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Reduced serum hepcidin levels in patients with chronic hepatitis C

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Abstract

Background/Aims—Patients with chronic hepatitis C (CHC) often have increased liver iron, a condition associated with reduced sustained response to antiviral therapy, more rapid progression to cirrhosis, and development of hepatocellular carcinoma. The hepatic hormone hepcidin is the major regulator of iron metabolism and inhibits iron absorption and recycling from erythrophagocytosis. Hepcidin decrease is a possible pathophysiological mechanism of iron overload in CHC, but studies in humans have been hampered so far by the lack of reliable quantitative assays for the 25-amino acid bioactive peptide in serum (s-hepcidin).

Methods—Using a recently validated immunoassay, we measured s-hepcidin levels in 81 untreated CHC patients and 57 controls with rigorous definition of normal iron status. All CHC patients underwent liver biopsy with histological iron score.

Results—S-hepcidin was significantly lower in CHC patients than in controls (geometric means with 95% confidence intervals: 33.7, 21.5–52.9 vs. 90.9, 76.1–108.4 ng/mL, respectively; $p < 0.001$). In CHC patients, s-hepcidin significantly correlated with serum ferritin and histological total iron score, but not with s-interleukin-6. After stratification for ferritin quartiles, s-hepcidin increased significantly across quartiles in both controls and CHC patients (chi for trend, $p < 0.001$). However, in CHC patients, s-hepcidin was significantly lower than in controls for each corresponding quartile (analysis of variance, $p < 0.001$).

Conclusions—These results, together with very recent studies in animal and cellular models, indicate that although hepcidin regulation by iron stores is maintained in CHC, the suppression of

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this hormone by hepatitis C virus is likely an important factor in liver iron accumulation in this condition.

Keywords

Chronic hepatitis C; Hemochromatosis; Hepcidin; Iron overload; Ferritin

1. Introduction

Persistent infection with hepatitis C virus (HCV) is a major cause of chronic liver disease, with an estimated 170 million infected people worldwide [1,2]. It is well established that about 20% of patients with chronic hepatitis C (CHC) will progress to cirrhosis within 20 years from infection, further resulting into an estimated annual risk of 3–7% of hepatocellular carcinoma (HCC) [3]. CHC patients frequently develop mild to moderate iron overload [4]. Many experimental and clinical studies [5–8], though not all [9,10], suggest that excessive iron in CHC is a cofactor promoting the progression of liver damage and increasing the risk of fibrosis, cirrhosis, and HCC. Hepatic iron concentration has been inversely associated with the response to antiviral therapy [11,12]. Iron removal by phlebotomy improves liver function tests [13, 14] and histology [15], increases the probability of sustained HCV eradication with antiviral therapy [16–18], and decreases HCC development in CHC patients [19]. Moreover, HCV transgenic mice that are overloaded with iron are prone to develop HCC [20]. Elucidating the mechanism(s) of iron accumulation in CHC may thus provide new tools for the management of CHC or for the prevention of its complications, or both. Many hypotheses have been advanced to explain the accumulation of iron in CHC, including local release of iron from necrotic hepatocytes, incidental carriage of hemochromatosis mutations, and HCV-induced perturbation of liver iron homeostasis, either directly or indirectly through immunologic and host response [21,22].

With the recent discovery of hepcidin [23,24], the liver has emerged as the central organ in the regulation of systemic iron homeostasis [25,26]. Hepcidin is a 25-amino acid peptide hormone primarily synthesized by hepatocytes, and it negatively controls two critical steps of iron homeostasis: duodenal absorption and the release from macrophages recycling iron through erythrophagocytosis [27]. At the molecular level, hepcidin binds to ferroportin, the membrane iron exporter highly expressed by enterocytes and macrophages [28]. This results in ferroportin internalization and degradation [28], and hence reduction of iron entry in the plasma compartment [29]. Hepcidin expression is modulated by iron stores, so that it decreases in iron deficiency to facilitate iron absorption while it increases in iron repletion to prevent pathological overload. Hepcidin expression is also induced by inflammation and suppressed by hypoxia and anemia [29–31]. Genetically determined hepcidin deficiency is now recognized as the key pathogenetic feature of iron overload in most types of hemochromatosis [32]. By analogy, disruption of hepcidin regulation has been postulated as a possible mechanism causing iron overload in acquired conditions, including alcoholic liver disease [33,34] and CHC [35]. Indeed, very recent studies in animal and cellular models have suggested that HCV infection may directly modulate hepcidin expression. In transgenic mice expressing the HCV polyprotein, hepcidin transcription was found to be downregulated through specific inhibition of the promoter by HCV-induced reactive oxygen species (ROS) [35]. Quite similar results were reported in hepatoma cell lines expressing HCV core and nonstructural proteins [36]. Until now, studies on hepcidin in human CHC patients have been hampered by the lack of reliable assays for the 25-amino acid bioactive peptide, especially in serum. To the best of our knowledge, only one study so far has investigated serum hepcidin in CHC, using a semiquantitative assay with a small number of controls [37]. In this study, we used a recently validated quantitative immunoassay for serum hepcidin [38] to evaluate correlations of hepcidin with disease status in CHC patients. The immunoassay is sufficiently discriminating

to reveal a gender difference in serum hepcidin concentrations of healthy subjects [38]; thus, we used sex-matched healthy controls with rigorous definition of normal iron status.

2. Materials and methods

2.1. Patients

The initial study population included 82 patients with CHC referred consecutively between 2006 and 2007 to the Gastroenterology Unit of the University of Verona and who fulfilled the following inclusion criteria: histologically proven CHC not previously treated with antiviral agents; Caucasian race; age between 18 and 65 years; absence of coexisting hepatitis B virus (HBV) or human immunodeficiency virus infections; absence of decompensated cirrhosis; absence of relevant concurrent medical conditions, including liver disease of etiologies other than HCV infection; absence of coexisting conditions that could influence the interpretation of iron parameters, such as chronic inflammatory diseases and haematological disorders, including porphyria and β-thalassemia trait; absence of known homozygosity for the C282Y mutation on the hemochromatosis (HFE) gene; absence of acute inflammatory disease, phlebotomy, or iron supplementation in the year preceding the liver biopsy; alcohol intake <25 g/day in the 6 months preceding liver biopsy; availability of the results of routine laboratory studies (complete blood count [CBC], transaminases, albumin) and serum samples (at least two distinct aliquots, 1 mL each) taken the same day of liver biopsy (or within the preceding or the following week), stored at −80°C, and never thawed previously. These serum samples were used to measure contemporaneously iron, transferrin, and ferritin (by routine laboratory methods), quantitative HCV RNA (RT-PCR COBAS Taqman Analyzer, Roche Molecular Diagnostic s.p.a, Milan, Italy), interleukin-6 (Human IL-6 ELISA BMS213/2CE Bender MedSystems GmbH, Vienna, Austria), and hepcidin (as described below). Liver biopsies were blindly reviewed by one of the authors (M.G.). A liver sample was considered adequate if it was longer than 1.5 cm and included more than seven portal tracts. A total of 79 out of 82 liver biopsies fulfilled these criteria. Activity and fibrosis were scored according to Ishak et al. [39]. Steatosis was recorded as absent, minimal (<5%), mild (5–33%), moderate (33–66%) and severe (>66%). For statistical analyses, three groups were considered: absent/minimal, mild, and moderate to severe. The histological quantification of hepatic iron in specimens stained with Perls' Prussian blue was carried out according to the Deugnier's Total Iron Score (TIS) [40]. This score has been used in both hemochromatosis and CHC patients [9,40]; it takes into account the relative scores attributed to hepatocytic, sinusoidal (Kupffer) cell, and portal tract iron deposits. Portal tract iron deposits were found almost exclusively in endothelial cells; therefore, they were grouped with Kupffer cells for analysis and reported as “mesenchymal” iron. Though TIS is a semiquantitative score as compared to the biochemical determination of hepatic iron concentration, the correlation between the two methods has been validated [9, 40,41], and TIS allows investigators to obtain information on the cellular and lobular distribution of iron.

2.2. Controls

Fifty-seven controls were enrolled from among healthy volunteers participating in a phase 2 trial at the Centre for Clinical Research of the Azienda Ospedaliera-Universitaria di Verona in Verona, Italy, as previously described in detail [38,42]. Briefly, at enrollment, the controls completed a questionnaire with specific items relevant to iron metabolism (e.g., any history of blood donations, previous pregnancies, menstrual losses) and were evaluated by laboratory studies, including CBC, serum iron, transferrin saturation (TS), ferritin, inflammatory markers (erythrocyte sedimentation rate [ESR], C-reactive protein [CRP]), liver function tests, and creatinine. To be considered as appropriate “normal controls” for the serum hepcidin assay, all these parameters were required to be normal. Although these controls were not specifically

selected only for this study, the gender distribution was not significantly different from that of the CHC patients (males 63% and 64%, respectively [$p = 0.8$]).

The study was approved by the Ethical Committees of the Azienda Ospedaliera-Universitaria of Verona, and all patients provided written informed consent.

2.3. Serum hepcidin

Hepcidin was measured in serum samples from all participants using a recently validated enzyme-linked immunosorbent assay (ELISA), as previously described [38]. In this paper, we also demonstrated the stability of hepcidin during storage at −80°C. In both CHC patients and controls, a blood sample was drawn between 7:30 and 8:30 a.m. after overnight fasting. Intra-assay precision coefficient of variation (CV) ranged from 5% to 19%, with higher variation at low hepcidin levels, while median CV for inter-assay reproducibility was 7% [38].

2.4. Statistical analyses

All calculations were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). As many of the continuous variables of interest, including serum hepcidin, iron, TS, ferritin, transaminases, and quantitative HCV RNA, showed a non-Gaussian distribution, their values were log-transformed and expressed as geometric means with 95% confidence intervals (CIs). Quantitative data were analysed using the Student's *t* test or by analysis of variance (ANOVA) with Tukey's post hoc comparison of the means, and with polynomial contrast for trend, when appropriate. Qualitative data were analyzed with the χ^2 test. Correlations between quantitative variables were assessed using Spearman's rho coefficient. Independent determinants of serum hepcidin levels were assessed, including all the variables significantly correlated with hepcidin at univariate analysis, as well as age, gender, and HFE status, in a stepwise multiple regression model estimating R^2 and standardized β-coefficients. To evaluate the different degree of correlation between hepcidin and ferritin in controls versus CHC patients, as well as in CHC patients with or without hepatic iron accumulation, data were analyzed in a general linear model by means of the F test for slopes. Two-sided p values < 0.05 were considered statistically significant.

3. Results

The main clinical and biochemical characteristics of CHC patients and controls are summarized in Table 1a, while liver histological features of CHC patients are reported in Table 1b. As expected, CHC patients had higher mean values of transaminases and serum iron than controls. However, mean serum hepcidin levels were significantly lower in CHC patients than in controls. To rule out a possible confounding factor, all CHC patients with available DNA samples (74 out of 82) were genotyped for HFE. One patient was found to be C282Y/C282Y homozygous (s-ferritin 711 μg/L, TS 100%) and was excluded from the study. We identified 1 C282Y/H63D compound heterozygote (1.4% of total genotyped), 3 H63D/H63D homozygotes (4.1%), 4 C282Y heterozygotes (5.5%), and 17 H63D heterozygotes (23.3%). The prevalence of HFE genotypes in tested patients was similar to that found in our geographic area [43,44]. TS, s-ferritin, and serum hepcidin levels were not significantly different across genotype categories (p by ANOVA = 0.28, 0.17, and 0.41, respectively; detailed data are given in Supplemental Table 1). Mean levels of TS, s-ferritin, and serum hepcidin in CHC patients for whom we were unable to do HFE genotyping were similar to those found in the CHC population genotyped (data not shown). Correlation analyses showed a strong positive association of hepcidin levels with serum ferritin (s-ferritin), both in controls and in CHC patients ($r = 0.741$, $p < 0.001$; and $r = 0.718$, $p < 0.001$; respectively). Hepcidin was lower in females than in males (geometric means in controls: 65.5 versus 109.9 ng/mL; in CHC: 10.6 versus 64.9 ng/mL; $p < 0.005$ for both), and correlated slightly with age in the controls (r =

0.294; $p = 0.03$) but not in CHC patients ($r = 0.186$, $p = 0.09$). At univariate analyses in CHC patients, hepcidin levels correlated positively also with serum iron ($r = 0.362$, $p = 0.001$), TS ($r = 0.394$, $p < 0.001$), hemoglobin ($r = 0.361$, $p = 0.001$), albumin ($r = 0.308$, $p = 0.006$), degree of steatosis ($r = 0.257$, $p = 0.02$), and particularly with TIS ($r = 0.488$, $p < 0.001$). Of note, TIS correlated significantly with s-ferritin (r = 0.545, $p < 0.001$). Similar significant correlations were found among hepcidin levels and either parenchymal (r = 0.428, $p < 0.001$) or mesenchymal (r = 0.423, $p < 0.001$) hepatic iron. Conversely, no significant correlation was found between hepcidin and viral loading, IL-6 levels, alcohol intake, serum alanine aminotransferase (ALT), histological grading, and staging. None of the variables significantly associated with hepcidin at univariate analyses remained statistically significant after adjustment for s-ferritin levels, except for TIS ($p = 0.03$). In a multivariate analysis including age, gender, HFE status, and all variables significantly associated with hepcidin at univariate analyses, only ferritin and positive TIS were independent predictors of serum hepcidin levels in CHC patients (Table 2). Overall, these data indicated that hepcidin was suppressed in CHC but was still regulated by iron. To further explore this hypothesis, we stratified the study population according to quartiles of ferritin (limits, calculated on the distribution of ferritin in controls: $1^{st} \leq 47.5$ μg/L; 2^{nd} 47.6–89.99 μg/L; 3^{rd} 90–149.5 μg/L; $4^{th} \geq 149.6$ mg/L). As shown in Fig. 1, both in controls and in CHC patients, mean hepcidin levels increased progressively with increasing ferritin quartiles (p value by ANOVA with polynomial contrast for linear trend <0.001 in both groups). Interestingly, the slope of hepcidin increase by ferritin levels was significantly different between controls and CHC patients (F = 9.699, $p = 0.002$, by F test for slope, adjusted for age and sex; see Supplemental Fig. 1). A similar trend (F = 3.6, $p = 0.059$) was observed for hepcidin increase by ferritin levels in CHC patients with hepatic iron accumulation compared to those without (see Supplemental Fig. 2), though formal statistical significance was not reached likely because of the relatively small number of patients in each group.

We also explored the relationship between viral loading and hepcidin levels after stratification for iron status. In this context, quantitative circulating HCV RNA correlated significantly and inversely with hepcidin in CHC patients with the lowest iron status, while this association gradually disappeared with increasing ferritin quartiles (Table 3).

4. Discussion

Measurement of hepcidin in biological fluids is a rapidly evolving field, with continuous efforts being made to overcome inherent technical difficulties (for an updated review on hepcidin assays, see Piperno et al. [45]). Indeed, such difficulties have hampered appropriate studies in human patients, including those with CHC. To the best of our knowledge, this is the first study that evaluated serum hepcidin levels in CHC by means of a validated quantitative assay [38]. Our results substantially confirm and refine those reported by Fujita et al. in Asian CHC patients, this study being the only other one on this topic published so far [37]. Indeed, Fujita et al. measured hepcidin using a first-generation surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) assay that was only semiquantitative, with data expressed in arbitrary units. Of note, in that study, hepcidin levels did not differ significantly from those in healthy controls, likely because of both methodological imprecision and the very low number of controls enrolled ($n = 10$). As a consequence, hepcidin downregulation could be indirectly documented only after normalization for ferritin values, by means of the so-called hepcidin:ferritin ratio [46]. In our study, we employed a second-generation immunoassay, capable of resolving gender differences in serum hepcidin in healthy subjects. Using gender-matched controls, we unequivocally demonstrated that CHC hepcidin concentrations are significantly lower than those of matched controls. Hepcidin downregulation is likely to contribute to liver iron accumulation in this condition. Of note, significant correlations of hepcidin levels with either s-ferritin or histologically proven hepatic

iron deposition highlighted that hepcidin regulation by iron burden is maintained in CHC. Nevertheless, stratification into ferritin quartiles clearly showed that hepcidin levels were much lower than expected in each CHC subgroup as compared to controls, suggesting that an as-yet undefined CHC-related factor opposes the effect of iron burden in these patients. Data shown in Supplemental Fig. 2 are also in agreement with the hypothesis that hepcidin upregulation was relatively deficient in patients who developed hepatic iron accumulation.

Although our study could not provide insights into the molecular mechanism(s) of hepcidin dysregulation in CHC, the results are in agreement with recent elegant studies in animal and cellular models that suggest a direct effect of HCV on liver hepcidin expression. Nishina et al. [35] studied transgenic mice expressing HVC polyprotein, which showed mild progressive hepatic iron accumulation. These mice had reduced hepcidin messenger RNA (mRNA) expression, which was attributed to HCV protein-induced ROS, with consequent upregulation of an inhibitor of the binding of the transcription factor CCAAT/enhancer-binding protein α (C/EBP-α) to the hepcidin promoter. Similar results were reported in hepatoma cells, where HCV-induced ROS were found to inhibit C/EBP-α through increased histone deacetylase activity [36]. A possible pitfall of these experimental models was that they could not take into account the effect of inflammation [47], which in CHC patients may counteract ROS-induced hepcidin suppression through the known hepcidin upregulation by proinflammatory cytokines, particularly IL-6 [48,49]. We therefore evaluated IL-6 levels in our CHC cohort. No significant relationship was found between serum IL-6 and hepcidin, a result in agreement with the lack of correlation between hepatic hepcidin mRNA expression and markers of CHC inflammatory activity observed by Aoki et al. [50]. While we cannot exclude a paracrine effect of local IL-6 release, current data argue that systemic inflammation does not prominently contribute to hepcidin regulation in CHC. According to the hypothesis of a direct suppressive effect of HCV on liver hepcidin expression, one could anticipate an inverse relationship between viral loading and circulating hepcidin levels. Such correlation was not observed in our CHC population when considered as a whole, in agreement with results from Fujita et al. using the semiquantitative hepcidin assay [37]. Nevertheless, when we analyzed data stratified for iron burden, we found a significant negative correlation between HCV RNA and serum hepcidin in CHC patients with the lowest iron burden, which gradually disappeared with increasing iron load. We speculate that hepcidin expression in CHC is determined by the opposing effects of hepcidin-suppressive viral factors and the hepcidin stimulation by iron load.

Theoretically, in the early phase of CHC, hepcidin may be prominently suppressed by HCV, but as iron accumulates, the negative influence of viral factors may be masked by the positive stimulation of iron. Because we had no reliable data on disease duration (difficult to obtain in clinical practice) on entry into this cross-sectional study, this hypothesis will require further exploration in studies with appropriate prospective design. However, very recent data suggesting liver iron and s-ferritin as surrogate markers of fibrosis [9] and, possibly, of disease duration [51] may indirectly support this view. Our study has other inherent limitations, including a relatively homogeneous cohort of CHC patients without overt advanced disease. Indeed, in advanced stages such as cirrhosis, hepcidin may be further decreased by impaired protein synthesis due to markedly reduced functional hepatic mass, contributing to additional, sometime massive [41,52] parenchymal iron overload. This is indirectly supported by preliminary data on hepatic hepcidin mRNA expression and semiquantitative urinary hepcidin in few cirrhotic patients of heterogeneous etiology undergoing liver transplantation [53]. Further studies are clearly needed to refine our knowledge on hepcidin regulation in the full clinical spectrum of CHC. Another limitation of this study may be represented by a lack of inclusion of patients with other chronic liver disease in the precirrhotic stage. Nevertheless, Fujita et al. recently demonstrated that liver hepcidin mRNA levels normalized to s-ferritin were significantly lower in CHC patients as compared to patients with chronic hepatic diseases caused by HBV, alcohol, and autoimmune conditions [54], again arguing in favor of a specific

effect of HCV on hepcidin modulation. In summary, our results, together with very recent studies in animal and cellular models, indicate that although hepcidin regulation by iron stores is maintained in CHC, the relative suppression of this hormone by HCV is likely an important factor in liver iron accumulation in this condition. Because of the potential negative effects of iron overload in CHC, in terms of either prognosis or response to available treatments, future treatments aimed at modulation [55] or supplementation of hepcidin may be beneficial in these patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CHC, chronic hepatitis C
HCV, hepatitis C virus
ANOVA, analysis of variance
CV, coefficient of variation
CIs, confidence intervals
CRP, C-reactive protein
ELISA, enzyme-linked immunosorbent assay
ESR, erythrocyte sedimentation rate
HFE, haemochromatosis
HBV, hepatitis B virus
HCC, hepatocellular carcinoma
mRNA, messenger RNA
ROS, reactive oxygen species
SELDI-TOF MS, surface-enhanced laser desorption/ionization time-of-flight mass spectrometry assay
TIS, Total iron score transferrin saturation

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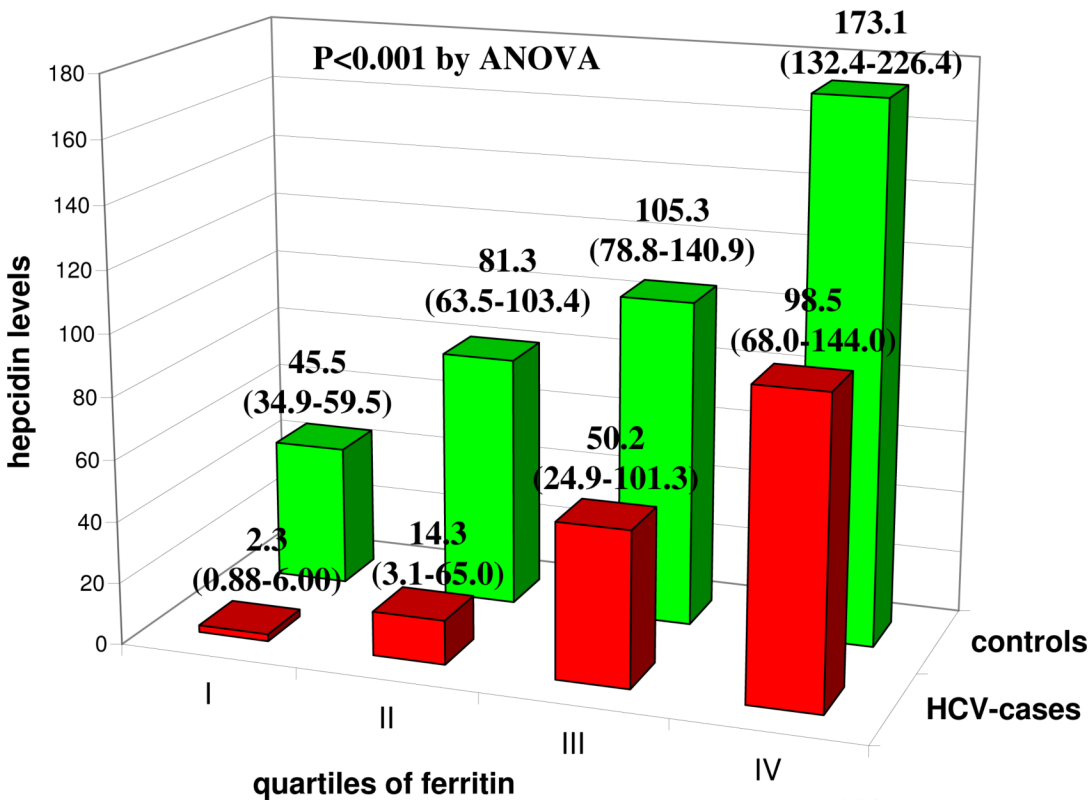

Figure 1.

Serum hepcidin levels in controls and in chronic hepatitis C (CHC) patients according to iron status (serum ferritin levels). Differences of mean hepcidin levels across ferritin quartiles in CHC patients ($P < 0.001$) and controls ($P < 0.001$). Differences of mean hepcidin levels between CHC patients and controls within each quartile (I: $P < 0.001$, II: $P = 0.006$, III: $P = 0.022$, IV: $P = 0.084$).

Table 1a

Clinical and biochemical characteristics of the study population.

	CONTROLS (N=57)	CHC (N=81)	P
Age (yr)[#]	35.0 (19.5–50.5)	42.2 (30.7–53.7)	0.002
Gender (M/F)	36/21	52/29	n.s.
Hemoglobin (g/dl)[#]	14.4 (13.2–15.6)	15.2 (13.7–16.7)	0.001
AST (IU/l)[*]	23.4 (22.1–24.8)	56.5 (49.8–64.1)	<0.001
ALT (IU/l)[*]	20.9 (18.8–23.1)	94.6 (81.2–110.3)	<0.001
S-Iron (μg/dl)[*]	96.4 (89.1–104.2)	118.6 (107.6–130.8)	0.002
S-Transferrin (g/l)[*]	2.5 (2.4–2.6)	2.8 (2.7–3.0)	<0.001
Transferrin Saturation (%)[*]	27.5 (25.4–29.9)	29.2 (25.9–32.9)	0.464
S-Ferritin (μg/1)[*]	85.3 (69.3–105.1)	122.4 (95.6–156.9)	0.037
S-Hepcidin (ng/ml)[*]	90.9 (76.1–108.4)	34.2 (21.6–53.9)	0.001
S-albumin (g/l)[#]	-	44.4 (43.7–45.2)	-
S-Interleukin 6 (pg/ml)	-	1.01 (0.66–1.33)[^]	-
S-HCV-RNA (IU/ml)[*]	-	5.94×10^5 ($4.13–8.53 \times 10^5$)	-
HCV-genotype (1/2/3/4)	-	42/11/24/4	-

[*] Geometric means with 95% CI

[#] Means with 95% CI

[^] reference range in normal subjects= 0.2–3.2 pg/ml

Table 1b

Histological findings in CHC patients.

Grading, mean (s.d.)	6.8 (6.3–7.4)
Staging, mean (s.d.)	2.6 (2.3–2.9)
Stage, *n* of patients (%)	
Stage 0	1 (1.3)
Stage I	12 (15.4)
Stage II	35 (44.9)
Stage III	14 (17.9)
Stage IV	5 (6.4)
Stage V	7 (9)
Stage VI	4 (5.1)
Steatosis	
***Steatosis, any degree, n* of patients (%)**	51 (65.4)
***Steatosis classes, n* of patients (%)**	
0	27 (34.6)
0–5	18 (23.1)
5–33	19 (24.4)
33–66	6 (7.7)
>66	8 (10.3)
Siderosis	
Positive iron stain (% of patients)	37 (47.4)
TIS (0–60), mean (s.d.)	4.2 (2.9–5.5)
***TIS score, n* of patients (%)**	
0	41 (52.6)
<7	14 (17.9)
7–15	21 (26.9)
>15	2 (2.6)

Histological staging and grading were assessed according to Ishak K et al[39]. Steatosis was classified as absent (0), minimal (<5%; grade I), mild (5–33%; grade II), moderate (33–66%; grade III) and severe (>66%; grade IV). Quantification of hepatic iron using liver samples stained with Perls' Prussian blue was carried out according to Total Iron Score proposed by Deugnier et al [40]. Adequate samples were available for 78/81.

Table 2

Predictors of serum hepcidin levels (dependent variable) in multiple linear regression analysis.*

Independent variables	Standardized β coefficient	SE	P
s-ferritin	0.74	0.15	<0.001
TIS positive	0.64	0.17	<0.001

adjusted R^2: 0.56, $P<0.001$

* variables included in the model were age ($P=0.3$), gender ($P=0.57$), HFE status (i.e. presence or absence of any HFE mutation, $P=0.91$), serum iron ($P=0.81$), transferrin saturation ($P=0.58$), hemoglobin ($P=0.47$), albumin ($P=0.17$), degree of steatosis ($P=0.4$).

Table 3

Correlation coefficients between serum hepcidin and viral loading (serum HCV-RNA levels) in CHC patients according to iron status (serum ferritin levels).

Quartiles of ferritin (μg/l)	r	P
1st (≤7.5)	**−0.526**	**0.036**
2nd (47.6–89.99)	−0.221	n.s.
3rd (90–149.5)	0.263	n.s.
4th (≥149.6)	0.017	n.s.