is calculated as the ratio of the number of successful acquisitions to that of the total number of acquisitions and a success rate of at least 60% or the interquartile range (IQR) $< 30\%$ were considered reliable.

New model score including the following clinical and laboratory parameters [body mass index (BMI), US of liver, Liver stiffness, LDL, HDL, TG, ALT, AST, AST/ALT ratio, Platelet, mean platelet volume (MPV), Ferritin and Fasting Blood Glucose]. the new scoring system for steatosis, where NAFLD score was calculated as per the following formula: $- 1.675 + 0.037 \times \text{age (years)} + 0.094 \times \text{body mass index (BMI, kg/m}^2) + 1.13 \times \text{impaired fasting glucose/diabetes (yes} = 1, \text{no} = 0) + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{platelet (X10}^9 \text{/L)} - 0.66 \times \text{Albumin (g/dL)}$ (table 1).

The basis of creation of the new score

BMI: Obesity is associated with a spectrum of liver abnormalities, known as nonalcoholic fatty liver disease (NAFLD), characterized by an increase in intrahepatic triglyceride (IHTG) content (i.e. steatosis) with or without inflammation and fibrosis (i.e. steatohepatitis) [10]

US of liver : Ultrasound is a non-invasive, widely available, and accurate tool in the detection of Non-alcoholic fatty liver disease (NAFLD) [11].

Liver stiffness: considered to be one of the direct consequences of the fibrotic evolution of chronic liver disease and function of the extent of hepatic fibrosis. [12]

Biochemical markers and lipid profile (LDL, HDL, TG, ALT, AST, AST/ALT ratio) are associated with NAFLD. Thus, it is indicated that in patients with NAFLD, there are considerable changes in biochemical markers. Thus, it seems essential that in clinical settings in cases in which biochemical and lipid changes are observed, sonography should be performed to examine individuals with NAFLD, since early diagnosis prevents further complications and delays them. [13].

Mean platelet volume: Patients with NAFLD have significantly higher values of MPV when compared to the healthy controls, and values of MPV could be used for prediction of the degree of liver steatosis and fibrosis in NAFLD patients and may be beneficial considering that they are simple, easy to measure, and cost-effective and are routinely checked in everyday practice [14].

Serum Ferritin: the level of Serum ferritin (SFL) can be an irrespective indicator to assess the progression of hepatic fibrosis in the patients with NAFLD because of its association with hepatic iron storage and hepatic inflammation. [15]

Fasting Blood Glucose : there is an independent nonlinear association between FBG and NAFLD, and the increase in FBG may indicate an increased risk of NAFLD. [16]

GULAB score: consists of five clinical and laboratory parameters: gender; US findings; fasting lipid levels; ALT levels; and BMI. The minimum and maximum scores were 1 and 7, respectively [17].

BARD score calculator (<http://www.pmidcalc.org>).

The BARD score was composed of 3 variables: AST/ALT ratio ≥ 0.8 – 2 points; a BMI ≥ 28 –1 point; and the presence of diabetes – 1 point. The possible score ranges from 0 to 4 points. According to the results of

Harrison et al. [18] BARD scores equaling 0 or 1 are of high (96%) negative predictive value (NPV) for advanced fibrosis.

Scoring of fibrosis:

APRI was defined: $[100 \times (\text{AST}/\text{upper limit of normality})/\text{platelet count } (10^9/\text{L})]$ [19].

FIB-4 values were calculated automatically using the formula $\text{age (years)} \times \text{AST [U/l]} / (\text{platelets } [10^9/\text{L}] \times (\text{ALT [U/l]})^{1/2})$, in which the age of the patient was the age at the time of the liver biopsy[20].

Statistical Analysis

Data were collected, tabulated and analyzed by SPSS 20 software [21]. According to the type of data, qualitative data was represented as number and percentage, quantitative continues group represent by mean \pm SD. The following tests were used to test differences for significance; difference and association of qualitative variables by Chi square test (X²). Differences between quantitative independent groups by t test. The Mann-Whitney U and Kruskal Wallis tests were used to compare non-normal distribution data between two or multiple groups, respectively. Univariate logistic regression analysis was carried out on variables of patients with or without NAFLD. A correlation analysis was done and Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off values. Area Under Curve (AUC) was also calculated. Variables significantly associated with the presence of NAFLD in univariate analysis (P < 0.05) were then subjected to multivariate logistic analysis to identify factors independently associated with NAFLD

RESULTS

Table (2), showed that there was a high statistically significant difference among both studied groups as regard age, weight, height and BMI. All non-NASH group were not diabetic versus 50% of NASH group and 16.7% of them were diabetic, with a high statistically significant difference among both of them. There was a high statistically significant difference among both studied groups as regard MPV and fasting blood sugar, which was higher among NASH risky group, while there was no significant difference as regard WBCs, RBCs, platelet

count and hemoglobin. There was a high statistical significant difference among both studied groups as regard liver function tests, lipid profile and ferritin. There was a high statistical significant difference among both studied groups as regard APRI and FIB-4, which was all higher among cases with NASH.

Table (3), showed that there was a high statistically significant difference among both studied groups as regard LMS and severity of fibrosis, which was all higher among cases with NASH. **Table (4)**, showed that in NASH cases there was a statistically significant positive correlation between the new studied score and BARD score, also there was a positive correlation with BMI, Hb, FBS, RBCs, platelet count, MPV, HDL, TG, albumin, ferritin, NAFLD and GULAP score but not reach significant level. While there was a negative correlation with age, WBCs, LDL, total cholesterol, ALT, AST, GGT, FIB4 and APRI, but not reach significant

level. In non-NASH cases there was a positive correlation between the new studied score with BMI, Hb, WBCs, RBCs, TG, ALT, AST, GGT and GULAP score, but not reach significant level. While there was a negative correlation with age, FBS, Platelet count, MPV, LDL, HDL, total cholesterol, albumin, LSM, FIB4, APRI, BARD and NAFLD score but not reach significant level. **Table (5)**, showed that after applying Multivariate analysis of significant variables for the new studied score among studied NASH group, it was proven to be non-significant predictor. **Table (6) and Fig. (2)**, showed that the sensitivity of new score in detection of NASH was 97.9% versus 91.7%, 89.6% and 100% of NAFLD, BARD and GULAP scores, respectively, with ability of 59.4% to exclude truly negative cases versus 68.8%, 93.8% and 96.9% of NAFLD, BARD and GULAP scores, respectively. The accuracy of new score was the same as NAFLD score 82.5% versus 91.3% of BARD score.

Table (1): Suggested new scoring system for steatosis

	0	1	2
BMI (kg/m ²)	<25	25-29.9	≥30
US (of liver)	No steatosis	Focal steatosis	Diffuse steatosis
Liver Stiffness	<6 kPa (F0)	6-8.8 kPa (F1-2)	≥8.9 kPa (F3-4)
LDL	≤130 (mg/dL)	>130 (mg/dL)	N/A
HDL	≥40 (mg/dL)	<40 (mg/dL)	N/A
TG	≤150 (mg/dL)	>150 (mg/dL)	N/A
ALT	Normal	Raised	N/A
AST	Normal	Raised	N/A
AST/ALT ratio	<1	>1	N/A
Platelet	>200 (×10 ³ /cc)	≤200 (×10 ³ /cc)	N/A
MPV	Normal	Raised	N/A
Ferritin	Normal	Raised >1.5 upper limit	N/A
FBS	≤110 (mg/dL)	111-125.9 (mg/dL)	≥126 (mg/dL)

N/A : not applicable

BMI; body mass index, US; ultrasonography, LDL; low-density lipoprotein, HDL; high-density lipoprotein, TG; triglyceride, ALT; Alanine aminotransferase, AST; aspartate aminotransferase, MPV; mean platelet volume, FBS; fasting blood sugar.

Table (2): Baseline characteristics of enrolled participants:

	NASH group N=48	Non-NASH group N=32	<i>p</i> value
Age (year)	55.1 ± 9.3	35.2 ± 5.2	<0.001
Gender			
Male <i>N</i> (%)	23 (46.9%)	97 (78.9%)	<0.001
Female <i>N</i> (%)	26 (53.1%)	26 (21.1%)	
Weight (Kg)	89.4 ± 12.2	73.1 ± 10.3	<0.001
Height (Meter)	1.62 ± 0.1	1.71 ± 0.1	<0.001
BMI	33.9 ± 5.3	24.2 ± 2.3	<0.001
Diabetes			
Non-diabetic <i>N</i> (%)	24 (50%)	32 (100%)	<0.001
Diabetic <i>N</i> (%)	8 (16.7%)	0	
Pre-diabetic <i>N</i> (%)	16 (33.3%)	0	
WBC's (×10 ³ cells/cc)	6.8 ± 1.56	6.9 ± 1.57	0.55
Hb (g/dl)	12.2 ± 0.82	12.6 ± 0.85	0.06
Platelet (×10 ³ /cc)	262.4 ± 69.9	267.3 ± 68.8	0.758
RBCs	4.2 ± 0.32	4.3 ± 0.31	0.112
MPV	13.8 ± 1.89	10.5 ± 1.27	<0.001
FBS (mg/dl)	105.7 ± 16.88	77.7 ± 6.18	<0.001
ALT(IU/L)	43.6 ± 6.8	19.9 ± 3.64	<0.001
AST(IU/L)	42.9 ± 6.8	18.9 ± 3.3	<0.001
Albumin (g/dl)	3.2 ± 0.12	3.5 ± 0.1	<0.001
GGT (U/L)	35.7 ± 5.7	19.9 ± 3.4	<0.001
TC (mg\dl)	174.7 ± 13.5	147.8 ± 15.7	<0.001
TG (mg\dl)	188.9 ± 23.3	87.4 ± 19.8	<0.005
LDL (mg\dl)	92.1 ± 15.1	70.9 ± 16.2	<0.001
HDL (mg\dl)	44.8 ± 5.6	59.4 ± 3.02	<0.001
Ferritin (g/dl)	189.02 ± 35.3	69.5 ± 19.1	<0.001
APRI score	0.43 ± 0.13	0.19 ± 0.05	<0.001
FIB-4 score	1.44 ± 0.47	0.59 ± 0.17	<0.001

BMI; body mass index, WBC's; white blood cells, Hb; hemoglobin, RBCs; red blood cells, MPV; mean platelet volume, FBS; fasting blood sugar, ALT; Alanine aminotransferase, AST; aspartate aminotransferase, GGT; gamma-glutamyl transferase, TC; total cholesterol, TG; triglyceride, LDL; low-density lipoprotein, HDL; high-density lipoprotein, APRI; AST to Platelet Ratio Index, FIB-4; fibrosis-4.

Table (3): Difference among both studied groups as regards LSM, stage and severity of fibrosis.

	NASH group N=48	Non-NASH group N=32	<i>p</i> value
LSM	6.4 ± 1.59	5.7 ± 1.53	0.03
Stage of fibrosis <i>N</i> (%)			
1	38 (79.2%)	27 (84.4%)	0.559
2	10 (20.8%)	5 (15.6%)	
Correlated fibrosis severity <i>N</i> (%)			
F0-F2	11 (22.9%)	32 (100%)	<0.001
F3-F4	9 (18.8%)	0	
Indeterminate	28 (58.3%)	0	
DFL <i>N</i> (%)			
Yes	38 (79.2%)	13 (40.6%)	0.001
No	10 (20.8%)	19 (59.4%)	
FFL <i>N</i> (%)			
Yes	10 (20.8%)	19 (59.4%)	0.001
No	38 (79.2%)	13 (40.6%)	

LSM; liver stiffness measurement, DFL; diffuse fatty liver, FFL; focal fatty liver.

Table (4): Pearson's correlation between new studied score and other clinical data among studied groups :

Variables	New score (NASH cases)			New score (non -NASH cases)		
	r	P-value		r	P-value	
Age	-0.267	0.067	NS	-0.031	0.867	NS
BMI	0.192	0.921	NS	0.184	0.321	NS
Hb	0.158	0.299	NS	0.138	0.499	NS
WBCs	-0.180	0.232	NS	0.173	0.332	NS
RBCs	0.077	0.856	NS	0.247	0.156	NS
FBS	0.242	0.098	NS	-0.185	0.398	NS
Platelet count	0.022	0.882	NS	-0.02	0.982	NS
MPV	0.003	0.980	NS	-0.195	0.280	NS
LDL	-0.191	0.194	NS	-0.171	0.394	NS
HDL	0.101	0.494	NS	-0.025	0.944	NS
Triglycerides	0.202	0.169	NS	0.283	0.116	NS
Total cholesterol	-0.103	0.454	NS	-0.110	0.554	NS
ALT	-0.075	0.615	NS	0.178	0.315	NS
AST	-0.056	0.703	NS	0.116	0.532	NS
Albumin	0.045	0.763	NS	-0.05	0.763	NS
Ferritin	0.071	0.631	NS	0.00	1.0	NS
GGT	-0.064	0.663	NS	0.164	0.363	NS
LSM	0.094	0.523	NS	-0.224	0.243	NS
FIB4	-0.139	0.346	NS	-0.101	0.581	NS
APRI	-0.017	0.910	NS	-0.109	0.551	NS
BARD	0.300	0.038	S	-0.049	0.791	NS
NAFLD score	0.046	0.757	NS	-0.005	0.997	NS
GULAP score	0.05	0.735	NS	0.115	0.135	NS

NS: P-value >0.05 (not significant), S: P-value<0.05 (significant).

BMI: body mass index; Hb: Hemoglobin; WBCs: White blood cells; RBCs :Red blood cells; FBS: fasting blood sugar; MPV: mean platelet volume; LDL: low-density lipoproteins; HDL: High-density lipoproteins; ALT: Alanine transferase enzyme; AST: Aspartame transaminase; GGT: Gamma-glutamyl transferase; LSM: Liver stiffness measurement; FIB4: Fibrosis-4; APRI : AST to Platelet Ratio Index; BARD: BMI, AST/ALT ratio, and diabetes; NAFLD: Non-alcoholic fatty liver disease; GULAB: scoring system for predicting NASH.

Table (5): Multivariate regression analysis of significant predictors for new studied score among the studied NASH group.

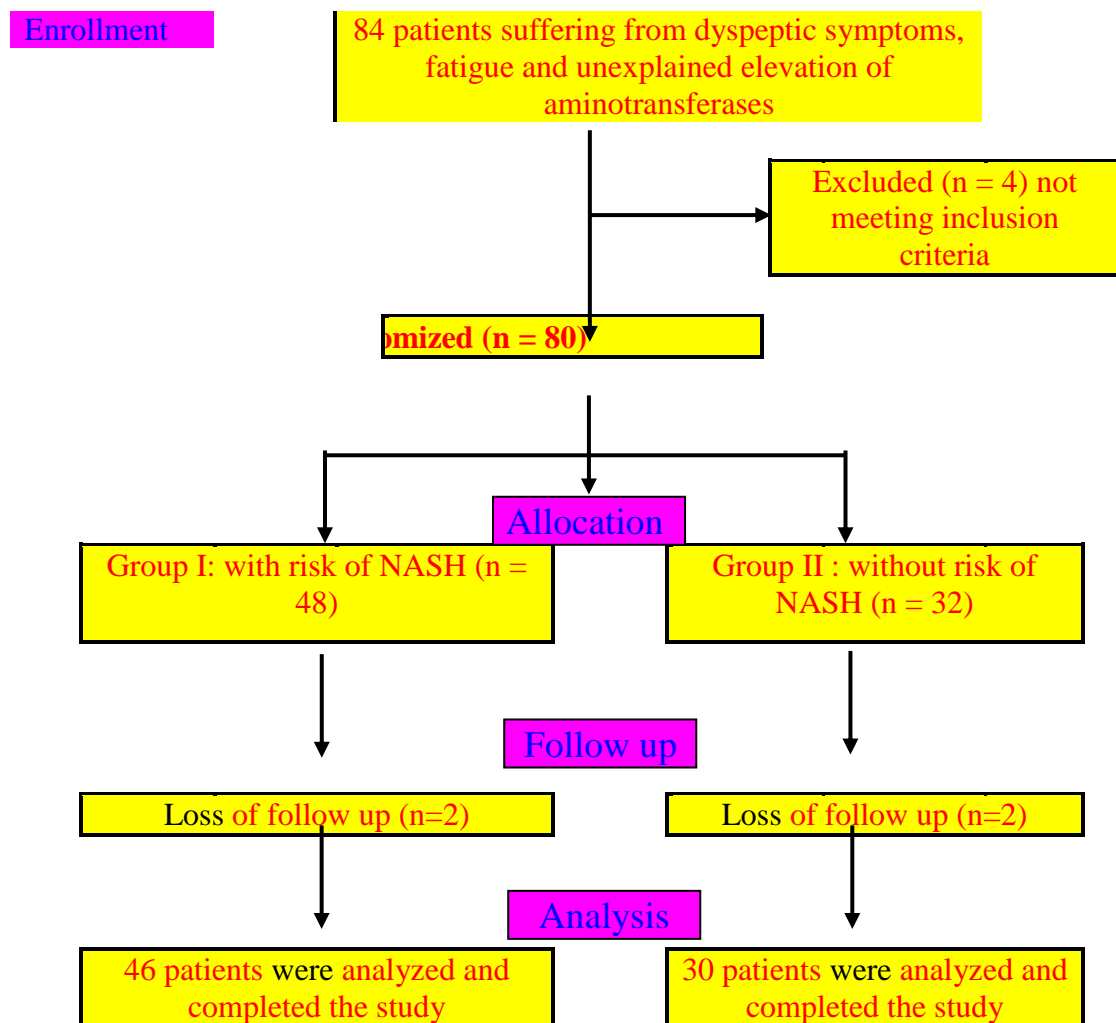
	Regression coefficient	SE	P-Value
BARD score	0.170	0.103	0.104 NS
$r=0.896$, $r^2 =0.803$ ANOVA $P<0.000^*$ Durbin-Waston ratio=1.768			

BARD score: BMI, AST/ALT ratio, and diabetes, SE= standard error

Table (6): Reliability data of standard and new scores as a predictor for NASH.

Variables	Cut off	AUC	P-value	PVP	PVN	sensitivity	specificity	accuracy
BARD	2.5	0.925	<0.001	95.6%	85.7%	89.6%	93.8%	91.3%
NAFLD score	-2.35	0.921	<0.001	81.5%	84.6%	91.7%	68.8%	82.5%
GULAB score	3.5	0.996	<0.001	98%	100%	100%	96.9%	98.8%
New score	1.5	0.789	<0.001	78.3	95	97.9	59.4	82.5

BARD: BMI, AST/ALT ratio, and diabetes, NAFLD: Non-Alcoholic Fatty Liver Disease, GULAB scoring system for predicting NASH based on five clinical and laboratory parameters: (Gender: male = 1, female = 0; Ultrasound abdomen: DFL = 2, FFL = 1; Lipid (fasting) levels: raised serum cholesterol or serum triglyceride or serum LDL = 1, normal lipid levels =0; ALT: raised =1, normal = 0; BMI: >27=1,<27=0).

**Fig. (1) :** Flow chart of patients in the studied groups

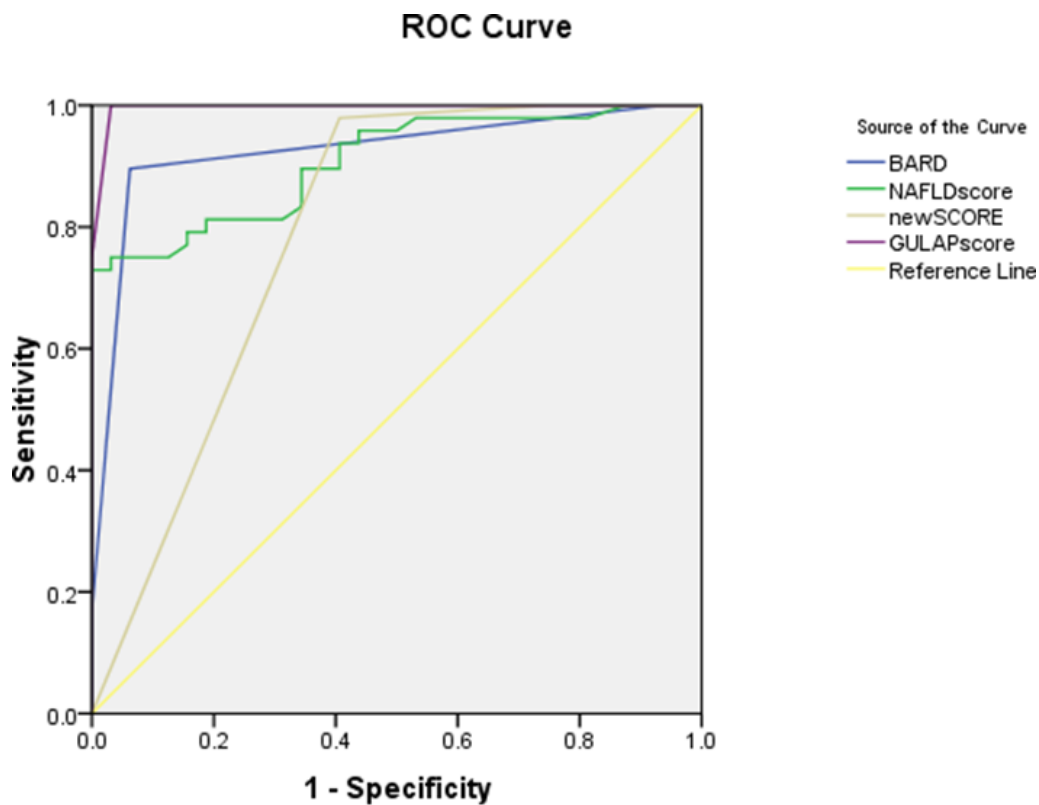


Fig. (2): Receiver operating characteristics (ROC) curve for standard and new scores as predictors of NASH

DISCUSSION

NAFLD/NASH are currently emerging as the primary causes of chronic liver disease, namely cirrhosis, and hepatocellular carcinoma worldwide. While in the era in which effective hepatitis C therapy is a reality and alcohol abuse is being curbed in some populations, the increased prevalence of obesity, type 2 diabetes and NAFLD/NASH affect both the developed and developing countries [4].

Conventionally, NASH diagnostics relies on a liver biopsy (LB) when all other causes of liver damage have been discarded in which hepatocyte ballooning, inflammation, and fibrosis are demonstrated. Alternatively, non-invasive strategies include liver stiffness measurement (LSM) using transient elastography (TE) [22].

This cross-sectional study was carried on (80) Egyptian patients presenting to the hepatology outpatient clinic, Zagazig University Hospitals suffering from dyspeptic symptoms, fatigue and unexplained elevation

of aminotransferases and having fatty liver by ultrasound.

In the present study, NASH was recorded in 48 (60%) of patients while 32 (40%) of patients had steatosis without proof of steatohepatitis. This came in agreement with **Tasneem et al. [17]** who reported that NASH was found in 78 (81.3%) patients while the remaining 18 (18.7%) patients had steatosis without evidence of steatohepatitis.

In the present study, the mean age of the patients in the study was 55.1 ± 9.3 years old in NASH group while it was 35.2 ± 5.2 in No NASH group with the statistically significant difference in both groups as regard the age. This result was in accordance with the result in the study of **Sepulveda-Villegas et al. [4]** who found that the patients in NASH group were older than those in the non-risk group (39.5 ± 13 vs. 33.9 ± 13.5 years, $p = 0.0021$). This may indicate the direct relation between old age and high incidence of NASH.

In the current study, the male distribution was predominant in the current

study (65%) with a significant difference between both groups. This was consistent with **Angulo et al. [23]**, where the male distribution was 53%, denoting the relationship between male gender and metabolic syndrome.

In the present study, the BMI was 33.9 ± 5.3 (kg/m^2) in NASH group while BMI in No NASH group was 24.2 ± 2.3 (kg/m^2) with the statistically significant difference among both groups. This result was in agreement with the result in the study of **Sepulveda-Villegas et al. [4]** who found that the patients in NASH group were obese (average BMI 32.7 ± 9.3 versus 25.2 ± 6.2 kg/m^2 , $p = 0.0012$), and in disagreement with our study, the study of **Beymer et al. [24]** showed no difference regarding BMI between both groups with and without NASH. This difference may be due to different inclusion and exclusion criteria in their study and ours.

In the current study, the co-morbid conditions including obesity, DM and dyslipidemia were highly significant in the NASH group. All cases in No NASH group were non-diabetic while 33.3% and 16.7% were pre-diabetic and diabetic respectively. Such risk factors were significantly related to NASH development. This was in agreement with the study by **Hashemi et al. [25]**, which revealed a significant increase in the rate of DM and BMI as steatosis being advanced.

Also, **Park et al. [26]** found that patients with NASH had a highly significant incidence of diabetes and obesity comparing to non-NASH patients

In this work, we assessed the relation between several laboratory markers and the risk of NASH, and we found that elevated blood sugar, high lipid profile and elevated liver enzymes (denoting ongoing necro-inflammation) are all considered risk factors of developing NASH.

In agreement with our study, **Sepulveda-Villegas et al. [4]** who found that regarding ALT, AST, TG, TC, HDL, LDL and glucose there was a statistically significant difference between both studied groups (with and without NASH).

In the present study, there was no significant difference regarding platelet count

in both groups. This came in agreement with **Park et al. [26]** who found no significant difference regarding platelet count ($p = 0.48$).

In the current study, there was a high statistically significant difference among both studied groups as regard ferritin with increased its level in the NASH group. This came in agreement with **Kowdley et al. [27]** who found that increased risk of NASH was associated with higher serum ferritin values.

In the present study, there was a highly significant difference between both studied groups as regard GGT. This came in agreement with **Sakugawa et al. [28]** who found a significant difference between GGT level and liver fibrosis severity.

In the current study, diffuse fatty liver was more evident in the NASH group (79.2%) than in the No NASH (40.6%). While focal fatty liver changes were significantly more common features among the No NASH group (59.4%) than in the NASH group (20.8%). This came in agreement with **Pulzi et al. [29]** who found that presenting normal (or non-steatosis) more frequently in Non-NASH (26/49) than in NASH (3/13) ($p = 0.048$). Abdominal US presented sensitivity and specificity of 76.9% and 46.9%, respectively, to identify NASH.

In this study, we proposed a diagnostic model using clinical, laboratory and imaging data to improve the differential diagnosis of patients who have NASH from those who don't have it. Patients with a score of more than 1.5 are more likely to have NASH (sensitivity 97.9%, specificity 59.4%, NPV 95%, PPV 78.3%). This composite index seems to be a good discriminator to identify NASH patients with more severe disease.

In the present study in comparison between the new score and other scores, sensitivity of new score in detection of NASH was 97.9% versus 91.7%, 89.6% and 100% of NAFLD, BARD and GULAB scores respectively, with ability of 59.4% to exclude truly negative cases versus 68.8%, 93.8% and 96.9% of NAFLD, BARD and GULAB scores respectively. The accuracy of the new score was the same as NAFLD score 82.5% versus 91.3% and 98.8% of BARD and GULAB scores respectively

As can be seen here, the sensitivity, specificity, PPV and NPV obtained by the measurements we proposed in this study are to some extent similar to the ones previously reported [30].

Despite the low specificity (we added it to the limitations of the study), the new score revealed a high sensitivity compared to the previously known scores that can be used as a good negative test to exclude NASH in the screening of high risk patients

Conclusion: The new model score considered simple, non-invasive and low-cost tool and can be used as a good negative test to exclude NASH in the screening of high risk patients and markedly reduce the need for liver biopsies in NAFLD patients.

Limitations : All auxiliary biomarker/scores for NAFLD/NASH diagnosis have pros and cons, and some do not consider the full spectrum of metabolic risk factors related to NASH. The limitation of imaging is that it cannot differentiate bland steatosis from steatohepatitis which is possible only with liver biopsy.

Recommendation : Wide-scale studies are recommended to evaluate the role of the new score model compared to liver biopsy in assessing the risk of NASH. Periodic monitoring of old patients with risk of NASH could aid to improve the quality of life and prevent the appearance of co-morbidities in the next decades of life.

REFERENCES

- 1- Younossi Z, Tacke F, Arrese M, Chander S, Mostafa I, Bugianesi E et al. Global Perspectives on Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. *Hepatology* 2019; 69 (6): 2672-82.
- 2- Jelenik T, Kaul K, Séquaris G, Flögel U, Phielix E, Kotzka J et al. Mechanisms of insulin resistance in primary and secondary nonalcoholic fatty liver. *Diabetes* 2017; 66 (8) : 2241-53.
- 3- Chalasani N, Younossi Z, Lavine J, Charlton M, Cusi K, Rinella M et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018; 67 (1): 328-57.
- 4- Sepulveda-Villegas M, Roman S, Rivera-Iñiguez I, Ojeda-Granados C, Gonzalez-Aldaco K, Torres-Reyes L et al. High prevalence of nonalcoholic steatohepatitis and abnormal liver stiffness in a young and obese Mexican population. *PloS One* 2019; 14 (1): e0208926.
- 5- Byrne C, Targher G. EASL–EASD–EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease: is universal screening appropriate?. *Diabetologia* 2016, 59(6): 1141-1144.
- 6- Abenavoli L, Milic N, Di Renzo L, Preveden T, Medić-Stojanoska M, De Lorenzo A. Metabolic aspects of adult patients with nonalcoholic fatty liver disease. *World J Gastroenterol* 2016; 22:7006–16.
- 7- Cichoż-Lach H, Celiński K, Prozorow-Król B, Swatek J, Słomka M, Lach T. The BARD score and the NAFLD fibrosis score in the assessment of advanced liver fibrosis in nonalcoholic fatty liver disease. *Med Sci Monit* 2012;18 (12) :CR 735-40.
- 8- Watanabe S, Yaginuma R, Ikejima K, Miyazaki A. Liver diseases and metabolic syndrome. *J Gastroenterol* 2008; 43(7) : 509-18.
- 9- Sandrin L, Fourquet B, Hasquenoph J, Yon S, Fournier C, Mal F et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; 29: 1705-13.
- 10- Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology* 2010, 51(2): 679-689.
- 11- Khov N, Sharma A, Riley TR. Bedside ultrasound in the diagnosis of nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; 20 (22): 6821- 6825.
- 12- Abenavoli L, Beaugrand M. Transient elastography in non-alcoholic fatty liver disease. *Ann Hepatol.* 2012; 11 (2):172-178.
- 13- Mansour-Ghanaei, R., Mansour-Ghanaei, F., Naghipour, M., & Joukar, F. (2019). Biochemical markers and lipid profile in nonalcoholic fatty liver disease patients in the PERSIAN Guilan cohort study (PGCS), Iran. *J Family Med Prim Care.* 2019 Mar; 8(3): 923–928.
- 14- Milovanovic Alempijevic T, Stojkovic Lalosevic M, Dumic I, Jovic N, Pavlovic Markovic A, Dragasevic S, et al. Diagnostic Accuracy of Platelet Count and Platelet Indices in Noninvasive Assessment of Fibrosis in Nonalcoholic Fatty Liver Disease Patients. *Can J Gastroenterol Hepatol.* 2017;2017:6070135.

- 15- Du SX, Lu LL, Geng N, Victor DW, Chen LZ, Wang C et al.** Association of serum ferritin with non-alcoholic fatty liver disease: a meta-analysis. *Lipids Health Dis.* 2017; 16 (1): 228-236
- 16- Zou Y, Yu M, Sheng G.** Association between fasting plasma glucose and nonalcoholic fatty liver disease in a nonobese Chinese population with normal blood lipid levels: a prospective cohort study. *Lipids Health Dis.* 2020 20; 19(1):1-11
- 17- Tasneem A, Luck N, Majid Z.** Factors predicting non-alcoholic steatohepatitis (NASH) and advanced fibrosis in patients with non-alcoholic fatty liver disease (NAFLD). *Trop Doct* 2018; 48 (2): 107-112.
- 18- Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri B.** Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut* 2008; 57 (10): 1441-47.
- 19- Wai C, Greenson J, Fontana R, Kalbfleisch J, Marrero J, Conjeevaram H et al.** A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; 38 (2) :518–26.
- 20- Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, et al.** Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; 43 (6) : 1317-25.
- 21- Liang G, Fu W, Wang K.** Analysis of t-test misuses and SPSS operations in medical research papers. *Burns Trauma* 2019; 7(1): s41038-019.
- 22- Torres-Valadez R, Roman S, Jose-Abrego A, Sepulveda-Villegas M, Ojeda-Granados C, Rivera-Iñiguez I et al.** Early detection of liver damage in Mexican patients with chronic liver disease. *J Transl Int Med* 2017; 5 (1): 49–57.
- 23- Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell G et al.** The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; 45(4): 846-854.
- 24- Beymer C, Kowdley KV, Larson A, Edmonson P, Dellinger E, Flum D.** Prevalence and predictors of asymptomatic liver disease in patients undergoing gastric bypass surgery. *Arch Surg* 2003; 38 (11):1240-44.
- 25- Hashemi S, Alavian S, Gholami-Fesharaki M.** Assessment of transient elastography (FibroScan) for diagnosis of fibrosis in non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Caspian J Intern Med* 2016; 7 (4): 242-52.
- 26- Park C, Tsai N, Wong L.** Implications of worse renal dysfunction and medical comorbidities in patients with NASH undergoing liver transplant evaluation: impact on MELD and more. *Clin Transplant* 2011, 25(6), E606-E611.
- 27- Kowdley KV, Belt P, Wilson LA, Yeh M, Neuschwander-Tetri B, Chalasani N et al.** NASH Clinical Research Network. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2012; 55 (1) : 77-85.
- 28- Sakugawa H, Nakayoshi T, Kobashigawa K, Yamashiro T, Maeshiro T, Miyagi S et al.** Clinical usefulness of biochemical markers of liver fibrosis in patients with nonalcoholic fatty liver disease. *World J Gastroenterol* 2005; 11(2) : 255-59.
- 29- Pulzi FB, Cisternas R, Melo M, Ribeiro C, Malheiros C, Salles J.** New clinical score to diagnose nonalcoholic steatohepatitis in obese patients. *Diabetol Metab Syndr* 2011; 3(1): 3.
- 30- Pulzi FB, Cisternas R, Melo MR, Ribeiro CM, Malheiros CA, Salles JE.** New clinical score to diagnose nonalcoholic steatohepatitis in obese patients. *Diabetol Metab Syndr* 2011; 3 (1): 3.

How to Cite

Elhamdi, Y., Farag, A., Mohammed Sadek, A., Bayoumi, S. New Screening non- invasive tool for Nonalcoholic Steatohepatitis in High-Risk Individuals. *Zagazig University Medical Journal*, 2021; (538-550): - . doi: 10.21608/zumj.2020.33313.1883