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ORIGINAL ARTICLE

Serum ferritin as a non-invasive marker in the prediction of hepatic fibrosis among Egyptian patients with non-alcoholic fatty liver disease

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Abstract

Background and Aim: Many studies have found a relationship between hepatic iron, serum ferritin, and non-alcoholic fatty liver disease (NAFLD) or its progress. The aim of this study is to assess the value of serum ferritin as a non-invasive marker in the prediction of hepatic fibrosis in NAFLD.

Methods: This study included 113 subjects who were classified into three groups. Group I included 30 healthy subjects as control with no clinical, radiological, and histological features of NAFLD. Group II included 31 NAFLD patients without hepatic fibrosis. Group III included 52 patients with hepatic fibrosis on top of NAFLD.

Results: Serum ferritin was determined using ferritin ELISA kit. Fibrosis 4 score was calculated. Liver biopsy was conducted for included patients. Significantly higher levels of serum ferritin were found in patients with hepatic fibrosis on top of NAFLD than controls. Receiver operating characteristic curve analysis revealed that an optimum cutoff level of 51.95 ng/mL was the best to predict fibrosis on top of NAFLD with diagnostic sensitivity and specificity of 65% and 60%, respectively, and area under the curve = 0.658.

Conclusion: Higher serum ferritin was found in patients with hepatic fibrosis on top of NAFLD. Serum ferritin was found to be a predictor of fibrosis on top of NAFLD with moderate sensitivity and specificity.

Introduction

Fatty liver or hepatosteatosis is characterized histologically by the accumulation of triglycerides within the cytoplasm of the hepatocytes[1] and refers to the fat accumulation in the liver exceeding 5–10% by weight.[2]

When hepatosteatosis is present in the absence of excessive alcohol consumption, it is termed non-alcoholic fatty liver disease or NAFLD, which is considered the hepatic manifestation of the metabolic syndrome, a constellation of frequent abnormalities involving insulin resistance, visceral obesity, diabetes, hypertension, and other additional factors.[1]

NAFLD encompasses a spectrum of disorders ranging from simple steatosis to inflammatory steatohepatitis (NASH), with increasing levels of fibrosis that can progress to liver cirrhosis, portal hypertension, and hepatocellular carcinoma.[3] Of those who develop NASH, about 20% of patients will develop cirrhosis during their lifetime.[4] Therefore, a diagnosis of NASH and early hepatic fibrosis may result in a more aggressive therapeutic approach toward the metabolic risk factors.[5]

The diagnosis of NAFLD needs the confirmation of hepatic steatosis based on either imaging studies or liver biopsy, together with the clinical exclusion of individuals who regularly consume >20 g of ethanol per day.[4] The diagnosis of NAFLD should be strongly suspected in the presence of features such as obesity, diabetes and obstructive sleep apnea or patients with asymptomatic and persistent elevation of aminotransferase, radiological finding of fatty liver and unexplained persistent hepatomegaly. Alternative diagnoses should be excluded by the history and serological testing, including the viral hepatitis, hemochromatosis, autoimmune liver disease, alpha-1 antitrypsin deficiency, Wilson's disease, and drug-induced liver dysfunction.[5]

In the clinical setting, there is still no consensus about whether liver biopsy is required to confirm a diagnosis of NAFLD.[6] Due to the many potential errors, due to sampling, inter/intra-observer variability, biopsy is the "best" not the "gold" standard for diagnosis.[6]

Recently, hepatic iron overload and its correlation with chronic liver disease have been considered.[7] With a recent progress in understanding iron metabolism, accumulating evidence suggests a link between altered iron metabolism and NAFLD. In the last decade, many studies have found a relationship between hepatic iron, serum ferritin, and NASH or its progress.[8,9]

This study was designed to assess the relation between serum ferritin and hepatic fibrosis on top of NAFLD.

Patients and methods

This case–control study was conducted at the Ain Shams Center for Organ Transplant (ASCOT), the Internal Medicine Department, the Tropical Department, at Ain Shams University and Specialized Hospitals, Cairo, Egypt, after approval from the Research and Ethics Committee of the Faculty of Medicine, Ain Shams University was obtained in accordance with local research governance requirements. All procedures conducted in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. This study is registered at https://clinicaltrials.gov/ct2/show/study/NCT02330731?show_desc=Y#desc and the registration identification number is NCT03250572.

Inclusion criteria: Patients with NAFLD with or without hepatic fibrosis. The patients and controls were collected from the ASCOT, Ain Shams Specialized Hospital, from donors for living-related liver transplantation, for whom liver biopsy was routinely conducted. Liver biopsies were also carried out for patients with clinical and radiological evidence of NAFLD with elevated liver enzymes in Ain Shams University Hospital.

Exclusion criteria: Patients with medical history, clinical or laboratory evidence of any other liver diseases, such as alcoholic or viral hepatitis, schistosomiasis, autoimmune hepatitis, drug induced hepatitis, hereditary liver disease, and those with decompensated liver cirrhosis.

This study included 113 patients who were classified into three groups:

- Group I included 30 healthy subjects enrolled as control with no clinical, radiological, or histological features of hepatic steatosis in the liver biopsy.
- Group II included 31 patients with hepatic steatosis ≥5%, with no histological evidence of hepatic fibrosis in the liver biopsy.
- Group III included 52 patients with hepatic fibrosis on top of NAFLD evidenced in liver biopsy.

All patients and healthy controls were subjected to the following:

- Complete medical history and clinical examination, including abdominal examination, waist circumference measurement, and body mass index (BMI).
- Laboratory assessment: Venous blood (8 mL) was withdrawn aseptically into a sterile disposable syringe from each patient and control, where 2 mL was placed in EDTA Vacutainer for conducting complete blood count (CBC), 2 mL of blood was collected on citrate for prothrombin time (PT) and international normalized ratio (INR) determination, and 4 mL was collected in two plain Vacutainers to be clotted and centrifuged (1500 g for 15 min) for the measurement of biochemical markers, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, albumin, creatinine, blood urea nitrogen (BUN), and serum ferritin. The laboratory work was conducted at Clinical Pathology Department.
- CBC was done using Coulter counter (T660) (Beckman Coulter, Inc., Brea, CA, USA).
- AST, ALT, bilirubin, albumin, creatinine, and BUN were measured on Synchron CX9 autoanalyzer (Beckman Instruments Inc.; Scientific Instruments Division, Fullerton, Brea City, CA, USA).
- PT and INR were determined on Diagnostica Stago (Asnieres, France)
- Full lipid profile, including total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), was analyzed using Beckman Coulter AU480 System (Beckman Coulter, Inc., Brea, CA, USA).

For exclusion of other liver diseases, viral markers, including hepatitis B surface antigen (HBsAg) and hepatitis C virus antibody (HCVAb), were tested using qualitative third-generation enzyme linked immunosorbent assay (ELISA) method.

Autoimmune markers, including antinuclear antibody (ANA) and antismooth muscle antibody (ASMA), were analyzed using indirect immunofluorescence technique. Bilharzial antibodies were examined using ELISA. Serum and urinary copper were measured using an atomic absorption spectrophotometry by Perkin Elmer Instruments Analyst 800 atomic absorption device. Ceruloplasmin was measured using the photometric method with an RA-1000 autoanalyzer.

Serum ferritin levels were determined for all samples using ferritin quantitative test kit based on a solid-phase ELISA. Serum samples were obtained under complete aseptic technique. They were collected in serum separator tube (SST) and were left to clot for 30 min at room temperature before centrifugation for 15 min at 1000×*g*. Serum was removed, aliquoted, and stored at ≤−20°C. Repeated freeze–thaw cycles were avoided. Reference ranges were 20–300 ng/mL for adult male, 10–100 ng/mL for premenopause female, and 20–200 ng/mL for postmenopause female.

Abdominal ultrasound was conducted using a Toshiba Aplio XV scanner (Toshiba, Japan) equipped with a broadband 2.5–5 MHz curved array probe to assess the presence of liver steatosis (bright liver), which was defined and graded as follows: (i) a diffuse hyperechoic echo texture (bright liver); (ii) increased liver echo texture compared with the kidney; (iii) vascular blurring; and (iv) deep attenuation. Steatosis was graded using this semiquantitative scale from 1 to 4. Fibrosis, when present with noticeable steatosis, was identified by a coarse echo pattern. Therefore, the grade fibrofatty indicated the presence of a bright liver, with a coarse texture.

Ultrasound-guided liver biopsy and histopathologic examination: Ultrasonography-guided liver biopsies were conducted under conscious sedation using a 16-gauge Klatskin needle. The length of the histological specimens was no less than 2.5 cm.

The histological examination of liver biopsy was conducted by the same pathologist. The samples were fixed in 10% neutral-buffered formalin, embedded in paraffin blocks, and then cut into 5-μm-thick sections and stained with hematoxylin and eosin. They were examined under a light microscope for histopathologic evaluation. Grading and staging of steatosis, microinflammation and fibrosis were done. Steatosis was graded on a scale from 0 to 3, where 0 = no steatosis, 5–33% = S1, 34–66% = S2, and >66% S3. Fibrosis staging was graded on a scale from 0 to 4, where 0 = no fibrosis and 4 = cirrhosis, as per the classification of Brunt *et al.*[10,11]

Fibrosis 4 score (FIB4) was measured for all patients using the equation:

$$\text{Age (years)} \times \text{AST}\left(\frac{\text{U}}{\text{L}}\right)/\text{platlet count}\left(10^9/L\right) \times \sqrt{\text{ALT}}\left(\frac{\text{U}}{\text{L}}\right).$$

Interpretation:

- FIB4 < 1.45 exclude hepatic fibrosis.
- FIB4 > 1.45 < 3.25 ranging from mild to moderate (F1–F2) hepatic fibrosis.
- FIB4 > 3.25 advanced fibrosis (F3–F4).

The results of different parameters were statistically analyzed.

Statistical analysis. The data collected were revised, coded, tabulated, and introduced to a PC using Statistical Package for Social Sciences (IBM SPSS version 20.0; Chicago, IL, USA). Data were presented, and a suitable analysis was carried out as per the type of data obtained for each parameter. Data were described as mean ± SD, range for parametric numerical data, and as frequency and percentage for non-numerical data. ANOVA test was used to assess the statistical significance of the difference of a parametric variable between means of more than two study groups. Chi-square test was used to examine the relationship between two qualitative variables, while Fisher's exact test was used when the expected count is less than five in more than 20% of the cells. Pearson's correlation coefficient (r) was used as a measure of the strength of a linear association between two quantitative variables. Receiver operating characteristic (ROC) curve analysis was carried out to test the diagnostic performance of a test or the accuracy of a test to discriminate diseased cases from normal cases. In a ROC curve, the true-positive rate (sensitivity) is plotted in function of the false-positive rate (100-specificity) for different cutoff points of a parameter. Each point on the ROC curve represents a sensitivity/specificity pair corresponding to a decision threshold. The area under the ROC curve (AUC) is a measure of how well a parameter can distinguish between two diagnostic groups (diseased/normal). Logistic regression analysis was conducted to find out significant predictors for the occurrence of NAFLD and fibrosis in the studied patients. Level of significance: P value > 0.05 is non-significant (NS), $P \leq 0.05$ is significant (S), while $P \leq 0.01$ is highly significant (HS).

Results

The mean ages of the studied groups were 28.03 ± 6.99, 29.94 ± 9.27, and 32.92 ± 12.66 for groups I, II, and III, respectively, with no significant difference between them regarding the age and sex. Thirty-seven (71.2%) of group III patients had F1 stage of hepatic fibrosis, 5 (9.6%) had F2 stage, and 10 (19.2%) had F3 stage. Twenty-five (80.6%) of group II patients had steatosis from 5 to 32%, and 6 (12%) had steatosis from 33 to 66%. Forty (76.9%) of group III patients had steatosis from 5 to 32%, and 12 (23%) had steatosis 33–66%. No patients in both groups had steatosis >66%, with an insignificant difference between both groups regarding grade of steatosis.

There was significantly higher levels of total cholesterol and triglycerides among NAFLD patients without fibrosis (group II) when compared with controls (group I). Alkaline phosphatase was significantly higher in group II and group III patients when compared with controls (group I). Significantly higher levels of AST were found in patients with NAFLD and hepatic fibrosis

Table 1 Comparison between groups I, II, and III as regards laboratory investigations

Variables	Group			ANOVA	*P* value	Post hoc test
	I Mean ± SD	II Mean ± SD	III Mean ± SD			
Total cholesterol	161.00 ± 21.51	180.84 ± 27.62	171.75 ± 45.06	2.364	0.099	(1,2)*
TG	78.27 ± 31.91	112.10 ± 52.13	99.21 ± 53.80	3.794	0.026*	(1,2)*
HDL	97.13 ± 23.51	112.48 ± 25.64	107.85 ± 36.84	2.008	0.139	
LDL	48.10 ± 12.93	45.68 ± 13.23	45.37 ± 10.90	0.521	0.596	
FBS	86.10 ± 9.68	100.65 ± 29.88	103.25 ± 54.58	1.788	0.172	
2hPP	91.70 ± 19.45	118.32 ± 71.42	116.15 ± 65.47	2.035	0.136	
HbA1c	4.82 ± 0.52	5.31 ± 1.40	5.24 ± 1.27	1.689	0.190	
AST	20.57 ± 5.31	20.32 ± 4.67	26.83 ± 15.46	4.609	0.012*	(1,3) *, (2,3)*
ALT	22.27 ± 10.51	22.39 ± 11.41	24.17 ± 22.79	0.157	0.855	
Total bilirubin	0.60 ± 0.31	0.59 ± 0.26	0.55 ± 0.28	0.432	0.650	
Direct bilirubin	0.19 ± 0.10	0.18 ± 0.08	0.19 ± 0.15	0.008	0.992	
Albumin	4.44 ± 0.34	4.47 ± 0.47	4.43 ± 0.52	0.075	0.928	
Creatinine	0.74 ± 0.14	0.85 ± 0.18	0.83 ± 0.26	2.214	0.114	
Alkaline ph.	61.37 ± 21.36	76.42 ± 24.11	73.14 ± 23.64	3.654	0.029*	(1,2)*, (1,3)*
HB	14.23 ± 1.17	13.94 ± 1.48	14.00 ± 1.57	0.344	0.709	
TLC	5.82 ± 1.93	6.91 ± 1.87	6.88 ± 2.25	2.951	0.056	(1,2)*, (1,3)*
Platelets	224.57 ± 55.15	242.97 ± 56.28	232.65 ± 72.96	0.630	0.535	

*Statistically significant at $P < 0.05$.

2hPP, 2 hours postprandial blood sugar; Alkaline Ph., alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBS, fasting blood sugar; HB, hemoglobin; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides; TLC, total leucocytic count.

Table 2 Comparison between groups I, II, and III as regards FIB4, iron study, and grade of steatosis by liver biopsy

Variables	Group			ANOVA	*P* value	Post hoc test
	I Mean ± SD	II Mean ± SD	III Mean ± SD			
FIB4	0.60 ± 0.25	0.57 ± 0.21	1.14 ± 1.62	3.506	0.033*	(1,3)*, (2,3)*
Serum ferritin	51.95 ± 39.38	76.94 ± 57.41	114.55 ± 120.85	4.915	0.009**	(1,3)**
Serum iron	100.77 ± 30.15	115.19 ± 60.26	108.42 ± 34.77	0.887	0.415	
Steatosis % by liver biopsy	0.00 ± 0.00	15.5 ± 19.09	16.28 ± 22.1	2.493	0.087	(1,2)*,(1,3)*

*Statistically significant at $P < 0.05$.
**Highly statistically significant at $P < 0.01$.
FIB4, fibrosis 4 score.

(group III) when compared with patients with NAFLD without hepatic fibrosis (group II) and control (group I). Total leukocytic count was significantly higher in NAFLD groups in comparison with controls (Table 1).

Serum ferritin was significantly higher in group III in comparison with group I. No significant difference was found between the three groups regarding serum iron. Significantly higher scores of FIB4 were found among NAFLD patients with

Table 3 Correlation between serum ferritin and laboratory variables in groups I, II, and III

Variables	Serum ferritin in group II		Serum ferritin in group III	
	Pearson's correlation	Significance (two-tailed)	Pearson's correlation	Significance (two-tailed)
Age	0.112	0.548	0.437	0.001**
Weight	0.441	0.013*	0.525	0.000**
BMI	0.292	0.111	0.481	0.000**
Waist circumference	0.457	0.010*	0.501	0.000**
Hb	−0.147	0.431	−0.059	0.678
TLC	0.075	0.688	−0.061	0.666
ANC	0.212	0.252	−0.031	0.825
Lymphocytes	0.168	0.367	−0.126	0.373
Platelets	−0.449	0.011*	−0.543	0.000**
CRP	0.016	0.932	0.108	0.448
Total cholesterol	0.361	0.046*	0.194	0.167
TG	0.180	0.334	0.508	0.000**
LDL	0.320	0.079	0.177	0.210
HDL	−0.033	0.861	−0.282*	0.043*
FBS	0.425	0.017*	0.424	0.002**
2hPP	0.350	0.054	0.517	0.000**
HbA1C	0.444	0.012*	0.394	0.004**
AST	0.209	0.259	0.659	0.000**
ALT	0.522	0.003**	0.471	0.000**
Total bilirubin	0.191	0.303	0.466	0.000**
Direct bilirubin	0.156	0.402	0.639	0.000**
Albumin	0.122	0.514	−0.315	0.023*
TP	−0.035	0.851	−0.229	0.102
PT	0.000	0.999	0.277	0.046*
PTT	−0.171	0.358	0.362	0.008**
INR	−0.353	0.051	0.397	0.004**
Creatinine	0.133	0.475	0.216	0.124
FIB4	0.251	0.173	0.849	0.000**
Serum iron	−0.018	0.923	0.439	0.001**
Alk. Ph.	0.102	0.586	0.212	0.131
Steatosis	0.009	0.962	0.745	0.000**

*Statistically significant at $P < 0.05$.
**Highly statistically significant at $P < 0.01$.
2hPP, 2 hours postprandial blood sugar; ANC, absolute neutophilic count; Alk. Ph., alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, c-reactive protein; FBS, fasting blood sugar; FIB4, fibrosis 4 score; HB, hemoglobin; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; INR, international normalized ratio; LDL, low-density lipoprotein; PT, prothrombin time; PTT, partial thromboplastin time; TG, triglycerides; TP, total proteins; TLC, total leucocytic count.

fibrosis (group III) when compared with groups I and II (Table 2).

In NAFLD patients, there was a significantly positive correlation between serum ferritin and age, weight, waist circumference, BMI, blood sugar, total cholesterol, triglycerides, liver enzymes, serum bilirubin, PT, INR, partial thromboplastin time (PTT), serum iron, FIB4 score, and grade of hepatic steatosis; and a significantly negative correlation with platelet count, HDL, and serum albumin (Table 3).

ROC curve analysis revealed that the best cutoff value of serum ferritin to assess its diagnostic performance in the prediction of the occurrence of NAFLD was ≥39.5 ng/mL with a sensitivity of 74.2% and a specificity of 66.7% (Fig. 1), while the cutoff value of serum ferritin ≥51.95 ng/mL was the best in predicting the occurrence of fibrosis in NAFLD patients with a sensitivity of 65.4% and a specificity of 40% (Fig. 2).

Logistic regression analysis was conducted to find out the significant predictors for the occurrence of NAFLD and fibrosis in the studied patients. High BMI, total leucocytic count (TLC), and alkaline phosphatase were found to be independent predictors of the occurrence of NAFLD, while high TLC was the only independent predictor of the occurrence of fibrosis on top of NAFLD (Tables 4–5).

Discussion

Increased ferritin with normal transferrin saturation is frequently found in patients with hepatic steatosis. The elevated ferritin is thought to be due to the combination of disrupted glucose, lipid, and iron metabolism.[12]

Serum ferritin levels >1.5 over the upper limit level of normal range were associated with worsened NAFLD histological activity and were an independent predictor of advanced hepatic fibrosis among NAFLD patients.[13] Serum ferritin, but not serum iron, transferrin saturation, or hepatic iron concentration has been proposed to be higher in patients with severe (stages 3–4) than with mild (stages 1–2) fibrosis, but not steatosis or inflammation, and could independently predict severe fibrosis.[14]

In the present study, there was a significantly higher waist circumference and BMI among the NAFLD patients when compared with the controls. These findings match with the results of Hsiao *et al.*[15] who concluded that waist circumference is independently associated with fatty liver. BMI was found to be an independent predictor of occurrence of NAFLD in the current study. These results were in congruence with Loomis *et al.*[16] who found that the prevalence of NAFLD increases with increased BMI.

In the current study, patients with NAFLD and hepatic fibrosis had higher age, waist circumference, and BMI in comparison with the control. Similarly, Moon *et al.*[9] and Angulo *et al.*[17] stated that age and BMI were predictors of fibrotic severity in NASH. Also, Chandok *et al.*[18] stated that age in association with AST/ALT ratio predicted the stage of NAFLD-induced liver disease.

The current study showed significantly higher total leukocytic count among NAFLD patients with or without hepatic fibrosis when compared with the control group. TLC was found to be an independent predictor of the occurrence of NAFLD in the studied patients and the occurrence of fibrosis among NAFLD patients in the current study. This agrees with Kim HL

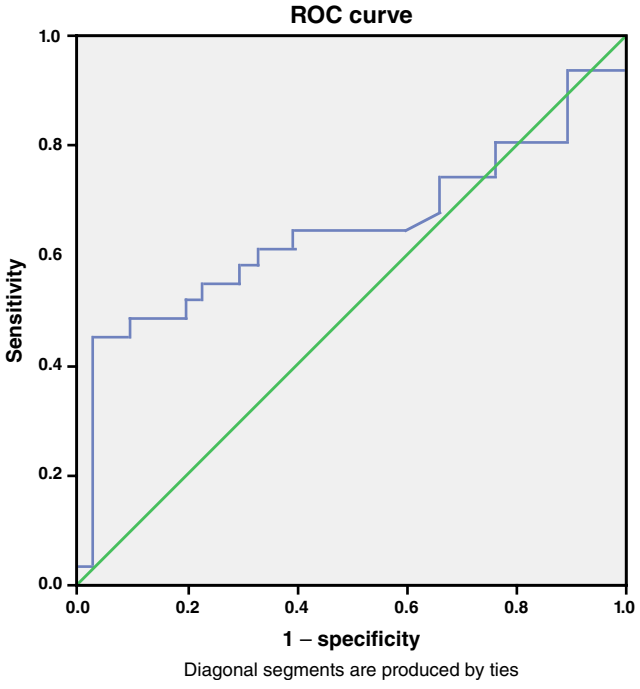

Figure 1 Receiver operating characteristic (ROC) curve displaying the diagnostic accuracy of serum ferritin to predict the occurrence of non-alcoholic fatty liver disease in the studied patients.

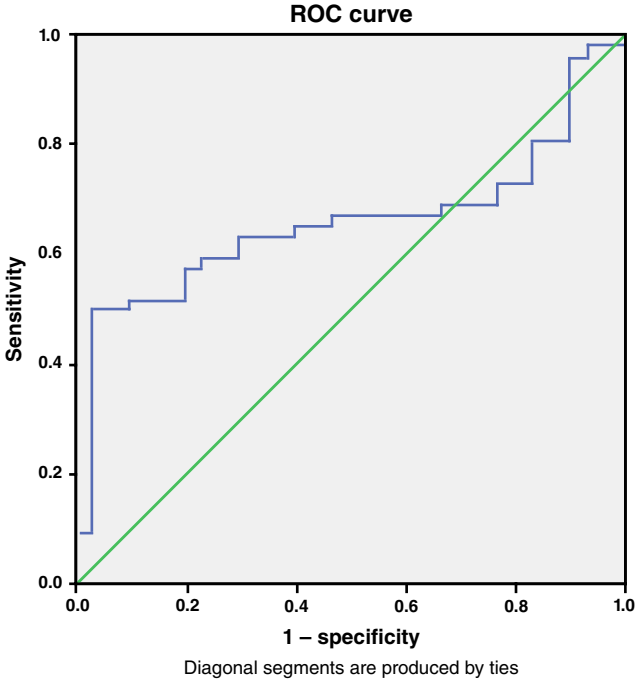

Figure 2 Receiver operating characteristic (ROC) curve displaying the diagnostic accuracy of serum ferritin to predict the occurrence of fibrosis in the studied patients.

Table 4 Logistic regression analysis displaying the independent predictors of the occurrence of non-alcoholic fatty liver disease in the studied patients

Variables	B	SE	Wald	Significance	Exp (*B*)
Weight	0.040	0.082	0.233	0.629	1.040
BMI	0.547	0.207	7.000	0.008**	1.728
Waist circumference	−0.004	0.089	0.003	0.960	0.996
TLC	0.506	0.222	5.185	0.023*	1.659
Total cholesterol	0.031	0.017	3.501	0.061	1.032
TG	0.016	0.011	2.172	0.141	1.016
Alk. ph	0.038	0.018	4.245	0.039*	1.039
Constant	−28.951	8.855	10.690	0.001	0.000

*Statistically significant at $P < 0.05$.

**Highly statistically significant at $P < 0.01$.

Alk. ph., alkaline phosphatase; BMI, body mass index; TG, triglycerides; TLC, total leucocytic count.

et al.[19] and Chung GE *et al*.[20] and could be explained by the state of systemic inflammation in NAFLD patients and endotoxin production from the fat stores. The accumulation of lipophilic persistent organic pollutants (POPs) in the adipose tissue leads to its slow release into the bloodstream. Thus, adipose tissue constitutes a continual source of internal exposure to POPs. Some POPs induce a proinflammatory state, which may lead to detrimental metabolic effects.[21] There are several explanations linking elevated total leukocyte counts to NAFLD and hepatic fibrosis on top. The insulin resistance and hyperinsulinemia serve as oxidative stress and thus stimulate inflammatory process, with the resultant activation of the transforming growth factor-beta pathway, dysregulation of multiple adipokines, apoptosis or activation of liver stellate cells, and hepatic inflammation.[22] Adipose tissue is an active endocrine organ with the capacity to synthesize and secrete various adipokines and cytokines. Adipose tissue imbalances caused by obesity increase the secretion of proinflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin-6 (IL-6), and reduce the secretion of anti-inflammatory factors, such as IL-10 and adiponectin.[23] Gut microbiome alterations play a pathological role in NAFLD through exposing the liver to high concentrations of harmful intestinal contents. Certain microbiome members, such as Escherichia, produce endogenous alcohol resulting in reactive oxygen species production and liver inflammation. In addition, microbial products, such as lipopolysaccharides, are potentially cytotoxic and induce inflammation.[24,25]

In the present study, there were higher levels of total cholesterol and triglycerides among NAFLD patients when compared with controls. This agrees with Kleeman *et al*.,[26] Lee *et al*.,[27] and Ma *et al*.,[28] who investigated the relation between NAFLD and hypercholesterolemia.

The significantly higher AST levels among NAFLD patients with hepatic fibrosis in the current study agree with the results of Mcpherson *et al*.[29], who stated that AST: ALT ratio was higher in hepatic fibrosis patients and has a negative predictive value in ruling out the presence of advanced fibrosis in NAFLD.

Alkaline phosphatase was significantly higher among NAFLD patients with or without hepatic fibrosis of the present study. Moreover, alkaline phosphatase was found to be an independent predictor of occurrence of NAFLD in the studied patients. These results agreed with those of Pantsari and Harrison.[30]

In this study, higher FIB4 scores were found among hepatic fibrosis patients in comparison with those without hepatic fibrosis and controls. Shah *et al*.[27] compared FIB4 with 7 other non-invasive markers of fibrosis in patients with NAFLD. FIB4 was found to be superior to NAFLD fibrosis score, Goteborg University Cirrhosis Index, AST: ALT ratio, AST: platelet ratio, body mass index, AST: ALT, diabetes (BARD) score, and cirrhosis discriminant score in the prediction of hepatic fibrosis among NAFLD patients.[31]

In this study, serum ferritin showed significant positive correlation with weight, waist circumference, total cholesterol, TG, fasting blood sugar (FBS), 2 hours postprandial blood sugar (2hPP), hemoglobin A1c (HbA1c), AST, ALT, serum iron, FIB4, and steatosis and significantly negatively correlated with platelets and albumin among NAFLD patients. Similarly, Zelber-

Table 5 Logistic regression analysis displaying the independent predictors of the occurrence of fibrosis in the studied patients

Variables	B	SE	Wald	Significance	Exp (*B*)
Age	0.028	0.037	0.548	0.459	1.028
BMI	0.089	0.120	0.544	0.461	1.093
Waist circumference	−0.005	0.033	0.022	0.881	0.995
TLC	0.286	0.139	4.258	0.039*	1.331
AST	0.058	0.039	2.235	0.135	1.060
Serum ferritin	0.006	0.006	0.947	0.330	1.006
Alk. ph	0.020	0.013	2.544	0.111	1.020
Constant	−6.906	3.085	5.012	0.025	0.001

Alk. ph., alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; TLC, total leucocytic count.

Sagi *et al.*[32] and Kowdley *et al.*[13] stated that elevated serum ferritin >1.5 × upper limit of normal (i.e., >300 ng/mL in women and >450 ng/mL in men) was significantly associated with elevated serum ALT, AST, iron, decreased platelets, and worsened histological activity, including steatosis, in patients with NAFLD complicated with fibrosis.

There was a positive correlation between serum ferritin and PT, PTT, and INR among NAFLD patients. These findings agreed with Na *et al.*[33] who stated that FIB4, AST, ALT, INR, cholesterol, and triglyceride showed a significant correlation with serum ferritin in NAFLD patients. However, Ghamarchehreh *et al.*[34] found no correlation between serum ferritin and PT in NAFLD patients.

The best cutoff value of serum ferritin was found to be 51.95 ng/mL, for predicting the occurrence of fibrosis on top of NAFLD in the current study, with a 65% sensitivity and a 60% specificity with AUC 0.658. Similarly, Parikh *et al.*[35] found that the cutoff value of 48 ng/mL was the best to predict fibrosis in NAFLD with AUC 0.779. On the other hand, Manousou *et al.*[8] found that the cutoff value of serum ferritin 240 ng/mL or more combined with BMI >28.2 identified patients at risk of developing fibrosis with an 82% sensitivity and a 79% specificity.

In the present study, serum ferritin was not one of the independent predictors of fibrosis in NAFLD patients. This agreed with the results of Moon *et al.*[9] However, Manousou *et al.*[8] and Fracanzani *et al.*[36] found that serum ferritin is an independent predictor of fibrosis in NAFLD patients. Similarly, Canbakan *et al.*[33] concluded that serum ferritin level has some prognostic significance in liver damage and fibrosis. Bugianesi *et al.*,[14] Kowdley *et al.*,[13] and Fracanzani *et al.*[36] found that ferritin level is an independent predictor of severe fibrosis.

Conclusion

Higher serum ferritin was found in patients with hepatic fibrosis on top of NAFLD. Serum ferritin was found to be a predictor of fibrosis on top of NAFLD with moderate sensitivity and specificity.

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