Serum Ferritin Levels Do not Predict the Stage of Underlying Non-Alcoholic Fatty Liver Disease

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Abstract

Background & Aim: Hepatic endothelial cells release ferritin in response to increased oxidative stress and lipid peroxidation. The principal aim of this study was to determine if serum ferritin levels predict the underlying stage of non-alcoholic fatty liver disease (NAFLD). **Methods**: The clinical, biochemical, radiologic and histologic findings of consecutive adult NAFLD patients accessed at a tertiary care center over a 15-year period were analyzed. Those with concurrent liver diseases were excluded. Patients were stratified into three groups based on their histologic stage of disease: simple steatosis, non-alcoholic steatohepatitis (NASH) or cirrhosis. Analysis of Variance and Tukey-Kramer Multiple Comparison tests were used to assess the relationship between serum ferritin levels and stages of NAFLD. **Results**: 482 patients fulfilled inclusion criteria, including 60 subjects with biopsy proven simple steatosis, 28 subjects with steatohepatitis (NASH) and 20 subjects with histologic or radiologic evidence of cirrhosis. Mean serum ferritin levels were similar in all three groups (simple steatosis: 223.9 ug/L; NASH: 240.7 ug/L; cirrhosis: 271.3 ug/L; p=0.84). NAFLD-induced cirrhotic patients were significantly older, more often diabetic and hypertensive, and had more frequent evidence of splenomegaly and hepatic dysfunction. Following univariate and multivariate modeling, only AST/ALT ratio, diabetes, splenomegaly and age accurately predicted the stage of underlying NAFLD-induced liver disease. **Conclusion**: Hyperferritinemia is common in patients with NAFLD but the extent of serum ferritin elevations do not predict the stage of underlying NAFLD disease.

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Key words

Non-alcoholic fatty liver disease (NAFLD) – cirrhosis – ferritin – liver steatosis.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a growing global epidemic. In the United States alone, it afflicts an estimated 20-30% of the population, and is the most common cause of elevated liver enzymes in western societies [1, 2]. The prevalence of NAFLD overlaps with related medical epidemics of obesity, diabetes mellitus, and hypercholesterolemia [3]. Non-alcoholic fatty liver disease is the hepatic manifestation of the pervasive metabolic syndrome, and its clinical relevance is its association with cardiovascular mortality and, in a minority of patients, end-stage liver disease [4, 5].

Non-alcoholic fatty liver disease encompasses a broad spectrum of stages of disease severity; these stages include simple steatosis, steatohepatitis (NASH), and cirrhosis. Accurate staging of NAFLD is necessary to prognosticate patients and identify those who require aggressive treatment of their metabolic syndrome and follow-up for monitoring of disease progression. Natural history studies suggest that patients with simple steatosis have a minimal risk for the development of liver-related complications, but up to 1 in 4 patients with NASH are at risk for developing cirrhosis over time, and cirrhotic patients are also at risk for developing hepatocellular carcinoma at a rate of 2 to 4% per annum [5, 6].

Non-invasive methods for the objective assessment of disease severity in NAFLD are the focus of intense research. While ultrasound elastography is reliable in estimating disease stage in viral hepatitis, it has not been validated in NALFD, and there are legitimate concerns that adiposity reduces the accuracy of hepatic stiffness measurements [7, 8]. Similarly, magnetic resonance elastography, while purported to be an accurate tool in distinguishing simple steatosis from NASH [9], is ultimately impractical given that it is not widely available, and requires technical expertise

which may not be available outside of tertiary care settings. Laboratory tests such as the FibroTest, SteatoTest and NashTest hold promise as reliable predictors of fibrosis and disease stage in NAFLD [10, 11]. However, such testing is expensive, can only be measured at validated laboratories, and remain under patent, thus limiting their utility as a method for mass population screening.

In addition to reflecting tissue iron stores, serum ferritin levels have been implicated in the adaptive response of hepatic endothelial cells to the oxidative stress that occurs with NAFLD-associated insulin resistance and lipid peroxidation [12]. Given the lack of consensus in the literature on the utility of serum ferritin as a predictor of disease severity in NAFLD, the aim of this study was to determine the relationship between serum ferritin and the stage of fatty liver disease.

Methods

Patient Population and Data Collection

Prospective, consecutive adult patients ≥ 18 years of age seen in the Liver Diseases Clinic at the University of Manitoba from 1995 to 2010 with a diagnosis of NAFLD were assessed. The diagnosis of NAFLD was made by hepatologists on the basis of liver imaging or biopsy compatible with fatty liver; the clinical presence of ≥1 feature of metabolic syndrome (i.e. diabetes mellitus, hypercholesterolemia, hypertriglyceridemia, obesity, hypertension), with or without abnormal liver enzymes; and chronic alcohol consumption ≤ 20 g/day. All subjects had serum ferritin and fasting transferrin saturation levels drawn. Genetic testing for hereditary hemochromatosis was performed in subjects with transferrin saturation ≥45% and serum ferritin ≥200 µg/L in females or ≥ 300 µg/L in males. Subjects with documented hereditary hemochromatosis were excluded. In addition, all patients had complete blood counts, serum alanine (ALT) and aspartate (AST) aminotransferase, alkaline phosphatase, gamma-glutamyl transferase (GGT), albumin, bilirubin, international normalized ratio (INR) for prothombin time, metabolic panel (total cholesterol, triglycerides, fasting glucose) and alpha-feto protein (AFP) levels as well as radiologic imaging of the liver (ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI)) performed at baseline.

Patients were excluded from the analysis if they had evidence of a concurrent overlapping liver disease (e.g. viral hepatitis, drug-induced liver disease, hereditary hemochromatosis, etc.) or concomitant severe inflammatory or infectious diseases known to cause hyperferritinemia (e.g. severe rheumatoid arthritis). Exclusion criteria for the latter were a compatible clinical history and physical examination, and, where appropriate, elevation in acute phase reactants (C-reactive protein and erythrocyte sedimentation rate), and/or positive blood cultures. To exclude overlapping liver disease(s), the following tests were performed: fasting transferrin saturation, genetic testing for hereditary hemochromatosis (done when transferrin saturation >45% with serum ferritin >200 µg/L in females or >300 µg/L in males), alpha-1 antitrypsin phenotype and quantification, serum ceruloplasmin, immunoglobulins, autoantibodies (nuclear, mitochondrial and smooth muscle antibodies) and serologic testing for hepatitis B and C viruses. Alcoholic liver disease was excluded on the basis of a patient interview revealing a history of ≤ 20 g/day ethanol consumption using a standard conversion scale (1 liter of wine = 100 g ethanol; 1 L beer = 50 g ethanol; 1 standard shot glass (30 mL) of spirits = 15 g of ethanol). Serum ferritin was considered elevated using the criteria from the World Health Organization (>300 µg/L in men or >200 µg/L in women).

In the absence of liver biopsy, the diagnosis of cirrhosis was made on the basis of a nodular appearance of the liver on medical imaging (either liver ultrasound, CT or MRI) with enlargement of the spleen (>12 centimeters), and two or more of the following: esophageal varices, ascites or thrombocytopenia (platelet count < $150x10^9$ cells/mL) with or without evidence of synthetic dysfunction (INR > 1.1, bilirubin > 18 mmol/L and serum albumin < 36 g/L).

Liver biopsies were obtained at the discretion of the hepatologist following informed consent from the patient. Histologic evaluation of liver specimens were performed by pathologists specialized in liver disease at the University of Manitoba.

This study was reviewed and approved by the institutional review board at the University of Manitoba.

Statistical methods

Continuous variables were reported as means, standard deviation and/or 95% confidence intervals, and categorical variables were reported as frequencies and percentages. Patients were stratified into three groups based on their histologic severity of disease and radiologic findings: simple steatosis, NASH or cirrhosis. Chi-square test of association (or F-test when warranted) and Armitage test for trend in proportions were used to examine differences in demographic factors and clinical variables (such as presence or absence of certain conditions) and severity of NAFLD. To assess for quantitative differences between the groups, Analysis of Variance (ANOVA) and the Tukey-Kramer Multiple Comparison tests were performed. Univariate regression analysis was used to determine which factors were associated with the biopsy staging of NAFLD. The Bonferroni adjustment was used to correct type one error when performing ANOVA. Factors found to be significant in univariate analysis were included in a multivariate regression analysis. Selection of the best predictors was carried out by means of forward and backwards stepwise regression modeling. Statistical analysis was performed using SAS Version 9.1 (SAS Inc, Cary, N.C.). For all analyses, statistical significance was set at less than 5%.

Results

A total of 482 subjects fulfilled inclusion criteria. Baseline clinico-demographic characteristics are shown in Table I. There were 227 males and 255 females in the

Table I. Baseline clinico-demographic characteristics of patient population

Variable	NAFLD without cirrhosis or biopsy (N=374)		non-NASH (N=60)		NASH (N=28)		Cirrhosis (N=20)		P	Overall (N=482)	
	n	%	n	%	n	%	n	%		n	%
Demographics											
Age^	46.7	(13)	44.7	(11)	46.2	(12)	58.8	(9)	0.00027*	46.9	(3.1)
Females	206	55.1	23	38.3	15	53.6	11	44.0	0.12	255	52.9
Follow-up (yr)^	2.0	(2.8)	4.7	(3.1)	6.0	(3.4)	6.3	(4.1)	0.000000**	2.7	(3.1)
Metabolic syndrome and related features											
BMI^	33.3	(5.7)	33.6	(5.4)	36.6	(5.8)	37.0	(8.1)	0.06	33.8	(5.9)
Diabetes	50	13.4	9	15	9	32.1	15	75.0	0.000000	83	17.2
Hyperlipidemia	83	22.2	29	48.3	12	42.9	7	35.0	0.00006	131	27.2
Splenomegaly	9	2.4	3	5.0	3	10.7	11	55.0	0.000000	26	5.4
HCC	1	0.3	0	0	0	0.0	3	15.0		4	0.8
Death	1	0.3	0	0	0	0.0	1	5.0		2	0.4

^Mean (SD) values; *Cirrhosis vs. NAFLD without cirrhosis or biopsy, non-NASH and NASH each; **NAFLD without cirrhosis or biopsy vs. non-NASH, NASH, and cirrhosis each

cohort (male to female ratio of 0.9), with a mean age of 46.9 years at the time of initial assessment. The mean body mass index (BMI) was 33.8 (SD 5.9). The mean AST, ALT, alkaline phosphatase, total cholesterol, and ferritin were 57.6 U/L, 82.5 U/L, 116.6 U/L, 6.8 mmol/L, and 199.6 µg/L, respectively.

Of the 482 subjects, 93 (19.3%) underwent liver biopsy, and 15 (3%) had clinical and radiologic evidence of cirrhosis without the need for liver biopsy. Table II summarizes the histologic characteristics of the patients who underwent liver biopsy. Of the 93 subjects with liver biopsy, 60 subjects (64.5%) had simple steatosis, 28 subjects (30%) had NASH, and another 5 subjects (5.4%) had cirrhosis.

Baseline laboratory values are depicted in Table III. The mean transferrin saturation was 29.1% (SD 13.3%) in the subjects without cirrhosis or liver biopsy; 29.4% (SD 17.1%) in the subjects with simple steatosis; 31.3% (SD 10.4%) in the subjects with NASH; and 13.8% (SD 18.6%) in the subjects with cirrhosis. The mean ferritin was 223.9 (SD 204.6) µg/L in the simple steatosis group, 240.7 µg/L (SD 228.5) in the NASH group, and 271.3 µg/L (SD 536.8) in the group with cirrhosis (Fig. 1). Among the 293 subjects without liver biopsy, the mean ferritin was 189.2 (SD 220.7) µg/L. Overall, 26 subjects (5.4%) had serum ferritin ≥ 500 µg/L.

Table II. Histopathology

Variable		n (%)
Biopsy done	93 (19.3)	
Grade	0	42 (45.2)
	1	33 (35.5)
	2	10 (10.8)
	3	3 (3.2)
	4	0 (0.0)
	Not graded	5 (5.4)
Stage	0	56 (60.2)
	1	17 (18.3)
	2	9 (9.7)
	3	6 (6.5)
	4	5 (5.4)
Steatosis	1	50 (53.8)
	2	30 (32.3)
	3	13 (14.0)
Biopsy diagnosis		
Non-NASH steatosis		60 (64.5)
NASH		28 (30.1)
Cirrhosis		5 (5.4)

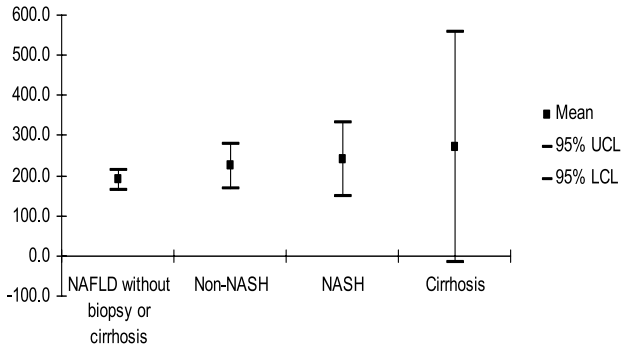

Fig 1. Ferritin values in various NAFLD groups.

As shown in Table IV, univariate regression analysis revealed that 15 parameters were significantly associated with the biopsy staging of NAFLD into the three predefinied patient groups (simple steatosis, NASH and cirrhosis). ALT/AST Ratio, diabetes and splenomegaly were the strongest single predictors of biopsy staging. Other significant clinical and demographic variables were weight, hypertension, imaging findings of hepatomegaly and/or splenomegaly, hemoglobin, AST, albumin, glucose, platelet count, and total bilirubin. In the multivariate analysis, only AST/ALT ratio,

Table III. Results of laboratory investigations of NAFLD patients

	NAFLD without cirrhosis or biopsy N=374			Non-NASH N=60			NASH N=28			Cirrhosis N=20			P
Variable	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	
Albumin	39.9	6	41	41.4	4.2	41	41.3	6	40	34.5	6.7	34.5	0.00007*
Alk Phosphatase	112.4	59.2	98	125.8	59.3	107.5	115.7	58.8	101	168.2	114	134	0.0009*
ALT	76.9	108.9	58	91.7	56.5	79.5	131.8	108	104.5	90.4	139	41.5	0.052
AST	53.7	83.9	38	47.9	28.5	40	68	45.3	54	148.5	295	48	0.0003*
Creatinine	74	22.7	75	77.8	18.5	77	84.7	48.9	76	80.1	19.2	77	0.18
Transferrin Saturation	29.1	13.3	27.7	29.4	17.1	27.9	31.3	10.4	31	13.8	18.6	20.8	0.52
Ferritin	189.2	220.7	141	224	204.6	161	240.7	228.5	151	271.4	134.2	112.5	0.34
GGT	115.3	168.5	62.5	155.4	163	95.5	147.1	262	74.5	139.8	121	113	0.32
INR	1.5	7.5	1	1.01	0.3	1	1.01	0.1	1	1.1	0.2	1.1	0.92
Platelets	248.8	66.7	244.5	263.5	90.1	250.5	238.9	64.3	243.5	172.1	73.4	171.5	0.00001*
Total Bilirubin	10.9	8.4	9	11.2	8.9	10	19.4	51.9	8.5	47.5	128.7	10	0.000004*
Total Cholesterol	7.2	18.8	5.55	5.4	1.4	5.75	5.6	1.3	5.54	5	1.4	4.9	0.88
Trglycerides	5.2	20.2	2.2	6.2	21.2	2.3	2.6	1.3	2.4	4.5	0.9	2.7	0.86

*Cirrhosis vs. NAFLD without cirrhosis or biopsy, non-NASH, and NASH each; *All groups different except non-NASH and NASH

platelet count, and diabetes were statistically significant in predicting the stage of the fatty liver disease on biopsy.

Discussion

There is conflicting evidence of the usefulness of serum ferritin as a prognosticator in observational studies of adults with fatty liver.

In a prospective study of 105 adult patients with NAFLD, Canbakan et al found that serum ferritin levels were higher in patients with NASH than in those with simple steatosis (p=0.016) [13]. In another prospective study of 50 NAFLD patients, Puljiz et al reported a statistically significant correlation between serum ferritin and NAFLD stage, with a mean serum ferritin of 309.4 ug/L in a group with NAFLD assessment score of ≥5, versus mean serum ferritin of 159.5 ug/L in subjects with NAFLD assessment score <5 (p=<0.001) [14]. As a proof of concept that iron stores correlate with disease severity of NAFLD, venesection among 198 Italian subjects with NAFLD resulted in a significant improvement in liver biochemistry and insulin resistance (as measured by Homeostatic Model Assessment) [15]. However, to date there have been no robust studies showing a histologic improvement in NAFLD following phlebotomy.

There is also evidence refuting the reliability of serum ferritin in predicting the stage of disease in NAFLD. In a study of 144 patients with NAFLD who underwent liver biopsy, Angulo et al found that serum ferritin, when controlled for age, obesity, diabetes and the AST/ALT ratio, did not correlate with the severity of NAFLD [16]. Likewise, in a study of 65 subjects with NAFLD in whom hepatic iron stores were studied, ferritin was not associated with pathologically advanced disease [17]. Finally, in a case-control study by Lonardo et al, no indices of iron metabolism were found to be independent predictors of NAFLD or severity of NAFLD [18].

Our study demonstrated that hyperferritinemia is common among patients with NAFLD, but increasing serum ferritin does not correlate with advancing stage of fatty liver disease. Despite the lack of a statistically significant correlation, there was a non-significant tendency toward increasing serum ferritin with progressive stage of disease. As such, it is conceivable that serum ferritin may be a useful metric in combination with other non-invasive markers to better prognosticate patients with NAFLD.

There is strong biological plausibility that serum ferritin may be a useful marker in assessing the stage of NAFLD. In the metabolic syndrome, insulin resistance leads to glucose intolerance, diabetes mellitus, low high-density lipoprotein, elevated uric acid, hypertension and atherosclerosis. Ultimately, lipid peroxidation promotes steatosis within the liver, and further oxidative stress and alteration in fatty acid metabolism culminates in steatohepatitis and fibrosis though activation of stellate cells. Through an adaptive response against the oxidative damage elicited by lipid peroxidation, endothelial cells in the liver up-regulate the anti-oxidant molecule heme-oxygenase-1, as well as ferritin [19]. Iron, released following the reaction between heme-oxygenase and porphyrin, generates substantial redox reactions, thus causing hepatic injury. However, through sequestration and ferroxidase activity, the destructive potential of free iron can be contained [12]. This latter homeostatic mechanism is effective in shielding the endothelium against the deleterious effects of heme and other oxidants. An inability to adapt to an iron-laden environment can culminate in extensive endothelial damage [12]. Heme-oxygenase-1 expression

Table IV. Results of Univariate and Multivariate modeling of predictors of NAFLD severity on biopsy

	Univariate regression			Multivariate regression		
Variable	Regression Coefficient	P	R2	Regression Coefficient	P	Model R2
ALT/AST Ratio	-0.6476	0.00000	0.27	-0.554	0.00000	
Diabetes Mellitus	0.7952	0.00000	0.22	0.483	0.0003	
Splenomegaly	0.9981	0.00000	0.22			
Age	0.0257	0.00002	0.16			
Albumin	-0.0523	0.00003	0.15			
Glucose	0.0845	0.0001	0.15			
Platelets	-0.0034	0.00006	0.14	-0.0025	0.0003	
Coarse liver	1.4095	0.002	0.09			
Hemoglobin	-0.0148	0.002	0.09			
Weight (kg)	0.0105	0.04	0.08			
AST	0.0015	0.01	0.07			
Hepatomegaly	0.3931	0.02	0.05			
Follow-up (yr)	0.0491	0.03	0.05			
Total Bilirubin	0.0026	0.03	0.04			
Hypertension	0.3690	0.04	0.04			
Multivariate model						**0.48**

correlates with ferritin, and given evidence that heme-oxygenase-1 parallels the severity of disease in NAFLD, one could speculate there would be a positive correlation between serum ferritin and the various stages of fatty liver disease. As we did not find such a correlation the intriguing possibility arises that those patients with more advanced stages of NAFLD (NASH and cirrhosis) have an intrinsic inability to up-regulate endothelial protective mechanisms and thereby are predisposed to develop more advanced disease. Clearly, further research is required to test this hypothesis.

There are several strengths and limitations associated with this study that warrant further discussion. The strengths include the prospective design and large sample size. Furthermore, all patients underwent a comprehensive evaluation to exclude overlapping liver diseases which may effect serum ferritin values and independently contribute to steatosis. Moreover, the regression analysis was adjusted for variables known to correlate with the severity of NAFLD, including liver transaminases, splenomegaly, presence of diabetes mellitus and age of the patient. On the other hand, there were several weaknesses, including a possible selection bias among patients seen at a tertiary referral center, and among patients who proceeded to liver biopsy. Another limitation was the relatively low number of patients with NASH or cirrhosis. Additionally, there were relatively few biopsies performed in this study (19.2% of the study population), although we maintain that biopsies are not necessary in all subjects with NAFLD, and most patients are not keen to undergo them. Finally, although patients were stable and without concurrent inflammatory diseases, serum ferritin levels were only recorded at baseline on one occasion and therefore may have differed somewhat from values at the time liver biopsies were performed.

The management of NAFLD is a major challenge in public health given that one quarter of western populations are afflicted with the disease. As such, non-invasive ways to assess disease severity that can be easily applied on a population level are urgently required. Specifically, methods to distinguish those patients with simple steatosis, the majority of whom will have no disease progression, from those patients with NASH and advanced fibrosis, will afford clinicians the ability to rapidly triage which patients require aggressive interventions and ongoing follow-up.

Conclusion

This study indicates that serum ferritin levels are increased in the majority of NAFLD patients but the extent of the increase does not predict the stage of the underlying liver disease. Further research is required to determine whether this lack of correlation reflects an intrinsic defect in NASH and NAFLD-induced cirrhosis patients to protect the liver from increased oxidative stress.

Conflicts of interest

No conflict to declare.

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