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HYPERFERRITINAEMIA IS A RISK FAKTOR FOR STEATOSIS IN CHRONIC LIVER DISEASE

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ABSTRACT

The aim of the present study was to investigate the relationship between ferritin and steatosis in patients with chronically abnormal liver function tests (LFTs) and high ferritin level.

One hundred and twenty-four consecutive patients with hyperferritinemia (male > 300 ng/mL, female > 200 ng/mL) were evaluated; clinical, biochemical and serological data, iron status parameters were obtained. Steatosis was graded by ultrasound as absent or present. Histology was available in 53 patients only.

Mean level of ferritin was 881 ± 77 ng/mL in men and 549 ± 82 ng/mL in women. The diagnosis was chronic hepatitis C in 53 (42.7%), non-alcoholic fatty liver disease/non-alcoholic steatohepatitis in 57 (45.9%), and alcoholic fatty liver disease in 14 (11.3%). Hepatic siderosis on liver biopsy was present in 17 of 54 (32%) patients; grade 1 in eight and grade 2 in nine. Overall, 92 patients (74.2%) had steatosis. By logistic regression, ferritin and γ-glutamyltransferase were independent predictors of steatosis. Ferritin levels were significantly related to low platelet count, steatosis and hepatitis C virus infection.

The obtained results show that in patients with chronically abnormal LFTs, high serum ferritin level is a risk factor for steatosis.

Key words: steatosis, ferritin, liver disease, hepatitis C, γ-glutamyltransferase

INTRODUCTION

There may be high serum ferritin levels in systemic inflammatory conditions and in renal, liver and neoplastic diseases(1, 2). Among patients with chronic liver disease, high serum ferritin, besides being a hallmark of hereditary hemochromatosis (HH), is frequently found in chronic hepatitis C, in alcoholic or non-alcoholic steatohepatitis (NASH), and in non-alcoholic fatty liver disease (NAFLD).

Patients with chronic hepatitis C virus (HCV) infection often have elevated serum iron indices, but these do not reflect accurately hepatic iron content, nor are they able to predict clinically important endpoints, such as progression of fibrosis and responsiveness to interferon-based regimen(3, 4). Studies attempting to link iron and the course of chronic hepatitis C have been inconclusive (5, 6, 7, 8). In chronic hepatitis C, steatosis is a common histological finding and occurs in 30%-70% of patients(9). The biological mechanism underlying steatosis in HCV infection is not definitively understood and is considered to be multifactorial with metabolic mechanisms, including insulin resistance (IR) and iron overload (10, 11). In fact, steatosis in patients infected by HCV genotype 1 is linked to a raised γ-glutamyltransferase (GGT) and to IR as a result of lipid peroxidation in the liver. The high prevalence of diabetes in subjects chronically infected with HCV has been ascribed to an increase in IR mediated by an increase in iron deposits (3, 12).

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In NAFLD, recent studies (8) have reported conflicting data on the role of iron in causing liver damage. Bonkovsky et al. (11) have shown that patients with NAFLD and iron overload have more severe liver disease, whereas other authors (5, 6, 7, 8, 9) did not observe any relationship between iron and clinical or pathological outcomes in patients with NAFLD.

We analyzed in a study patients with compensated chronic liver disease characterized by elevated serum ferritin levels, of varying etiology, to reassess the link between hiperferritinemia and other markers of the metabolic syndrome, mainly steatosis.

MATERIAL AND METHODS

Patients

We studied all patients consecutively referred to our Gastroenterology & Hepatology Unit, a tertiary referral center, between January 2004 and January 2009. Patients were included in the study if they had abnormal liver function tests and a high serum ferritin level.

Serum ferritin was considered raised according to the WHO criteria if > 300 ng/mL in men and > 200 ng/mL in women. Patients were excluded if they had transferrin saturation > 45%. Alcohol intake and drug use or abuse was evaluated through the administration of a questionnaire. Concomitant inflammatory diseases potentially capable of causing hyperferritinemia were ruled out on the basis of the absence of clinical signs or abnormal blood test results (erythrocyte sedimentation rate, rheumatoid factor, and C reactive protein).

All patients had liver ultrasound (US); liver biopsy was performed only when clinically appropriate and in patients who did not refuse. Steatosis on US was assessed as present or absent; when present, it was graded as mild, moderate or severe by two experienced ultrasonographers (always the same throughout the study period), who were unaware of the clinical and laboratory results. The presence of steatosis was determined in a qualitative manner according to standardized criteria.

Histological examination

Biopsies were evaluated for grade and stage according to Ishak and, on Perl's Prussian-blue-stained sections, for iron content. Stainable iron was scored as: grade 0, no detectable iron; grade 1, granules of iron visible at 400 × magnification; grade 2, discrete iron granules visible at 100 × magnification; grade 3, iron visible at 25 × magnification, and grade 4, masses of iron visible at 10 × magnification.

Statistics analysis

Continuous variables were summarized as mean ± SD and categorical variables as frequency and percentage. Multiple logistic and linear regression models were used to assess the relationship of steatosis, high ferritin and chronic liver disease. The dependent variable was steatosis on US, coded as 0 (absent) or 1 (present).

As candidate risk factors for steatosis, we selected age, sex, BMI, presence of cirrhosis, baseline alanine aminotransferase (ALT) /aspartate aminotransferase (AST), platelets, GGT, ferritin, serum iron, transferrin, transferrin saturation, glucose, bilirubin, and diabetes. Multiple logistic regression analysis was performed to identify independent predictors of steatosis. Multiple linear regression analysis was performed to identify independent predictors of ferritin levels as a continuous dependent variable. Variables found to be associated with the dependent variables on univariate logistic or linear regression at $P \leq 0.10$ were included in multivariate regression models. Regression analyses were performed using PROC LOGISTIC and PROC REG subroutines.

RESULTS

Features of the patients included in the study are shown in Table 1. The 124 patients (34 women and 90 men) had a mean age of 51.3 ± 1.3 years. The mean value of ferritin was 799 ± 75 ng/mL and that of serum iron was 126 ± 6.3 μg/dL.

HCV infection was detected in 53 patients (42.7%), 35 of whom (28.2%) had NAFLD without overt diabetes, 11 (8.8%) had NAFLD, and 11 had NASH at histology. Finally, 14 patients (11.3%) were classified as having AFLD.

Overall, 92 patients (74.2%) had steatosis on US: 46 moderate and 46 severe. The etiological pattern of the patients with steatosis was as follows: 35 (38%) subjects were infected with HCV, 35 (38%) had NAFLD, 11 (12%) were with NAFLD, and 11 (12%) had a diagnosis of NASH at histology.

Table 1. *Demographic, laboratory, ultrasound, etiological and histological features of patients..*

Variable

Demography	**(mean ± SD)**	**Laboratory values**	**(mean ± SD)**	**Etiology**	**n (%)**
Mean age (year)	51.3 ± 1.3	ALT-UNL	3.0 ± 1.0	Anti-HCV	53 (42.7)
Age (year)	**n (%)**	AST-UNL	2.0 ± 1.0	NAFLD	35 (28.2)
≤ 45	56 (45.2)	GGT-UNL	2.0 ± 0.3	ASH	11 (8.8)
> 45	68 (54.8)	Ferritin (ng/mL)	799.7 ± 75.6	NASH	11 (8.8)
Sex	**n (%)**	Serum iron (μg/dL)	126 ± 6.3	AFLD	14 (11.3)
Male	90 (72.5)	Platelet count × 10^3/cmm	186 ± 74.33	**Histology**	**n (%)**
Female	34 (27.5)	**Ultrasound**	**n (%)**	Chronic hepatitis C	27 (50)
BMI (kg/m^2)	**n (%)**	Steatosis present	92 (74.2)	AFLD	9 (16.6)
< 25	74 (59.6)	Steatosis absent	32 (25.8)	NAFLD	7 (12.9)
25-29.9	50 (40.3)			NASH	11 (20.3)

BMI - Body Mass Index; ALT - alanine aminotransferase; AST - aspartate aminotransferase; GGT - γ-glutamyltransferase; HCV - chronic hepatitis C virus;
NAFLD - non-alcoholic fatty liver disease; ASH - alcoholic steatohepatitis; NASH - non-alcoholic steatohepatitis; AFLD - alcoholic fatty liver disea

HCV infection was detected in 53 patients (42.7%). All these were infected by HCV genotype 1b; 36 (68%) had steatosis, nine were detected by US and 27 by liver biopsy.

At liver biopsy, performed in 54 patients out of 124 (43.5%), 27 (50%) had chronic hepatitis C and nine (16.6%) had micronodular cryptogenic cirrhosis. Seven patients (12.9%) had NAFLD (macrovesicular steatosis) and 11 (20.3%), NASH (macrovesicular steatosis and lobular inflammation). Seventeen patients (31.5%) had siderosis: eight, grade 1 and nine, grade 2.

Univariate and multivariate analyses were performed to identify predictors of steatosis. By univariate analysis age (P = 0.06), ferritin (P = 0.0006), GGT (P = 0.03) and anti-HCV positivity (P = 0.02) were associated with steatosis (P < 0.10) **(Table 2)**.

Table 2. *Univariate analysis of risk factors for absent/present liver steatosis in patients with high serum level ferritin.*

Variable	**Steatosis**		
	Absent (n = 32)	**Present (n = 92)**	**P value**
Age (year) (mean ± SD)	50.9 ± 3.1	54.2 ± 1.3	0.06
Sex n (%)	18 (56.2)	72 (78.2)	0.14
BMI (kg/m^2) (mean ± SD)	24.4 ± 3.2	25.2 ± 3.1	0.30
ALT-UNL (mean ± SD)	47.7 ± 7.7	117.5 ± 11.2	0.1
AST-UNL (mean ± SD)	35.7 ± 5.3	89 ± 10.8	0.3
GGT (mean ± SD)	93.1 ± 20.7	174.1 ± 19.7	0.03
Anti-HCV positivity n (%)	24 (75)	28 (30.4)	0.02
Ferritin (ng/mL) (mean ± SD)	464 ± 183	1060.8 ± 79	0.0006
Serum Iron (μg/dL) (mean ± SD)	96.3 ± 7.5	137 ± 7.7	0.8
Platelet count × 10^3/cmm (mean ± SD)	217.8 ± 16.1	176.9 ± 8.26	0.24

BMI – Body Mass Index ;ALT - alanine aminotransferase; AST - aspartate aminotransferase; GGT - γ-glutamyltransferase; HCV - chronic hepatitis C virus

By multivariate analysis, ferritin (OR: 1.002; 95% CI: 1.001-1.004), and GGT (OR: 1.007; 95% CI: 1.001-1.013) were the only independent predictors of steatosis **(Table 3)**.

Table 3 . *Predictors of steatosis in patients by logistic regression model.*

Predictor	**Univariate analysis**		**Multivariate analysis**	
	OR (95% CI)	**P value**	**OR (95% CI)**	**P value**
Age (year)	0.962 (0.923-1.002)	0.060	0.97 (0.94-1.14)	0.23
Sex	0.455 (0.160-1.297)	0.14	-	-
BMI (kg/m^2)	1.050 (0.96-1.14)	0.23	-	-
ALT-UNL	0.997 (0.993-1.001)	0.12	-	-
AST-UNL	0.998 (0.994-1.002)	0.36	-	-
GGT-UNL	1.007 (1.001-1.013)	0.030	1.007 (1.003-1.014)	0.0043
Anti-HCV positivity	0.274 (0.110-0.682)	0.005	0.40 (0.20-1.10)	0.08
Platelet count × 10^3/cmm	1.000 (1.000-1.000)	0.24	-	-
Ferritin (ng/mL)	1.002 (1.001-1.004)	0.0006	1.003 (1.002-1.004)	0.0009
Serum Iron (μg/dL)	0.999 (0.991-1.008)	0.84	-	-

To identify predictors of ferritin levels, univariate and multivariate linear regression analyses were performed. Univariate analysis showed that male sex, anti-HCV positivity, platelet count, AST, ALT, GGT level and steatosis were significantly associated with ferritin levels. The model for the independent predictors of ferritin levels as a continuous variable by multiple linear regression analysis **(Table 4)** included anti-HCV positivity (P = 0.0028), platelet count (P = 0.0161) and steatosis (P < 0.0001)

Table 4. *Multivariate analysis of risk factors for high serum ferritin levels in patients by linear regression model.*

Variable	**β**	**SE**	**P**
Male	93.183	183.13	0.612
ALT-UNL	-0.07174	1.09734	0.948
AST-UNL	1.06027	1.05841	0.319
GGT-UNL	0.92457	0.5746	0.111
Anti-HCV positivity	521.964	169.40	0.0028
Platelet count × 10^3/cmm	-0.00250	0.0010	0.0161
Steatosis	933.7287	180.437	< 0.0001

DISCUSSION

Hyperferritinemia is frequent in patients with chronic liver disease, whatever the etiology of the underlying damage. In patients with high serum ferritin levels, the cause of liver disease was chronic HCV infection in 42.7%, NAFLD/NASH in 45.9%, and AFLD in 11%. Steatosis on US was predicted independently by ferritin and GGT levels. High ferritin levels were associated with HCV infection and with more advanced liver disease, shown by low platelet counts.

Chronic hepatitis C, with or without cirrhosis, often presents with abnormal iron indices, particularly with raised levels of ferritin, which does not necessarily represents iron overload. Several mechanisms have been hypothesized to explain the altered iron indices and possible liver siderosis, including an excess of oxygen free radicals, increased fibrogenesis through activation of stellate cells and impairment of the host immune response (8). Among our 29 patients with chronic liver disease caused by HCV genotype 1b, in whom liver biopsy was performed, only 17 had siderosis (eight mild, nine moderate, none severe). Theoretically, serum ferritin could be elevated as an acute phase reaction linked to the necroinflammatory process of chronic hepatitis C, but the moderate increase in ALT and the degree of activity typically observed in these patients negates this interpretation, even if in our analysis chronic HCV infection was independently linked to higher ferritin levels at multivariate analysis. It is however difficult to

disentangle the role of HCV from that of steatosis, which is commonly associated with raised levels of ferritin, and is a common finding in HCV infection, even when caused by HCV genotype 1 (3, 4, 5, 6, 12).

In our study, HCV-infected patients also showed a moderate degree of steatosis. NAFLD is known to be by itself strongly associated with the metabolic syndrome, which may explain the strong relationship between HCV infection and diabetes.

In NAFLD, lipid peroxidation promotes transition from steatosis to steatohepatitis, which involves multiple cellular adaptations and evokes biomarkers of the oxidative stress that occurs when fatty acid metabolism is altered (3, 4, 12). The induction of heme-oxygenase 1 is an adaptive response against oxidative damage elicited by lipid peroxidation, and may be critical in the progression of the disease. The association we found between ferritin and moderate/severe steatosis supports the concept that serum ferritin is a risk factor for fatty liver. Further support for this hypothesis is lent by the data of Zelber-Sagi et al who demonstrated that NAFLD is the major determinant of increased serum ferritin levels at a population-based level.

An important finding of this work is the association we found between raised ferritin and reduction in platelet counts, a known marker of portal hypertension. We confirmed the observation by other authors who demonstrated that serum ferritin, but not iron stores, was a significant predictor of severe fibrosis in patients with NAFLD. All these data provide further evidence that hyperferritinemia might be another surrogate marker of advanced liver disease of any etiology.

According to recent reports, GGT is an independent predictor of liver steatosis. Our data indicate that patients with elevated GGT levels have the greatest likelihood of having moderate/severe steatosis. The administration of a questionnaire regarding alcohol intake and drug use or abuse makes us confident in excluding any role of these potential confounders on GGT levels. Lack of data on smoking, however, could affect the accuracy of the results. The association between GGT levels and steatosis is likely the result of the association between regional body fat distribution and fatty liver, irrespective of total body fat quantity, which is consistent with the assumption that GGT is a surrogate marker of central fat accumulation. Therefore, the GGT level may be a simple and reliable marker of visceral and hepatic fat and, by inference, of hepatic IR. Thus, patients with elevated serum ferritin and GGT levels are at risk of developing liver steatosis. Modelling the indication for US scanning on these predictors would maximize its cost effectiveness.

The main limitation of the current study, as well as of other cross-sectional studies, is that it is unable to distinguish the temporality of the associations between hyperferritinemia, steatosis and chronic hepatitis C. Lack of histological data in a proportion of subjects, particularly on intra-hepatic iron deposition, could also affect the interpretation of our findings. We are aware that the use of a more sensitive imaging technique such as magnetic resonance imaging could improve the rate of steatosis detection. In addition, we cannot exclude the possibility that denied alcohol abuse may be responsible for the observed prevalence of steatosis. A further methodological issue arises in the potential limitation of the generalizability of our results to new populations and settings.

In **conclusion**, this study shows that:

1. Steatosis and chronic HCV infection are the main causes of hyperferritinemia.
2. The finding of high ferritin levels represents a risk factor for steatosis and has clinical relevance, being associated with low platelet count.
3. Patients with chronic HCV infection often have elevated iron indices, but they do not reflect the iron content in the liver, nor are they able to provide important clinical endpoints such as progression of fibrosis and response to interferon therapy.
4. Hyperferritinemia can be used as markers of steatosis in non-obese and non-alcoholic patients.

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