Prohepcidin Level Is Decreased In Patients with Chronic Viral C Hepatitis, and Has No Correlation with Disease Progression

Usama Khalil; Monkez Motae; Abdullah Abelaziz; Alsayed Ahmed Alnahal and Mahmoud Ashour

Internal Medicine Department, Faculty of Medicine.Zagazig University hospital
kamel_ashour2025@hotmail.com

Abstract: Background and Study Aims: iron is deemed to play a crucial role in the path physiology of liver damage in patients with chronic viral C hepatitis (CHC), Hepcidin has recently emerged as the key hormone in the regulation of iron balance and recycling. Because of technical difficulties we assessed plasma prohepcidm (hepcidin prohormone) levels in patients with CHC to investigate the association of this molecule with iron parameters (serum iron, serum ferritin, total iron binding capacity and transferrin saturation), disease activity as well as its relation to the development of hepatocellular carcinoma(HCC). **Patients and Methods**: We enrolled 45 patients with chronic hepatitis C, 10 of them were complicated by hepatocellular carcinoma, and 15 healthy controls. Plasma levels of prohepcidin were measured by enzyme-linked immunosorbent assays. serum iron parameters were assessed. Liver biopsies were taken for assessment of necro-inflammatory and fibrotic arranges according to Metaver Scoring System. **Results:** Mean prohepcidin levels were significantly lower in patients with chronic hepatitis C (CHC) and those with hepatocellular carcinoma (HCC) than in healthy comparison controls (P<0.05). In patients with CHC and those with HCC there was a significant increase in iron parameters compared to the controls (P <0.001).Also there was no significant correlation between serum prohepcidin or iron parameters and grade of inflammation or stage of fibrosis in chronic hepatitis C patients. No significant difference regarding serum prohepcidin and iron parameters was found between CHC patients with and without HCC. **Conclusion:** Significantly lower plasma prohepcidin levels and increased serum iron and ferritin were observed in patients with chronic hepatitis C with and without HCC compared to the controls, this may open the way for the use of hepcidin analogues in CHC patients to improve the response to antiviral therapy.
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1. Introduction

Chronic HCV infection is accompanied by so many changes in the liver, many of which are pathognomonc, that HCV is characterized not only as a virul infection but also as a HCV-induced metabolic syndrome. Liver iron overload is a well described metabolic abnormality of HCV infection though not completely understood [(1)]. Many experimental and clinical studies [(2)] though not all. Nabon *et al.*,[(3)] suggest that excessive iron in CHC is a cofactor promoting the progression of liver damage and increasing the risk of fibrosis, cirrhosis, and HCC. Many hypothesis have been advanced to explain the accumulation of iron in CHC, including local release of iron from necrotic hepatocyte, incidental carriage of hemochromatosis mutations, and HCV- induced perturbation of liver iron homeostasis, either directly or indirectly through immunologic and host response[(4)]. Iron removal by phlebotomy improves liver tests, histology[(5)] and increases the probability of sustained HCV eradication with antiviral therapy [(6)] and decreases HCC development in CHC patients-.In 2001, Park *et al*.,[(7)] discovered an iron regulatory hormone, hepcidin, synthesized predominantly in the liver, had changed the understanding of iron metabolism regulation. Hepcidin negatively controls two critical steps of iron homeostasis :duodenal absorption and the release from macrophages recycling iron through erythrophagocytosis [(8)].At the molecular level, hepcidin binds to ferroprotein, the membrane iron exporter highly expressed by enterocytes and macrophages, This results in ferroprotein internalization and degradation, and hence reduction of iron in the plasma compartment. Hepcidin expression is modulated by iron stores, so that it decreases in iron deficiency to facilitate HCV, hepatocellular carcinoma, iron overload, ferritin 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 [(9)]. By analogy, disruption of hepcidin regulation has been postulated as a possible mechanism causing iron overload in acquired conditions, including alcoholic liver disease and CHC[(10)]. In transgenic mice expressing the HCV polyprotein, hepcidin transcription was found to be down regulated through specific inhibition of the promoter by HCV-induced reactive oxygen species (ROS), Quite similar results were reported in hepatoma cell lines expressing HCV core and nonstructural proteins **[(11)]**.Until now, studies on hepcidin in human CHC patients have been hampered by the lack of reliable assays for the 25-ammo acid bioactive peptide,

especially in serum. One study has investigated serum hepcidin in CHC patients, using a semi quantitative assay with a small number of controls [12]

Prohepcidin, a hepcidin precursor protein predominantly synthesized in the liver undergoes two cleavages and is rapidly secreted from the hepatocytes as mature,25 amino acid peptide hepcidin. It has been shown that, to some extent, prohepcidin is also secreted by hepatocytes. The aim of this study was to assess the serum concentration of this hepcidin prohormone, prohepcidin, in CHC patients and evaluate its possible association with **the** disease progression and development of HCC.

2. Patients and Methods:

This cross-sectional study was carried out in HCV Treatment Center in AL- Ahrar General Hospital, Internal Medicine and Clinical Pathology Departments, Faculty of Medicine, Zagazig University, Sharkia Governorate, Egypt, from February 2009 to February 2010.

It included 60 subjects classified into groups:

Group 1, included 45 CHC patients who were classified into two subgroups Subgroup **la,** included 35 patients (32 males and 3 females) with a mean age ± Standard deviation of 40 ± 10.1 years- Subjects were recruited randomly from native patients attending the HCV treatment Center before starting antiviral therapy.

Subgroup Ib, included 10 male patients with a mean age of 54 ± 7.6 years With CHC complicated with HCC.

Group II (control group) included 15 healthy volunteers (13 males and 2Prohepcidin level is decreased in patients with chronic viral C hepatitis, and has no correlation with disease progression, all of them were judged to be in good health and with normal liver function tests.

All patients were selected according to HCV treatment protocol implemented by "Egyptian Committee for Control of Viral Hepatitis ". All participants gave written consents to share in the study, The following were the inclusion criteria for patients with CHC: age 18 years or older; WBC > 4000/mm^3; neutrophil count >2000/mm^3; platelets >100,000/mm^3; HB >12 gm for females and >13 gm for males; PT within 2 seconds above Upper Limit Normal (UNL); albumin >3.5 gm/dl; AST and ALT within 20 % above ULN; direct bilrubin within 20 % of UNL(0.4mg/dl); indirect bilrubin within 20% UNL(l mg/dl); absence of coexisting conditions that could influence the interpretation of iron parameters, such as chronic inflammatory disease, phlebotomy, hematological abnormalities or iron supplementation in the year preceding the study; serum creatinine within normal limit(WNL); HBsAg negative;ANA<1:160; positive ant-HCV and HCV PCR; Alfa fetoprotein < 100 ng / ml, patients with normal CT abdomen were selected.; Female patient practicing adequate contraception.The exclusion criteria were: any other causes of liver diseases other than HCV diagnosed by liver biopsy; decompensated liver disease; drug- related liver disease and pregnancy. Patients with HCC had high Alfa fetoprotein > 100 ng /ml with positive triphasic CT abdomen for hepatic focal lesion.

Methods:

All persons were subjected to the following: complete history taking; full clinical examination; laboratory investigations including: complete blood picture; liver function tests; blood urea; serum creatinine; thyroid function tests (TFT); ANA; Alfa fetoprotein;; HCV antibody using third generation EL1SA and HCV-RNA by Cobas TaqMan Real-time PCR from Roche with lower limit of detection 15 IU / ml,Bilharzial antibody, abdominal ultrasonography; triphasic abdominal CT. Serum iron, ferritin, total iron binding capacity (TIBC) and transferrin saturation (TSAT) were calculated., Serum prohepcidin levels were measured by enzyme-linked immunosorbent assay. The kit was supplied from DRG International, Inc., USA.Liver biopsies were taken for assessment of necroinflammatory and fibrotic changes according to Metaver Scoring System for grading of inflammatory Activity (A) and staging of fibrotic changes (F) [13].

Statistical analysis;

Values were expressed as mean ± standard Deviation around the mean (SD). The significance of differences was calculated using ANOFA tests. **Student** "t" test was used for comparing two means. Correlation between variables was done using correlation coefficient “r " test.*P* values of <0.05, and< 0.01 were considered significant and highly significant respectively.

3. Results:

The results of this study, as shown in table (1), revealed that serum prohepcidin values and TSAT were significantly lower in CHC patients compared to the healthy controls, while values of serum iron, serum ferritin were significantly higher in CHC patients than those in controls (*P* values were < 0.05 and 0.001 respectively), Table(2) revealed that there were no statistically significant difference between CHC patients with and without HCC regarding serum prohepcidin and iron parameters (*P* > 0.05). Also table (3) revealed that there were no significant correlation between serum prohepcidin and hepatic fibrosis or inflammatory activity according to Metaver Scoring System in CHC patients (*P*>0.05). There were no significant correlation between serum iron parameters and different inflammatory grades or stages of fibrosis among CHC patients as seen in table (4) & (5) respectively (*P* > 0.05).

Table (1): Comparison in the mean ±SD of prohepcidin, iron, ferritin and HB between CHC patients and controls

	Control(N=15)	CHC (n=45)	t	P
Serum Prohepcidin (ng/ml)	227.2 ±42.7	176.52± 89.1	2.11	0.036
Iron (ug/dl)	107.3 ±32.7	187.4 ±38.3	6.68	0.001
Ferritin (ng/ml)	183.3 ±88.5	428.9 ± 288.9	16.5	0.001
TIBC	328.4± 48.8	834.2± 195.9	9.51	0.001
TSAT	35.4± 7.8	22.9± 6.3	6.08	0.001

This table showed a significant decrease in serum prohepcidiru and TSAT and significant increase in serum iron, and ferritin and TIBC in CHC patients compared to control group.

Table (2): Least significance difference (L.S.D) to compare between serum prehepcidin and iron status parameters among CHC, HCC patients, and Control group of the study

		CHC	HCC
Serum Prohepcidin (ng/ml)	Control	<.01	<.05
	CHC	>.05(NS)	
Iron (ug/dl)	Control	<.001	<.05
	CHC	>.05(NS)	
Ferritin (ng/ml)	Control	<.001	<.001
	CHC	>.05(NS)	
TIBC	Control	<.001	<.001
	CHC	>.05(NS)	

Table (2) shows the serum prehepcidin statistically lower in CHC,HCC patients as compared to control group, but no statistical difrernce could be found between CHC and HCC patients.also iron,ferritin and TIbC, were statistically higher in CHC and HCC patients as compared to the control group, while no statistically significant difference between CHC and HCC patients as regards to iron status parameters**.**

Table (3): Correlation coefficient between serum prohepcidin and "fibrosis and inflammatory activity" using Metaver Scoring System among CHC patients.

	r	P
A (inflammatory activity)	**0.27**	>0.05(NS)
F (fibrosis)	0.14	>0.05(NS)

This table showed no significant correlation between serum prohepcidin level and either grade of inflammation or stage of fibrosis in CHC patients.

Table (4): Comparison in the mean±SD of serum iron indices in different inflammatory grades in CHC patients.

	Al (no =15)	A2 (no = 9)	A3 (no =11)	P
Iron (ug/dl) (Range)	174,4±418 (115.2-231,16)	189,8±29,3 (147.36 -240,72)	196.6±42 (140,5-269.24)	0.61
TIBC(ug/d[ ) (Range)	805.7±178 7 (501,1-115,7)	800,8±96,1 (675.7-965.4)	900,2 ± 226 (493.2 - 1384.7)	0.34
Ferrit!n(ug/dl) (Range)	475.9±292.1 (150-1)39)	388.6±287 (65,5 - 888)	529.7±302.5 (114.3-1037)	0.55

This table showed no significant difference of serum iron parameters in different inflammatory grades of CHC patients.

Table(5): Comparison in the mean ± SD of iron parameters in different stages of fibrosis in CHC patients.

	F1 (n=13)	F2 (n=12)	F3 (n=6)	F4 (n=4)	P
Iron (ug/dl) (range)	166,4±39,7 (115.2-223.2)	195.2±33.7 (144.19-250.5)	186.6±42.7 (140.5- 269.2)	218.9±31.4 (189.7-248.2)	0.24
TIBC(ug/dl) (range)	795.7±184.8) (501.1-1157)	798.9±147.8 (493,2-1051)	966.6±2I3 (715-1348.2)	761.4±8.7 (755,3-767,6)	0.11
Ferritin(ug/dl) (range)	488.9±324 150-1139)	386.3±248 (65.5-888)	610,8±309.6 (114.3-1037)	401±352.1 (152-650)	0.34

This table showed no significant difference between various stages of fibrosis regarding iron parameters in CHC patients.

4. Discussion:

Measurement of hepcidin in biological fluids is a rapidly evolving field, with continuous efforts being made to overcome inherent / technical difficulties. Such difficulties have hampered appropriate studies in human patients, including those with CHC. Because of this we planned to measure hepcidin prohormone, prohepcidin (60 amino acid), instead of hepcidin. Both groups of our study (CHC patients and healthy controls) were matched for age,sex and BMI. The results of our study revealed significantly decreased serum prohepcidin values in CHC patients compared to thecontrols (p <0.05) and this in agreement with Fujita *et al.* [14]; Olmez *et al.* [15]. but in contrast to the finding of Lin *et al.* [16] who reported significantly increased serum prohepcidin values in CHC patients compared to the control subjects. To explain the differences between our results and those of other investigators regarding serum prohepcidin and its relation to hepatic pathological changes we can, theoretically, say that 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 while, in advanced stages such as cirrhosis, hepcidin may be further decreased by impaired protein synthesis due to markedly reduced functional hepatic mass. Because we had no reliable data on disease duration on entry into this cross-sectional study, though we selected our patients with near normal liver function tests, this hypothesis will require further exploration in studies with appropriate prospective design. Another factor which may contribute to this discrepancy between the current study and others is that the assessment of serum prohepcidin does not totally reflect the level of biologically active mature hepidin. In the mean time, we did not find significant association between serum prohepcidin and the stage of liver fibrosis or grade of necro-inflammation in agreement with Lin *et al.,* [16] who said that expression of serum prohepcidin is independent of the degree of hepatic inflammation as measured by histopathological activity but this in contrast to the findings of Tsochatzis *et al.,* [17]. who Identified significant correlation between serum hepcidin and both hepatic necro-inflammation and fibrosis,possibly due to different HCV genotypes.

Also we found Significantly increased levels of serum iron and ferritin in our CHC patients compared to the controls confirming the results of Fujita *et al.* [18] and Domenico Girelli *et al.* [19], though we found no definite association between iron indices and hepatic pathological changes. Recent evidence showed that the proteolytic cleavage of prohepcidin to hepcidin is regulated by the hepatic prohormone convertase furin [20]. Of interest, the inhibition of furin activity prevented the conversion of prohepcidin to hepcidin. In situations of liver function impairment the prohepcidin synthesis as ‘well as activity or expression converting enzymes might be altered and affect prohepcidin concentrations. This finding could also suggest that HCV interference with hepcidin synthesis may occur at the step of prohormone synthesis or maturation in the liver- Our study revealed that,regarding serum prohepcidin and iron indices, there were no significant change between CHC patients without and with HCC.

Also there was no significant correlation between serum iron indices and hepatic necro-inflammation or fibrosis in contrast to the finding of Lin *et al.,* [16] that iron overload in the liver causes many changes including induction of reactive oxygen species (ROS) and oxidative stress, damage to lysosomes and mitochondria, stimulation of hepatocyte proliferation and expansion of portal tracts by fibrosis. This might be attributed to the fact that they measured liver iron not serum iron or they were in advanced stage of hepatic affection than our patients.

From this study we concluded that patients with HCV infection had decreased serum prohepcidin level, increased serum iron and serum ferritin compared to healthy population.Also there was no significant correlation between serum prohepcidin, serum iron indices and hepatic inflammatory activity, fibrosis staging or development of HCC in CHC patients. More studies including large number of CHC patients with and without HCC with measuring liver iron level to clarify the role of iron toxicity and pathogenesis of chronic hepatitis C and development of hepatocellular carcinoma. In addition, we recommend further work to evaluate the efficacy of hepcidin analogues in reducing iron overload in these patients and its impact on CHC disease progression and response to antiviral therapy.

Correspondence author
Mahmoud Ashour
Internal Medicine Department, Faculty of Medicine.Zagazig University hospital
kamel_ashour2025@hotmail.com

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