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Study of Serum Ferritin Levels in Patients with NAFLD and Its Relation to Male Gender and Smoking

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ABSTRACT

Background: The second-leading cause of cirrhosis and liver transplantation in the world is non-alcoholic fatty liver disease (NAFLD). NAFLD encompasses a wide spectrum of diseases, including simple steatosis, non-alcoholic steatohepatitis (NASH), cirrhosis, and its effects, including hepatocellular cancer and mortality. In individuals with NAFLD, serum ferritin levels have been proposed as a potential predictor of the frequency and severity of liver fibrosis. The aim of the current study is to evaluate serum levels of ferritin in NAFILD as predictor to disease severity and its relation to male gender and smoking.

Patients and methods: Ninety-nine adults enrolled in the study between January 2019 and May 2019. Patients had to have NAFLD, based on the NAFLD Liver Fat Score (NLFS Score) and the Hepatic Steatosis Index (HIS Score). Advanced fibrosis was defined as stages 3-4. Analyses were performed. Serum ferritin levels were assessed for each through venous sampling.

Results: On assessment of liver fibrosis by the FIB-4 index, there was 67 had F0 (67.7%), 27 had F1-2 (27.3%) and 5 had F3-4 (5.1%). there was a significant correlation between serum ferritin and disease severity. Male patients had higher significant values than females (p=0.003). Smoker patients showed highly significant values in comparison to non-smokers (p=0.014) serum ferritin where it was higher in males. No significant correlation was found between liver fibrosis and serum ferritin.

Conclusions: In NAFLD patients, higher serum ferritin was linked to male gender and smoking history. The findings suggest that NAFLD in a male smoker adult is more susceptible to liver disease development and should be treated aggressively.

Keywords: NAFLD, Serum ferritin, Male, Smoking, FIB-4 index, NLFS Score.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the second-leading cause of cirrhosis and liver transplantation worldwide. The global prevalence of NAFLD is rising as a result of the obesity pandemic [(1)]. NAFLD, on the other hand, is a multisystem disorder that raises the risk of diabetes, heart disease, and chronic kidney disease [(2)].

A fatty liver is defined as having fat accumulation in the liver that is larger than 5–10% of its weight and having triglycerides deposited inside the cytoplasm of hepatocytes [(3)].

Simple steatosis, non-alcoholic steatohepatitis (NASH), cirrhosis, and its consequences, such as hepatocellular cancer and mortality, are only a few of the several forms of liver illnesses that can affect people [(4)]. NAFLD is diagnosed by confirming hepatic steatosis with imaging modalities or a liver biopsy, as well as clinical exclusion of persons who use more than 20 grammes of ethanol on a regular basis [(5)].

Patients with asymptomatic and persistent aminotransferase increases, radiographic evidence of fatty liver, and unexplained persistent hepatomegaly should be strongly suspected of having NAFLD. The history and serological testing should rule out other diagnoses including viral hepatitis, hemochromatosis, autoimmune liver disease, alpha-1 antitrypsin deficiency, Wilson's disease, and drug-induced liver failure [(6)].

The association between insulin resistance and the development of NAFLD is widely known. Other factors that contribute to oxidative damage may play a role in the development of NASH [(7)].

Despite the fact that ferritin is an acute phase reactant, its presence in the blood of fatty liver disease patients may reflect more than just disease activity. The role of altered iron metabolism in the development of NAFLD and disease progression has piqued researchers' interest in various studies. In patients with NAFLD, serum ferritin levels have been suggested as a viable predictor of the existence and severity of liver fibrosis. Several studies, however, have revealed no consistent link between blood ferritin levels and the severity of NASH illness [(8)].

In the current study, we enrolled smokers and nonsmokers of different age groups with NAFLD and analyzed serum ferritin among them. The aim of this study is to evaluate serum levels of ferritin in NAFILD as predictor to disease severity and its relation to male gender and smoking.

PATIENTS AND METHODS

In this observational cross-sectional study a total of 99 patients with NAFLD were included. All patients were recruited from Minia University Hospital from January to June 2019.

Patients were included when they met the following criteria: diagnosis of NAFLD according to the NAFLD liver fat score (NLFS Score) and Hepatic steatosis index (HSI) and confirmation with ultrasonography [(9, 10)].

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Patients were excluded to participate if they matched with one or more of the following criteria: (1) Under eighteen years old. (2) Use of hepatic steatosis-inducing medicines (e.g., oestrogen, corticosteroids, methotrexate, or amiodarone) within 6 months of study entry. (3) Evidence of co-infection with hepatitis C, and or B. (4) Serum ferritin levels > 1000ng/ml. (5) Autoimmune liver disease. (6) Excessive alcohol intake or abuse, defined as consuming more than 10 grammes of alcohol per day. The Faculty of Medicine's Research and Ethics Committee gave its clearance in compliance with local research governance standards. Each participant in the study gave their informed consent.

NAFLD Diagnosis:

The NAFLD liver fat score enables to diagnose NAFLD using readily available clinical and laboratory data. The ideal cut-off value for NAFLD was determined at -0.640, indicating that NAFLD may be ruled out in patients who scored below it and NAFLD is likely to be diagnosed in people who scored above it **(9).** The hepatic steatosis index (HSI) is a quick and easy way to check for nonalcoholic fatty liver disease. NAFLD can be ruled out if the HSI value is less than 30 (with a negative likelihood ratio of up to 0.186). NAFLD positive diagnosis is quite likely if the HSI value is 36 or higher (with a positive likelihood ratio starting from 6.069) **(10).**

Patient Information Collection:

Patient information was gathered, including demographics, physical examination, and laboratory test findings. Age, sex, and smoking history were among the demographic factors examined.

The findings of the physical examination, including height and weight, were noted. The patient's blood pressure was also recorded. The results of laboratory tests, such as platelet (PLT), serum aspartate aminotransferase (AST), and serum alanine aminotransferase ALT, bilirubin, albumin, creatinine, full lipid profile, fasting insulin, fasting blood sugar, HbA1c, and serum ferritin, were gathered using standard automated methods. The BMI was computed by dividing the weight (kg) by the square of height (meters). Standing at the level of the umbilicus, the waist circumference (WC) was measured. The smoking index is calculated as follows: daily tobacco consumption smoking duration. Fasting blood glucose > 126 mg/dL is used to diagnose diabetes mellitus.

Assessment of liver fibrosis:

The relationship between serum ferritin levels and liver fibrosis was assessed using the Fiborsis-4 (FIB-4) score for all patients, the Fibrosis 4 score (FIB4) was calculated using the following formula: Age years AST (U/L)/platelet count (109=L) ALT (U/L). FIB4 1.45 indicates that hepatic fibrosis is not present, FIB4 > 1.45 and 3.25 indicates mild to moderate (F1–F2) hepatic fibrosis, and FIBl 4 > 3.25 indicates advanced fibrosis (F3–F4). Assessment of ferritin five mL of peripheral venous blood was obtained then centrifuged and then serum was kept under –70 ^{0}C till time of assessment human ferritin solid-phase ELISA kits supplied by Thermo Fisher Co.

Ethical consent:

An approval of the study was obtained from Minia University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical Analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test.

Qualitative data were represented as frequencies and relative percentages. Chi square test (χ^2) to calculate difference between two or more groups of qualitative variables. Continuous variables were expressed in terms of means and standard deviations, whereas categorical variables were expressed in terms of percentages. For non-parametric quantitative data between two groups, the Mann Whitney test was utilized. For non-parametric quantitative data comparing the three groups, the Kruskal Wallis test was applied. To identify a link between two quantitative, continuous variables, Pearson's correlation coefficient was applied. P value $\leq$0.05 was considered significant.

RESULTS

A total of 99 individuals with NAFLD were participated in the research, with 32 men (32.3%) and 67 females (67.6%), with ages ranging from 20 to 80 years with an average of 49.2±11.5. Twenty-one (21.2%) of the patients had a significant smoking history of more than ten pack-years. In this study, 15 (15.2%) of the participants were bdiabetics. Patients were classified as normal weight in 11 cases, overweight in 17 cases, and obese in 71 cases based on their BMIs. Table 1 summarizes these and other features of the 99 patients.

Table (1): Descriptive data of studied patients (No. 99)

Variable		N (%) or Mean ± SD
Age	Range Mean ± SD	(20-80) 49.2 ± 11.5
Gender	Male Female	32 (32.3%) 67 (67.7%)
Occupation	Housewife Worker Retired Deriver Nurse Employee Farmer Student	65 (65.7%) 7 (7.1%) 4 (4%) 4 (4%) 1 (1%) 1 (1%) 16 (16.2%) 1(1%)
DM	No Yes	84 (84.8%) 15 (15.2%)
HTN	No Yes	79 (79.8%) 20 (20.2%)
Smoking	No smoking Current Smoker X-Smoker	78 (78.8%) 15 (15.2%) 6 (6.1%)
SBP	Range Mean±SD	(90-180) 122.4 ± 15.5
DBP	Range Mean±SD	(50-110) 78.6 ± 10.3
Pulse	Range Mean±SD	(65-100) 82.6 ± 5.7
Weight	Range Mean±SD	(53-160) 83.6 ± 18.7
Height	Range Mean±SD	(145-183) 161.2 ± 8.3
BMI	Range Mean±SD	(19-52) 31.9 ± 6
Waist	Range Mean±SD	(70-170) 101 ± 16.3

DM: diabetes mellitus, HTN: hypertension, SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index.

Advanced liver fibrosis (fibrosis F3-4) was present in 5 (5.1%) subjects, while 27 (27.3%) subjects have (Fibrosis F1-2) and 67(67.7%) have (fibrosis F0) as shown in table 2.

Table (2): Liver fibrosis assessed by the FIB-4 index in patients with NAFLD

Scores		N=99
Fibrosis	No Yes	67 (67.7%) 32 (32.3%)
Fibrosis stage	F0 F1-2 F3-4	67 (67.7%) 27 (27.3%) 5 (5.1%)

The laboratory test results of NAFLD patients are presented in table 3.

Table (3): Laboratory Characteristics of Patients of NAFLD.

Laboratory data		N=99
Hb (g/dL)	Mean±SD	13.4 ± 1.7
TLC	Mean±SD	7.1 ± 1.4
PLT (mcL)	Mean±SD	278 ± 6.71
INR	Mean±SD	1.1 ± 0.1
Creatinine (mg/dl)	Mean±SD	0.9 ± 00.2
RBS (mg/dL)	Mean±SD	118 ± 7.31
Fasting glucose (mg/dl)	Mean±SD	93.9 ± 21.4
Fasting insulin (mIU/L)	Mean±SD	22 ± 5.1
Ferrtitn (µg/L)	Mean±SD	290 ± 73.41
Cholesterol (mg/dL)	Mean±SD	164.3 ± 35
HDL (mg/dL)	Mean±SD	41.3 ± 8.6
LDL (mg/dL)	Mean±SD	91.6 ± 5
TG (ng/mL)	Mean±SD	120 ± 26.41
ALT (U/L)	Mean±SD	18 ± 4.21
AST (U/L)	Mean±SD	23 ± 4.61
Albumin (g/L)	Mean±SD	4.5 ± 0.5
Total Bilirubin (µmol/L)	Mean±SD	0.4 ± 0.1
Serum HbA1C	Mean±SD	5.9 ± 1.12

As shown in table 4, male patients had high significant ferritin values when compared to female patients (p=0.003).

Table (4): Relation between ferritin level and Sex

Variable		N	Serum ferritin level (µg/L) Median/(IQR)	P value
Sex	Male	32	331.5 ± 80.32	**0.003***
	Female	67	255 ± 60.21	

As regarding the smoking, there was a statistically significance between ferritin and smoking with p=0.014 (**Table 5).**

Table (5): Relation between ferritin level and Smoking

Variable		N	Serum ferritin level Median/(IQR)	P value
Smoking	No	78	259 ± 58.91 [a]	**0.014***
	Smoker	15	326 ±73.31 [b]	
	Ex-Smoker	6	257.5 ± 55.81	

Kruskal Wallis test for non-parametric quantitative data between the Three groups followed by Mann Whitney test between the two groups.
Superscripts with different small letters indicate significance between each two groups.
***: Significant level at P value < 0.05**

There was no significant association between serum ferritin and clinical or laboratory data. Despite the fact that there was a negative correlation between ferritin and SBP, (r=-0.034, p=0.736) and DBP (r=-0.032, p=0.753). There was no statistically significant difference in the relation between liver fibrosis stage and serum ferritin. There was weak positive correlation between serum ferritin and smoking index (p=0.043 and r=0.1) **(Table 6).**

Table (6): Relation between ferritin level and Fibrosis stage.

Variable		N	Serum ferritin level Median/(IQR)	P value
Fibrosis stage	F0	67	290 ± 70.11	0.835
	F1-2	27	293 ± 68.54	
	F3-4	5	205 ± 50.12	

DISCUSSION

NAFLD (non-alcoholic fatty liver disease) is the world's most common chronic liver condition [(11)]. Recognizing those with the metabolic syndrome (MS) is critical for identifying people at risk of NAFLD. MS is diagnosed when three of the five clinical risk markers are present: low serum HDL cholesterol, impaired fasting serum glucose, a waist circumference more than the cut-off value (varies by gender and ethnicity), raised serum triglycerides, and high blood pressure (HTN) [(12)]. NAFLD is becoming more common as a hepatic manifestation of the metabolic syndrome as a result of the obesity pandemic [(1)].

Despite the fact that the pathogenesis of NAFLD/NASH is not fully understood, there is substantial evidence associating insulin resistance to the development of NAFLD. Other variables that contribute to oxidative damage may be implicated in the development of NASH [(7)].

Because serum ferritin is an acute-phase protein, its level rises in cases of liver necrosis and inflammation [(13)]. Serum ferritin levels can be used as an independent indicator to monitor the advancement of hepatic fibrosis in people with NAFLD because of their association with hepatic iron storage and hepatic inflammation, according to some studies. Researchers revealed that people with NAFLD have higher ferritin levels in their blood, that may be related to insulin resistance and hepatocyte damage [(14, 15)]. However, some empirical evidence shows that serum ferritin cannot predict the stage of NAFLD, and results from observational studies were inconsistent [(16)].

The aim of this study was to evaluate serum levels of ferritin in NAFILD as predictor to disease severity and its relation to male gender and smoking. This study was done on NAFLD patients who attended the Tropical Medicine Department outpatient clinic at Minia University Hospital in Minia, Egypt, between January and May of 2019.

Patients had to have NAFLD, based on the NAFLD Liver Fat Score (NLFS Score) and the Hepatic Steatosis Index (HIS Score). The gold standard test for the diagnosis of NAFLD, liver biopsy, was not used in this study because patients strongly oppose it and clinicians have little inclination to refer patients with a likely diagnosis of NAFLD/NASH for a liver biopsy. Referrals like these are common when other causes of liver disease have not been ruled out or when therapy is ineffective. Therefore, the NAFLD liver fat score and the hepatic steatosis index (HIS) were used as a predictive for presence or absence of NAFLD.

Ninety-nine NAFLD patients were included in the research. They were offered a thorough medical history, followed by a physical examination, laboratory tests, and an abdominal ultrasound.

Males had significantly higher serum ferritin levels than females in our study. We found no significant correlation between serum level of ferritin and fibrosis stage or hepatic steatosis score. Also, there was no correlation between serum ferritin levels and age, BMI, liver enzyme levels, fasting plasma sugar, fasting insulin, or serum lipid levels.

Our findings were consistent with those of **Modares *et al.* [(17)],** who found no correlation between serum ferritin levels and the histopathological grade or stage of the disease. Furthermore, serum ferritin levels did not correlate with age, BMI, liver enzyme levels, fasting plasma sugar, or serum lipids.

According to a retrospective study conducted by **Angulo and colleagues[(18)]** on over 1400 patients with NAFLD, serum ferritin levels alone lack overall accuracy in distinguishing between patients with NAFLD with and without liver fibrosis, as well as between patients with and without severe or advanced liver fibrosis. They reported that serum ferritin was ineffective in detecting and staging NAFLD. They also discovered that adding serum ferritin levels to a variety of non-invasive scoring systems did not significantly impact their overall accuracy in distinguishing between individuals with and without advanced fibrosis.

Furthermore, our findings were consistent with those of **Chandock *et al.* [(16)],** who discovered that while hyper ferritinemia is common in NAFLD patients, rising serum ferritin does not correspond with disease progression. Despite the lack of a statistically significant link, there was a non-significant trend toward higher serum ferritin levels as disease progressed.

In contrast, our findings contradicted a research by **Kowdley and colleagues [(19)],** who discovered an independent relationship between serum ferritin levels and the likelihood of developing fibrosis in NAFLD. They theorized that values more than 1.5 upper normal limits may indicate liver fibrosis in these patients with extreme obesity and metabolic syndrome. Serum ferritin levels and BMI were correlated to fibrosis, portal and lobular inflammation, and fibrosis was linked to BMI in individuals with biopsy-proven NAFLD.

After evaluating the data of 1200 individuals with biopsy-proven NAFLD from a large Japanese cohort database, **Yoneda and colleagues [(20)]** found comparable results. They found that serum ferritin levels were connected with steatosis grade and fibrosis stage independently, but that it was ineffective as a predictor of liver fibrosis because other factors such as sex and metabolic characteristics could have interfered. When used alone, serum ferritin exhibited a low diagnostic accuracy for identifying fibrosis in patients with NAFLD, according to their findings.

Our findings contradict those of **El Nakeeb *et al.* [(21)],** who found increased serum ferritin in patients with hepatic fibrosis in addition to NAFLD. Serum ferritin was revealed to be a moderately sensitive and specific predictor of fibrosis in addition to NAFLD.

We assumed that Serum ferritin level could be affected by genetic, environmental factors, and diet habituates differences between the studies population.

In our study median serum ferritin levels were elevated in smokers than nonsmokers which were statistically significant and there was weak positive correlation between serum ferritin levels and smoking index. It's been reported that smoking raises the concentration of iron in alveolar macrophages and bronchoalveolar lavage fluid to extremely high levels, disrupting iron homeostasis in the lungs and throughout the body [(22)].

This study has certain strengths and limitations that should be examined in more detail. One of the advantages is the possible design. All patients were also given a comprehensive examination to rule out any overlapping liver diseases that may affect serum ferritin levels and cause steatosis on their own. On the other hand, it has several limitations. For example, it only covered a limited number of patients. Second, there is no control group.

CONCLUSION

In NAFLD patients, higher serum ferritin was linked to male gender and smoking history. The findings suggest that NAFLD in a male smoker adult is more susceptible to liver disease development and should be treated aggressively.

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Author contribution: Authors contributed equally in the study.

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