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doi:10.3748/wjg.15.2132

World J Gastroenterol 2009 May 7; 15(17): 2132-2138
World Journal of Gastroenterology ISSN 1007-9327
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BRIEF ARTICLES

Hyperferritinemia is a risk factor for steatosis in chronic liver disease

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Received: July 6, 2008 Revised: April 3, 2009
Accepted: April 10, 2009
Published online: May 7, 2009

Abstract

AIM: To investigate the relationship between ferritin and steatosis in patients with chronically abnormal liver function tests (LFTs) and high ferritin level.

METHODS: 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, *HFE* gene mutations and homeostasis model assessment score were obtained. Steatosis was graded by ultrasound as absent or present. Histology was available in 53 patients only.

RESULTS: 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 cryptogenic liver damage in 14 (11.3%). None was diagnosed as hereditary hemochromatosis (HH). 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.

CONCLUSION: In a non-obese cohort of non-alcoholic patients with chronically abnormal LFTs without HH, high serum ferritin level is a risk factor for steatosis.

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Key words: Steatosis; Serum ferritin; Chronic liver disease; Hepatitis C; γ-glutamyltransferase

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Licata A, Nebbia ME, Cabibbo G, Lo Iacono G, Barbaria F, Brucato V, Alessi N, Porrovecchio S, Di Marco V, Craxì A, Cammà C. Hyperferritinemia is a risk factor for steatosis in chronic liver disease. *World J Gastroenterol* 2009; 15(17): 2132-2138 Available from: URL: http://www.wjgnet.com/1007-9327/15/2132.asp DOI: http://dx.doi.org/10.3748/wjg.15.2132

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).

A raised ferritin level, with an increased transferrin saturation and liver iron concentration, is a typical presentation of HH, an autosomal recessive disorder linked to *HFE* gene mutations[3], which account for most cases of HH in northern Europe and the USA[4]. Epidemiological studies in Mediterranean populations have shown that C282Y occurs only sporadically, while H63D is found among 13.5% of the general population[5]. In this area, *HFE* polymorphism seems to have a modest diagnostic relevance, since many cases of HH do not display the classic pattern of mutations[5]. It

has been suggested that *HFE* mutations may be involved in cases of liver disease complicated by iron overload and in patients with type 2 diabetes[6].

Patients with chronic hepatitis C virus (HCV) infection often have elevated serum iron indices[7], 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 regimens[8-10]. Studies attempting to link iron and the course of chronic hepatitis C have been inconclusive[11]. In chronic hepatitis C, steatosis is a common histological finding and occurs in 30%-70% of patients[12-13]. 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)[14] and iron overload[8,11,15]. 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[14]. 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[16,17].

In NAFLD, recent studies[18-20] have reported conflicting data on the role of iron in causing liver damage. George *et al*[21] and Bonkovsky *et al*[22] have shown that patients with NAFLD and iron overload have more severe liver disease, whereas Younossi *et al*[19] and Angulo *et al*[20] did not observe any relationship between iron and clinical or pathological outcomes in patients with NAFLD. Mendler *et al*[23] have reported that patients with NAFLD have no more iron overload than patients with isolated steatosis, and that the *HFE* genotype does not influence liver damage, although unexplained hepatic iron overload is nearly always associated with metabolic abnormalities.

We analyzed in a cross-sectional study a cohort of non-obese, non-alcoholic patients with compensated chronic liver disease characterized by elevated serum ferritin levels, of varying etiology, excluding HH, to reassess the link between hyperferritinemia and other markers of the metabolic syndrome, mainly steatosis.

MATERIALS AND METHODS

Patients

We studied all patients consecutively referred to our Gastroenterology & Hepatology Unit, a tertiary referral center, between January 2001 and January 2004. Patients were included in the study if they had abnormal liver function tests and a high serum ferritin level, and if their clinical workup conclusively excluded a final diagnosis of HH. HH was excluded by measurement of transferrin saturation following an overnight fast, according to American Association for the Study of Liver Diseases practice guidelines[24].

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 a history of alcohol abuse (alcohol consumption > 30 g/d in men and > 20 g/d in women), obesity [body mass index (BMI) $\geqslant$ 30], transferrin saturation > 45%, hepatitis B surface antigen positivity, autoimmune hepatitis, celiac disease, Wilson disease, α-1-antitripsin deficiency, porphyria cutanea tarda, or previous antiviral treatment in patients with chronic HCV infection. 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).

One hundred and twenty-four consecutive patients fitting the above criteria were selected from about 1800 subjects admitted for evaluation of abnormal LFTs to our unit (2001-2004). Clinical features, biochemical data, HCV and HBV status, histological features and iron status parameters were registered at baseline. All patients were genetically tested for *HFE* gene mutations. IR was determined by the homeostasis model assessment (HOMA) method using the following equation: insulin resistance (HOMA-IR) = fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5.

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[25].

HFE mutation analysis

HFE gene mutations were evaluated by a reverse hybridization assay (Nuclear Laser Diagnostics) that assessed 11 *HFE* gene mutations: V53M, V59M, H63D, H63H, SC65C, C282Y, Q127H, E168Q, E168X, W169X, Q283P on DNA from peripheral blood mononuclear cells. Extracted DNA fragments were amplified by PCR and PCR products were hybridized with allele-specific oligonucleotide probes, and the hybridized probes were read by a colorimetric reaction.

Histological examination

Biopsies were evaluated for grade and stage according to Ishak[26] 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

Table 1 Demographic, laboratory and histological features of 124 patients (mean ± SD)

Variable	
Mean age (yr)	53.3 ± 1.2
Age (yr), *n* (%)	
≤ 50	51 (41.2)
> 50	73 (58.8)
Sex, *n* (%)	
Male	90 (72.5)
Female	34 (27.5)
BMI (kg/m^2)	
< 25	74 (59.6)
25-29.9	50 (40.3)
ALT-UNL	3.0 ± 1.0
AST-UNL	2.0 ± 1.0
GGT-UNL	2.0 ± 0.3
Ferritin (ng/mL)	799.7 ± 75.6
Serum iron (μg/dL)	126 ± 6.3
Platelet count × 10^3/cmm	186 ± 74.33
HOMA score	3.48 ± 1.80
Steatosis	92 (74.2)
Etiology	
Anti-HCV	53 (42.7)
NAFLD	35 (28.2)
NAFLD/diabetes	11 (8.8)
NASH	11 (8.8)
Cryptogenic	14 (11.3)
Histology (54)	
Chronic hepatitis C	27 (50)
Cirrhosis cryptogenic	9 (16.6)
NAFLD	7 (12.9)
NASH	11 (20.3)
HFE mutations	53 (42.7)
H63D heterozygous	49 (39.5)
C282Y heterozygous	2 (1.6)
C282Y/H63D compound het	2 (1.6)

ULN: Upper limit of normal.

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 (SAS Institute, Inc., Cary, NC, USA)[23].

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 53.3 ± 1.2 years. The mean value of ferritin was 799 ± 75 ng/mL and that of serum iron was 126 ± 6.3 μg/dL.

Table 2 Univariate analysis of risk factors for absent/present liver steatosis in 124 patients with high serum level ferritin

Variable	Steatosis		*P* value
	Absent (*n* = 32)	Present (*n* = 92)	
Age (yr)	50.9 ± 3.1	54.2 ± 1.3	0.06
Sex	18 (56.2)	72 (78.2)	0.14
BMI (kg/m^2)	24.4 ± 3.2	25.2 ± 3.1	0.30
ALT-UNL	47.7 ± 7.7	117.5 ± 11.2	0.1
AST-UNL	35.7 ± 5.3	89 ± 10.8	0.3
GGT	93.1 ± 20.7	174.1 ± 19.7	0.03
Anti-HCV positivity	24 (75)	28 (30.4)	0.02
Ferritin (ng/mL)	464 ± 183	1060.8 ± 79	0.0006
Serum Iron (μg/dL)	96.3 ± 7.5	137 ± 7.7	0.8
Platelet count × 10^3/cmm	217.8 ± 16.1	176.9 ± 8.26	0.24
HOMA	3.0 ± 2.25	3.5 ± 2.8	0.23
HFE mutations	14 (43.7)	40 (43.4)	0.63
Diabetes	11 (34.3)	11 (12)	0.2

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

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 diabetic with NAFLD, and 11 (12%) had a diagnosis of NASH at histology.

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.

Among the 11 *HFE* gene mutations analyzed, only two (H63D and C282Y) were present in our population, while the remaining nine mutations were not found in any patient. H63D and C282Y mutations were distributed as follows: 53 patients tested (42.7%) carried at least one *HFE* gene mutation. These were distributed as follows: 49 (39.5%) patients were H63D heterozygous, two (1.6%) were C282Y heterozygous, and two (1.6%) were C282Y/H63D, compound heterozygous. None were ultimately diagnosed with HH on clinical and laboratory criteria.

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). 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). The baseline ferritin

Table 3 Predictors of steatosis in 124 patients by logistic regression model

Predictor	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age (yr)	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	-	-
HFE mutations	0.796 (0.313-2.020)	0.63	-	-
Diabetes	0.995 (0.986-1.004)	0.28	-	-

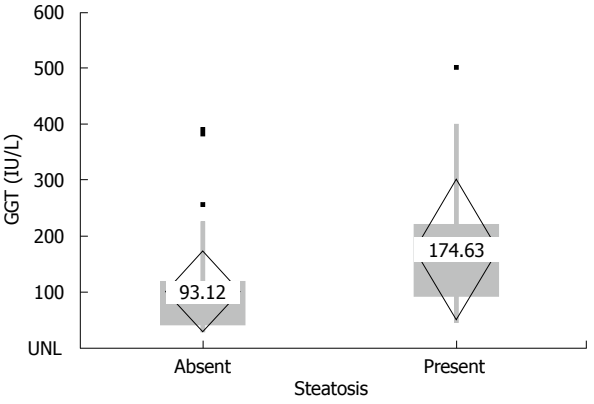

Figure 1 Baseline GGT levels according to steatosis in 124 non-obese, non-alcoholic patients without hereditary haemochromatosis (HH).

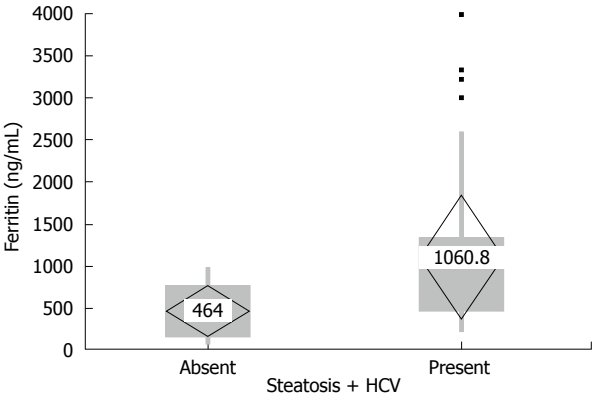

Figure 2 Baseline ferritin levels according to steatosis and HCV infection status in 124 non-obese, non-alcoholic patients without hereditary haemochromatosis (HH).

and GGT levels in patients with or without steatosis are shown in Figures 1 and 2.

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). Figure 2 outlines baseline ferritin levels according to HCV infection status.

Table 4 Multivariate analysis of risk factors for high serum ferritin levels in 124 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 this cohort of 124 non-obese, non-alcoholic patients with high serum ferritin levels, without HH, the cause of liver disease was chronic HCV infection in 42.7%, NAFLD/NASH in 45.9%, and untraceable 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.

In our study, no patients could be finally diagnosed with HH, although at least one of the characterized *HFE* gene mutations (C282Y and/or H63D, mostly the latter) was found in 50% of our patients in an heterozygote state. In fact, none of these carriers of *HFE* mutations had a transferrin saturation > 45%, liver siderosis beyond grade 1, or evidence of any other organ damage attributable to iron overload. It is noteworthy that an excess H63D allele frequency observed in our patients, as compared to the 12%-19% range observed in the normal population in our area[5,28], suggests that heterozygosity for this mutated allele may increase the appearance of high ferritin levels, once predisposing factors such as IR, steatosis and cirrhosis are operating.

Chronic hepatitis C, with or without cirrhosis, often presents with abnormal iron indices[29,30], 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[31-34]. 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[35,36], and is a common finding in HCV infection[37], even when caused by HCV genotype 1[14]. 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. The association between IR and moderate/severe steatosis in chronic hepatitis C is well supported[36-38]. In fact, IR could lead to the development of steatosis of the liver in HCV-infected patients[14], which makes them prone to the onset of 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. 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[39]. 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*[40] who demonstrated that NAFLD is the major determinant of increased serum ferritin levels at a population-based level. Moreover, they have shown that the association between serum ferritin and insulin is much more evident in the NAFLD group. Although recent studies have suggested that serum ferritin is a marker of IR[42-44], we could not provide evidence for a direct correlation between IR and elevated levels of serum ferritin. Consonant with Zelber-Sagi *et al*[40], we believe that the association found in previous studies between ferritin and IR may depend upon undiagnosed NAFLD.

Data from the third National Health and Nutrition Examination Survey (1988-1994) show a significant association between elevated serum ferritin and newly diagnosed diabetes mellitus[16]. We found that 17.7% of our patients had type 2 diabetes. In our study, however, ferritin levels were not significantly associated with IR, as evaluated by HOMA score, as well as by the presence of overt diabetes, probably as a result of the relatively small size of this sample, in which younger patients under evaluation for chronic hepatitis C predominated. Although a recent study has suggested that diabetes is the main factor accounting for the high ferritin level detected in chronic HCV infection[45], we could not provide evidence for a direct correlation between IR and hyperferritinemia.

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[46]. We confirmed the observation by Bugianesi *et al*[36] 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[14]. 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[47]. 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[48]. 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. Our study included a Mediterranean cohort of non-obese patients without HH, which limits the broad application of the results.

In conclusion, this study shows that in a non-obese cohort of non alcoholic patients, steatosis and chronic HCV infection are the main causes of hyperferritinemia. In Southern European populations, the finding of high

ferritin levels, after the exclusion of diagnosis of HH, represents a risk factor for steatosis and has clinical relevance, being associated with low platelet count.

ACKNOWLEDGMENTS

The authors thank Warren Blumberg for his forbearance in editing the manuscript.

COMMENTS

Background

Patients with chronic hepatitis C virus (HCV) infection often have elevated serum iron indices, but these do not accurately reflect hepatic iron content, nor are they able to predict clinically important endpoints, such as progression of fibrosis and responsiveness to interferon-based regimens.

Research frontiers

In this study, the authors showed that, in a non-obese cohort of non-alcoholic patients, steatosis and chronic HCV infection are the main causes of hyperferritinemia. In southern European populations, high ferritin levels, after exclusion of a diagnosis of hereditary hemchromatosis (HH), represent a risk factor for steatosis and have clinical relevance, being associated with low platelet count.

Innovations and breakthroughs

In a non-obese cohort of non-alcoholic patients with chronically abnormal liver function tests, without HH, serum ferritin high level is, therefore, a risk factor for steatosis.

Applications

Hyperferritinemia can be used as markers of steatosis in non-obese and non-alcoholic patients.

Peer review

The authors study the underlying liver disease in a cohort of individuals selected because they had both chronic liver disease as well as elevated serum ferritin levels. They found that most individuals had either chronic HCV or fatty liver disease. Additional analysis of clinicopathological data showed an association between ferritin and steatosis and GGT and steatosis. Overall the paper is well written.

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