

# The relationship between anemia, serum hepcidin levels, and chronic hepatitis C in chronic hemodialysis patients

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## Background

Hepcidin is a small peptide that is produced by hepatocytes and circulates in the plasma. It plays a central role in regulating the iron status in the body.

## Aim

The aim of this study was to measure serum hepcidin levels in maintenance hemodialysis (MHD) patients and identify a possible impact of chronic hepatitis C virus (HCV) infection on the severity of anemia.

## Patients and methods

This cross-sectional study was conducted on a cohort of 80 MHD patients (40 HCV positive and 40 HCV negative) and 20 healthy age-matched and sex-matched participants who participated as normal controls. Serum hepcidin and highly-sensitive C-reactive protein were measured in patients to study their possible effect on hematological and inflammatory parameters when compared with the control group.

## Results

MHD patients had significantly higher serum hepcidin levels than did controls. The HCV-positive group had significantly lower serum hepcidin and ferritin levels when compared with the HCV-negative group. All MHD patients had significantly higher levels of serum highly-sensitive C-reactive protein than did controls. Hepcidin was also found to correlate with age and serum ferritin levels among MHD patients.

## Conclusion

Changes in serum hepcidin levels are associated with iron status and microinflammation in MHD patients. If used as a diagnostic tool, it may improve targeting and timing of iron therapy by identifying patients during periods of reticuloendothelial blockage of iron transport. This is important to avoid iron overload, especially in HCV-positive patients, which may otherwise cause liver injury resulting in fibrosis, cirrhosis, and finally HCC. We also revealed the value of ferritin levels, which seemed to play an important role in determining the severity of liver disease related to liver fibrosis and microinflammatory activity.

## Keywords:

hepatitis C virus, hepcidin, inflammation, maintenance hemodialysis

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## Introduction

Anemia occurs in the majority of patients with end stage renal disease who require dialysis therapy, and it can be corrected effectively using erythropoiesis-stimulating agents (ESAs) [1]. However, a considerable proportion of patients exhibit a suboptimal response to ESA, and iron deficiency has been identified as the major cause of this hyporesponsiveness because of accelerated erythropoiesis driven by the ESA treatment [2]. On the other hand, the inflammation that is frequently seen in dialysis patients may also contribute to iron-restricted erythropoiesis by reducing the release of stored iron from the reticuloendothelial system to circulating transferrin, a condition that, unlike iron depletion, reduces the likelihood and extent of response to intravenous iron administration [3]. Hepcidin plays a central role in regulating the iron status in the body [4]. Production of hepcidin is induced by excess iron stores

and by inflammation and is suppressed by erythropoietic activity [5]. It has been hypothesized that measuring serum levels of hepcidin may be useful as an additional tool for predicting and monitoring the need for iron supplementation [6]. Chronic hepatitis is also a common problem in patients with CKD, particularly in those patients undergoing hemodialysis. Excessive iron in the liver of hepatic patients contributes to hepatic fibrosis, cirrhosis, and finally HCC, whereas iron depletion is considered beneficial. Iron supplementation should be avoided in CKD patients with chronic hepatitis treated with ESAs [7].

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## Aim

The aim of this study was to measure serum hepcidin levels in maintenance hemodialysis (MHD) patients and to determine the correlation between hepcidin and parameters of iron metabolism and inflammation and to identify a possible impact of chronic hepatitis C virus (HCV) infection on the severity of anemia in patients undergoing MHD.

## Patients and methods

A total of 80 hemodialysis patients from two hemodialysis units (Fayoum University Hospital and Fayoum General Hospital) were included in this study. The study was conducted in accordance with local ethical committee regulations and to standards set by faculty of medicine, Cairo University. Informed consent was obtained from every participant. All patients were undergoing 4-hour dialysis sessions, three times a week, with bicarbonate dialysate for at least 6 months. Patients were categorized into two groups: group I, consisting of 40 hemodialysis patients with chronic hepatitis C; and group II, consisting of 40 HCV-negative hemodialysis patients who were matched for age, sex, and duration under MHD. Group III consisted of 20 healthy participants who were matched for age and sex. We excluded patients with any other chronic infection or inflammation and patients who received iron or blood transfusion within the past 3 months. Blood samples were drawn from all patients before starting the mid-week hemodialysis session to assess the following: complete blood count, urea, creatinine, calcium and phosphorus, urea reduction ratio, iron profile (serum iron total iron-binding capacity and ferritin), alanine transaminase, aspartate aminotransferase, albumin and bilirubin (total and direct), and highly-sensitive C-reactive protein (hs-CRP). The levels of hepcidin were measured using the enzyme-linked immunosorbent assay kit (Human Heparin, ELISA kit, catalog no. E1979h; EIAab, East Lake Hi-Tech Development Zone, Wuhan, China), which has a detection range of 0.187–12 ng/ml and has demonstrated high sensitivity and excellent specificity for the detection of human hepcidin. In addition, an abdominal ultrasound was also carried out for all group I patients.

## Statistical analysis

Data were collected and coded to facilitate data manipulation and then double entered into Microsoft Access (www.eiaab.com). Data analysis was performed using SPSS software, version 18, in Windows 7 (SPSS Inc., Chicago, Illinois, USA). Simple descriptive analyses

in the form of numbers and percentages for qualitative data, arithmetic means as central tendency measurement, SD as measure of dispersion for quantitative parametric data, and inferential statistic test were performed. (a) For quantitative parametric data, the independent Student's test was used to compare the measures of two independent groups. (b) For quantitative nonparametric data, the Mann–Whitney test was used to compare two independent groups. (c) For qualitative data, the  $\chi^2$ -test was used to compare two or more than two qualitative groups; and the bivariate Pearson correlation test was applied to test the association between variables.

*P* value 0.05 or less were considered significant.

## Results

This study was conducted in Fayoum University Hospital and Fayoum General Hospital from February 2015 to November 2016 and included 100 participants who were divided into 40 HCV-positive MHD patients, 40 HCV-negative MHD patients, and 20 normal participants as a control group. Of the HCV-negative MHD patients, 19 (47.5%) were male and 21 (52.5%) were female, with a mean age of 48.7±11.7 years, whereas of the HCV-positive MHD patients 21 (52.5%) were male and 19 (47.5%) were female, with a mean age of 44.7±14.5 years. The control group comprised 10 (50%) women and 10 (50%) men, with a mean age of 41.6±9.6.

There is a statistically significant difference with *P* value 0.05 or less between hemodialysis patients with HCV and hemodialysis patients without HCV (219±180.3 vs. 460.3±310.8) and between the HCV-negative group and controls (460.3±310.8 vs. 80.9±36.3) as regards ferritin.

As shown in Tables 1 and 2 and in Fig. 1, our study showed that there was a statistically significant difference between hemodialysis patients with HCV and controls (12.5±1.8 vs. 2.1±0.54) ( $P<0.001$ ) and between hemodialysis patients without HCV and controls (12.3±3.7 vs. 2.1±0.54) ( $P<0.001$ ) as regards the mean values of hs-CRP. Regarding hepcidin level, there was statistically significant difference between the HCV-positive group and the HCV-negative group (2.8±2 vs. 3.9±2.1) ( $P<0.001$ ), between the HCV-positive group and controls (2.8±2 vs. 46±0.1) ( $P<0.001$ ), and between the HCV-negative group and controls (3.9±2.1 vs. 46±0.1) ( $P<0.001$ ).

There was a statistically significant positive correlation between hepcidin level and age among cases of hemodialysis ( $P<0.05$ ).

**Table 1 Comparison of hematological parameters in the different study groups**

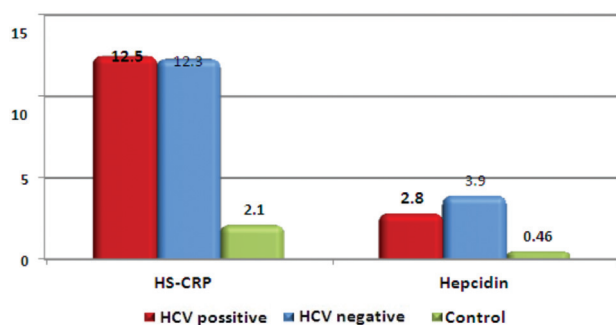
Variables	HCV positive (n=40) (mean±SD)	HCV negative (n=40) (mean±SD)	Control (n=20) (mean±SD)	P value	Significance
Complete blood count					
Hb (g/dl)	10.4±1.8	10.5±1.4	13.7±1.1	<0.001 <sup>b,c</sup>	HS
MCV	82.3±8.6	80.3±5.7	94±3.1	<0.001 <sup>b,c</sup>	HS
MCH	28.2±2.7	28.6±2.6	30.1±1.7	0.02 <sup>b</sup>	S
WBCs (×10 <sup>3</sup> )	5.5±0.86	5.1±0.92	6.5±0.99	<0.001 <sup>b,c</sup>	HS
PLT (×10 <sup>3</sup> )	211.2±62.5	203.9±68.8	372.5±24.6	<0.001 <sup>b,c</sup>	HS
Iron profile					
Serum iron (µg/dl)	69±32.7	78.7±29.4	81±12.9	0.2	NS
Ferritin (ng/ml)	219.9±180.3	460.3±310.8	80.9±36.3	<0.001 <sup>a,c</sup>	HS
TIBC (µg/dl)	366.8±103.2	368.3±131.0	321.8±12.5	0.2	NS
T-saturation %	20.7±13.9	22.6±13.7	24.8±3.3	0.5	NS

Hb, hemoglobin; HCV, hepatitis C virus; HS, highly significant; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; PLT, platelet count; S, significant; TIBC, total iron-binding capacity; WBC, white blood cell. <sup>a</sup>Between HCV positive and HCV negative. <sup>b</sup>Between HCV positive and control. <sup>c</sup>Between HCV negative and control.

**Table 2 Comparison of hepcidin level and highly-sensitive C-reactive protein level in the different study groups**

Variables	HCV positive (n=40) (mean±SD)	HCV negative (n=40) (mean±SD)	Control (n=20)	P value	Significance
HS-CRP (mg/l) (≥5)	12.5±1.8	12.3±3.7	2.1±0.54	<0.001 <sup>b,c</sup>	HS
Hepcidin (ng/ml)	2.8±2	3.9±2.1	0.46±0.1	<0.001 <sup>a,b,c</sup>	HS

HCV, hepatitis C virus; HS, highly significant; hs-CRP, highly-sensitive C-reactive protein. <sup>a</sup>Between HCV positive and HCV negative. <sup>b</sup>Between HCV positive and control. <sup>c</sup>Between HCV negative and control.

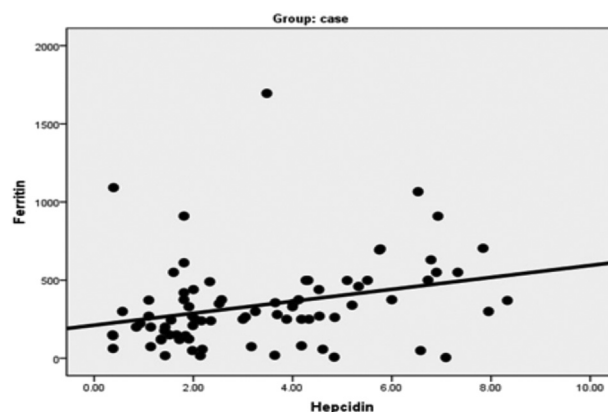
**Figure 1**

A statistically significant difference is seen between hemodialysis patients with hepatitis C virus (HCV) and controls (12.5±1.8 vs. 2.1±0.54) ( $P<0.001$ ) and between HCV-negative patients and controls (12.3±3.7 vs. 2.1±0.54) ( $P<0.001$ ) as regards the mean values of highly-sensitive C-reactive protein (hs-CRP). Regarding hepcidin levels there is a statistically significant difference between the HCV-positive group and the HCV-negative group (2.8±2 vs. 3.9±2.1) ( $P<0.001$ ), between the HCV-positive group and controls (2.8±2 vs. 0.46±0.1) ( $P<0.001$ ), and between the HCV-negative group and controls (3.9±2.1 vs. 0.46±0.1) ( $P<0.001$ ).

Tables 3–5 and Fig. 2 show a statistically significant positive correlation between hepcidin and ferritin among hemodialysis patients ( $P<0.05$ ).

## Discussion

Anemia in MHD patients is a multifactorial condition and its clinical management remains challenging. The interactions between iron metabolism, EPO deficiency, and chronic inflammation are difficult to

**Figure 2**

A statistically significant positive correlation is seen between hepcidin and ferritin in our hemodialysis patients ( $P<0.05$ ).

dissect, and new markers are urgently required to optimize treatment approaches [8]. The inflammation frequently seen in dialysis patients may also contribute to iron-restricted erythropoiesis by reducing the release of stored iron from the reticuloendothelial system to circulating transferrin [3]. The discovery of hepcidin and its role in iron homeostasis have revolutionized our understanding of the pathogenesis of iron overload and iron-restricted anemia and have stimulated the development of new diagnostic and therapeutic methods for these disorders [9]. Hepcidin levels are elevated in patients with chronic kidney disease, and thus high hepcidin levels are thought to be linked to anemia of renal failure [10]. In contrast,

**Table 3 Correlation between sex and hepcidin levels**

Sex	HCV positive (n=40)		P value	HCV negative (n=40)		P value	Control (n=20)		P value
	n	Mean hepcidin (ng/ml)		n	Mean hepcidin (ng/ml)		n	Mean hepcidin (ng/ml)	
Male	21	3.0	0.8	19	4.5	0.6	10	0.47	0.9
Female	19	2.7		21	3.5		10	0.45	

HCV, hepatitis C virus.

**Table 4 Correlation between hepcidin and different patients' variables**

Variables	Hepcidin (ng/ml)		
	r	P value	Significance
Age (years)	0.31	0.005	HS
Duration of dialysis (years)	0.09	0.4	NS
Hb (g/dl)	-0.03	0.8	NS
Albumin level (g/dl)	0.06	0.6	NS
URR%	0.03	0.8	NS
HS-CRP (mg/l)	0.21	0.07	NS
ESA dose (IU/week)	0.03	0.8	NS

ESA, erythropoiesis-stimulating agent; Hb, hemoglobin; HS, highly significant; hs-CRP, highly-sensitive C-reactive protein; URR, urea reduction ratio in hemodialysis.

lower serum hepcidin levels were shown to be related to the severity of liver fibrosis in chronic hepatitis patients without renal failure [11].

In our study we found that MHD patients had significantly higher serum hepcidin levels (mean: 3.9 ng/ml for the HCV-negative group and 2.8 ng/ml for the HCV-positive group) than did the controls (0.46 ng/l) ( $P=0.001$ ). This finding is in agreement with the observations made by Zhang *et al.* [12], who demonstrated that hepcidin in the MHD patients was significantly higher than in controls.

ESRD patients are always in a chronic inflammatory state. Especially in MHD patients, there is more severe inflammation with hemodialysis, which will strongly simulate the upregulation of hepcidin mRNA expression [13].

The present study demonstrated that levels of serum hepcidin were significantly lower in the HCV-positive group than in the HCV-negative group of MHD patients ( $P<0.001$ ). In agreement with these data, Sezgin and Zumurtdal [14] reported that the hepcidin levels in MHD patients with hepatitis who received no EPO were lower when compared with the levels in those patients without liver disease and receiving the maximum dose of EPO [14]. Inflammation is an important inducer of hepcidin synthesis [15]. In MHD patients, inflammation is also a well-known feature and, in our study, we found high levels of serum hs-CRP among MHD patients, with nonstatistically significant difference between the

**Table 5 Correlation between hepcidin and iron profile among hemodialysis patients**

Variables	Hepcidin (ng/ml)		
	r	P value	Significance
Serum iron ( $\mu$ g/dl)	-0.04	0.7	NS
Ferritin (ng/ml)	0.29	0.009	HS
TIBC ( $\mu$ g/dl)	0.06	0.6	NS
T-saturation %	-0.06	0.6	NS

HS, highly significant; TIBC, total iron-binding capacity.

HCV-positive group (12.5 mg/l) and the HCV-negative group (12.3 mg/l). However, there was a high statistically significant difference between MHD patients and healthy controls (2.1 mg/l) ( $P<0.001$ ).

Findings consistent with ours have been found by Caliskan *et al.* [16], who reported that the inflammatory markers including hs-CRP, interleukin-6, and tumor necrosis factor- $\alpha$  were higher in MHD patients than in the controls.

Regarding ferritin, we demonstrated higher levels of ferritin among MHD (HCV negative) patients when compared with the levels of ferritin among controls ( $P<0.001$ ). This finding is in agreement with the observations made by Zhang *et al.* [12].

However, serum ferritin levels in the HCV-positive group were significantly lower than those in the HCV-negative group ( $P<0.001$ ). This is in agreement with Nemeth *et al.* [17], who reported that some of their study patients (hemochromatosis patients) had normal ferritin with mid-normal hepcidin levels and others had high ferritin with low-end-normal hepcidin levels, thus concluding that hepcidin downregulation could be indirectly documented only after the normalization of ferritin values.

In contrast to our data, Fujita *et al.* [18] reported that serum ferritin levels were significantly higher in the HCV-positive group than in the HCV-negative group as the hepatic hepcidin expression levels in chronic liver diseases were strongly associated with the serum ferritin concentration. Therefore, HCV-positive patients had relatively low hepcidin expression levels with severe hyperferritinemia, suggesting the possibility of hepcidin dysregulation in these patients [18].



In contrast, Nabila *et al.* [19] demonstrated that there was no statistically significant difference in ferritin levels between HCV-positive MHD patients and HCV-negative MHD patients. Hs-CRP and ferritin are known as markers of inflammation, and both of them were significantly high in our MHD patients. It is important to note that the protocol of our study excluded patients with active illness or infection.

In the current study, we found a statistically significant positive correlation between age and hepcidin levels among our hemodialysis patients ( $r=0.31$ ,  $P=0.005$ ). In contrast, Xu *et al.* [20] demonstrated that there was no statistically significant correlation between hepcidin and patient age; however, it should be noted that their patient group size was nearly half of ours.

We also found a highly significant positive correlation between serum hepcidin levels and ferritin ( $r=0.29$ ,  $P=0.009$ ). Serum ferritin is a marker of iron stores in the liver and reticuloendothelial system, as well as being an acute-phase protein. This suggests that hepcidin plays a major role in regulating iron homeostasis in MHD patients. Findings consistent with ours have been seen in the study by Zhang *et al.* [12]. In addition, Caliskan *et al.* [16] reported that serum prohepcidin levels were positively correlated with ferritin ( $r=0.405$ ,  $P=0.001$ ).

In conclusion, serum hepcidin levels are associated with iron status and microinflammation in MHD patients. If used as a diagnostic tool, it may improve targeting and timing of iron therapy by identifying patients during periods of reticuloendothelial blockage of iron transport. This is important to avoid iron overload, especially in HCV-positive patients, which may cause liver injury resulting in fibrosis, cirrhosis, and finally HCC. Furthermore, we revealed the value of ferritin levels, which seemed to play an important role in determining the severity of liver disease related to liver fibrosis and necroinflammatory activity.

Further studies evaluating the hepcidin antagonist for therapeutic purposes are needed in the future. Currently, hepcidin antagonists, which are produced through different biotechnological techniques, including humanized antihepcidin antibodies, aptamers, anticalin, siRNAs, and the old traditional heparin, are in clinical trials, and perhaps in a few years we will know whether the downregulation of hepcidin really improves anemia in most or at least some chronic diseases.

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Nil.

#### Conflicts of interest

There are no conflicts of interest.

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