

## Role of Hepcidin in the Pathogenesis of Anemia Not Caused by Iron Deficiency

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### Abstract

**Background:** The aim of the study is to assess the role of hepcidin in the pathogenesis of some types of anemia not caused by iron deficiency and to find out a possible relationship between serum hepcidin level and iron profile studies.

**Subjects and methods:** The study was carried out on 80 subjects divided into 4 groups: Group I included 20 rheumatoid arthritis patients associated with anemia; Group II included 20 chronic liver disease patients associated with anemia; Group III included 20 patients with thalassemia and their results were compared with 20 apparently healthy subjects (Group IV) of matched age and sex as control group. Each individual was subjected to careful history taking, general examination, and routine laboratory investigations including iron profile in addition to hepcidin level assay.

**Results:** There were statistically significant increase in hepcidin levels in group I, II, III when compared to control group ( $P=0.002$ ,  $0.001$ ,  $<0.001$ ) respectively. Also, hepcidin levels were significantly higher in group III compared to group I and II ( $P\leq 0.001$ ,  $<0.001$ ) respectively. While no significant difference was found between group I and II ( $P=0.665$ ). There was significant strong positive correlation between hepcidin levels and serum ferritin levels in all patients groups ( $P\leq 0.001$ ), and significant strong positive correlation between hepcidin levels and serum iron levels ( $P\leq 0.001$ ), while there was significant negative correlation between hepcidin level and Hb level ( $P\leq 0.001$ ).

**Conclusion:** Hepcidin measurement is a useful tool in the work up of anemic patients associated with disturbed iron homeostasis. Hepcidin regulation must be taken into consideration in the full clinical spectrum of thalassemia and chronic hepatitis C patients.

**Keywords:** Hepcidin; Anemia of chronic inflammation; Thalassemia; Chronic liver disease

hemolysis, and dyserythropoiesis) suppress the production of hepcidin [4].

### Introduction and Aim of the Work

Hepcidin is a 25-amino acid peptide ( $\beta$ -defensin-like) produced by hepatocytes and considered a key regulator of systemic iron homeostasis. In concordance with this dual function, its expression is modulated by systemic iron requirements and in response to infectious and inflammatory stimuli. Studies of hepcidin provide novel insight into the molecular mechanisms involved in maintaining iron homeostasis in the healthy state and iron redistribution in response to chronic infections and inflammation [1].

Hepcidin is secreted into plasma, excreted in urine and has been shown to prevent the absorption of iron from the digestive tract, also inhibit the release of stored iron from macrophages and hepatocytes [2].

Serum iron levels are believed to influence how much hepcidin is produced in the liver; with low iron levels decreasing hepcidin production and high iron levels encouraging its production [1].

Anemia of chronic Inflammation leads to increased hepcidin production via IL-6 and decreased erythropoietin levels [3], whereas factors associated with increased erythropoiesis (hypoxia, bleeding,

Studies in anemic patients suffering from diseases such as rheumatoid arthritis, inflammatory bowel diseases, cancer, and end-stage renal disease are needed to validate the potency of hepcidin measurements under these conditions [5].

Hepcidin is considered a promising marker in the investigation of iron status; measurement of patient's hepcidin level not only would be useful in diagnosis of iron disorders but also will present new therapeutic hopes for our patients [6].

In iron loading anemias such as thalassemia, studies have suggested hepcidin or hepcidin/ferritin index values at the lower end of the reference range to be a result of suppressed hepcidin production due to high and less effective erythropoietic activity. These findings may be relevant in the search for non-invasive measures of iron burden and improved therapeutic interventions for these often congenital diseases [7]. Serum hepcidin concentrations have been measured in patients with CHC and relatively low levels were observed compared to HCV negative individuals. This effect of HCV on hepcidin expression was fully reversible after successful eradication of HCV. The consequences of this dysregulation of the hepcidin expression by HCV may be an important mechanism underlying the iron overload seen in CHC and may have significant implications for the management of chronic HCV infection. Also serum hepcidin concentrations were correlated

significantly with hepatic hepcidin mRNA levels in patients with CHC, indicating that serum hepcidin is a valid approach to evaluate hepatic hepcidin production [8].

The aim of the work is to assess the role of hepcidin in the pathogenesis of some forms of anemia not caused by iron deficiency, and to find out a possible relationship between serum hepcidin level and iron profile studies.

## Subjects and Methods

This study included 80 subjects; 60 patients selected from the outpatient clinic in Minia University Hospital in the period from June 2011 to November 2012. In addition to 20 apparently healthy subjects of matched age and sex chosen as control group. They were divided into the following groups: Group I: Included 20 rheumatoid arthritis patients associated with anemia; 9 (45%) males and 11 (55%) females, their ages ranged from 18 to 65 years. Group II: Included 20 patients with chronic liver disease associated with anemia; 12 (60%) males and 8 (40%) females, their ages ranged from 25 to 65 years. Group III: Included 20 patients with thalassemia, 13 (65%) males and 7 (35%) females, their ages ranged from 2.5 to 30 years. Group IV: Included 20 apparently healthy subjects of matched age and sex; 9 (45%) males and 11 (55%) females, their ages ranged from 18 to 65 years.

### Inclusion criteria

Rheumatoid, chronic liver disease, thalassemic patients with HB level less than 12 g/dl and normal or elevated serum iron and serum ferritin.

### All participants were subjected to the following

Careful history taking with special stress on anemia manifestations; duration, blood transfusion and family history, clinical examination and laboratory investigations including:

#### Routine investigations:

Complete blood count (CBC), renal function tests (blood urea, serum creatinine), liver function tests (ALT, AST, serum albumin and total protein), HB Electrophoresis, serum iron and serum ferritin assay, erythrocytes sedimentation rate (ESR), C-reactive protein (CRP) and Rheumatoid factor (RF).

#### Special investigations:

Hepcidin level assay by enzyme immunoassay (EIA).

### The methods used are described as follows

#### Routine Investigations: CBC:

Determined by automated cell counter Sysmex KX-21N (TAO Medical Incorporation, Japan).

#### Routine chemistry tests:

Urea, creatinine, albumin, total protein, AST, ALT, were assayed using fully automated clinical chemistry auto-analyzer system Konelab 20i (Thermo Electron Incorporation, Finland).

#### ESR:

Determined by Westergren method.

#### CRP:

Was determined using semiquantitative assay supplied by CRP Teco diagnostic latex agglutination test [9].

#### Ferritin:

The ferritin quantitative test is based on a solid phase enzyme linked immunosorbent assay (ELISA) [10]. (Kit supplied by BioCheck instruments Foster city, USA).

#### Iron and Total iron binding capacity (TIBC):

Was determined using quantitative coloremtric method. (Kit supplied by Stanbio instruments Texas City USA).

#### RF:

Was determined using semiquantitative assay supplied by Teco diagnostic latex agglutination test [11].

#### Hepcidin assay:

The DRG hepcidin hormone enzyme immunoassay kit provides materials for the quantitative determination of hepcidin hormone in human serum and urine. (Kit supplied by DRG instruments GmbH, Germany).

### Statistical analysis of the data collected

Analysis of data was done by using SPSS (statistical program for social science version 20) as follow: Description of quantitative variables as mean, SD and range. Description of qualitative variables as number and percentage. Chi-square test, independent sample t-test, Kruskal Wallis test, One Way Anova test and Mann Whitney test.

## Results

All obtained results of different groups were summarized in Tables 1-4 and Figures 1-3.

When comparing group I with group II there was no statistically significant difference ( $P=0.914$ ), while on comparing the remaining groups there were statistically significant increase in serum ferritin levels, in group I, II, III ( $p=0.001$ ,  $0.003$ ,  $<0.001$ ) respectively, and in group III compared to group I and II ( $p\leq 0.001$ ,  $<0.001$ ) respectively.

On comparing control group with group I, II and III, There were statistically significant increase in serum iron level in patients groups ( $P\leq 0.001$ ,  $0.027$ ,  $<0.001$ ) respectively. Also on comparing group III with group I and II, there were statistically significant increase in serum iron ( $P\leq 0.001$ ,  $<0.001$ ) respectively.

There were statistically significant increase in hepcidin levels in group I, II, III when compared to control group ( $P=0.002$ ,  $0.001$ ,  $<0.001$ ) respectively. Also, hepcidin level was significantly higher in group III compared to group I and II ( $P\leq 0.001$ ,  $<0.001$ ) respectively. While no significant difference was found between group I and II ( $P=0.665$ ).

The level of TIBC is significantly decreased in groups I, II, III, when compared with control group ( $P\leq 0.001$ ,  $<0.001$ ,  $<0.001$ ), while on comparing group I with group II, III and group II with III, there were no statistically significant difference between these groups ( $p=0.317$ ,  $0.655$ ,  $0.176$ ) respectively.

	Group I N=20	Group II N=20	Group III N=20	Group IV N=20
Ferritin (ng/ml)	39-922	29-909	415-5819	20-305
Range (M ± SD)	363 ± 273	385 ± 298	3378 ± 1471	128 ± 92
P-value	<0.001	<0.001	<0.001	
Iron (ug/dl)	100-310	15-390	295-704	66-140
Range (M ± SD)	186 ± 65	150 ± 88	455 ± 117	99 ± 21
P-value	<0.001	<0.001	<0.001	
TIBC (ug/dl)	52-274	45-290	39-220	277-395
Range (M ± SD)	140 ± 73	166 ± 76	132 ± 63	347 ± 39
P-value	<0.001	<0.001	<0.001	

Table 1: Comparison between different studied groups regarding serum ferritin, serum iron and TIBC.

	Group I N=20	Group II N=20	Group III N=20	Group IV N=20
Hepcidin (ng/ml)	15-308	15-352	227-3175	10-82
Range (M ± SD)	116.3 ± 99	138.6 ± 9.5	1209 ± 792	36.6 ± 19
P-value	0.002	0.001	< 0.001	

Table 2: Comparison of hepcidin levels in different studied groups.

Hepcidin (ng/ml)		r	P
	Iron (ug/dl)	0.843	<0.001**
	Ferritin (ng/ml)	0.825	<0.001**
	Hb (gm/dl)	-0.611	<0.001**

Table 3: Correlations of hepcidin with serum iron, serum ferritin and Hb levels in all patients.

*N.B.*

r=0-0.24 (weak correlation)

r=0.25-0.49 (fair correlation)

r=0.5-0.74 (moderate correlation)

r=0.75-1 (strong correlation)

\*\* =P-value <0.001

Hepcidin ng/ml)		Group I		Group II		Group III	
		r	P	r	P	r	P
	Iron(ug/dl)	0.802	<0.001**	0.782	<0.001**	0.666	<0.001**
	Ferritin(ng/ml)	0.970	<0.001**	0.790	<0.001**	0.512	0.021*
	Hb(gm/dl)	-0.798	<0.001**	-0.897	<0.001**	-0.787	<0.001**

Table 4: Correlations of hepcidin with serum iron, serum ferritin and Hb levels in different studied patients groups.

*N.B.*

\* =P-value <0.05

\*\* =P-value <0.001

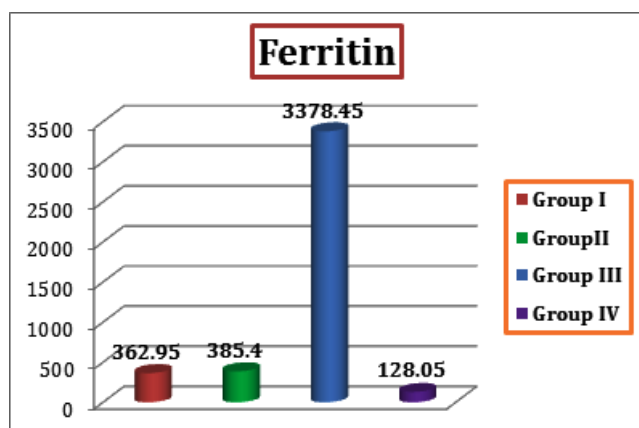


Figure 1: Comparison between different studied groups regarding mean values of serum ferritin showing the highest level in group III.

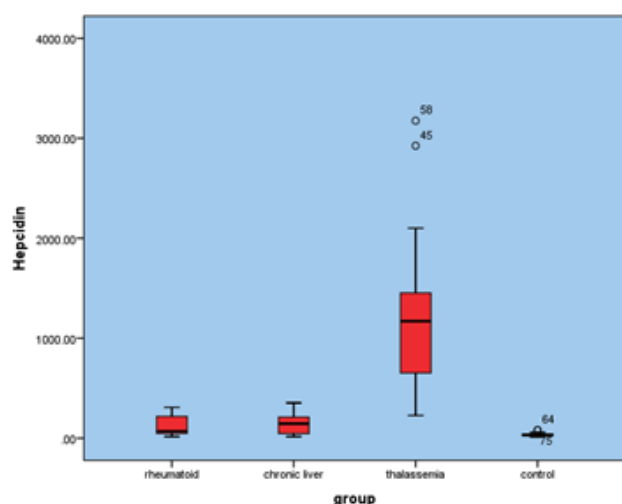


Figure 2: Distribution of hepcidin in different studied group, showing high levels in all groups especially group III.

## Discussion

Hepcidin is a hormone primarily synthesized by hepatocytes, and it mainly controls two critical steps of iron homeostasis: duodenal absorption and the release from macrophages recycling iron through erythrophagocytosis [12]. The discovery of hepcidin and the role that it plays as a negative regulator of intestinal iron absorption and macrophage iron release has significantly progressed the understanding of iron metabolism [13].

Disruption of hepcidin regulation has been postulated as possible important mechanism causing iron overload in acquired inflammatory conditions, like chronic hepatitis C (CHC) [14], inflammatory bowel disease and rheumatoid arthritis [15].

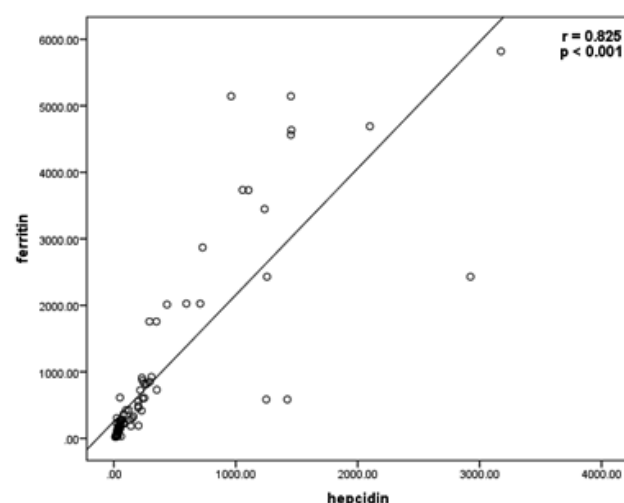


Figure 3: Significant strong positive correlation between ferritin levels and hepcidin levels in all patients.

In our study the level of hepcidin in chronic HCV group was higher than control group, these results were in agreement with [16] who documented that serum hepcidin was significantly higher in the CHC group than the control group, which could be attributed to two factors; inflammation and iron stores.

Inflammation increases hepcidin synthesis through inflammatory cytokines mainly IL6, a positive correlation was found between inflammatory markers and serum hepcidin [17], however, hepcidin level in CHC patients was found to be lower than control group in several studies [18,19].

Girelli et al. [18], speculated that hepcidin expression in CHC is determined by the opposing effects of; hepcidin-suppressive viral factors and by 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.

Hongyan et al. [19], demonstrates in his study that hepcidin effectively inhibits HCV replication in cell culture and HCV reduces hepcidin expression. It is possible that hepcidin is a mediator in innate immunity and HCV has developed a strategy to suppress its expression. It is also possible to develop a therapy using hepcidin. Besides its antiviral effect, the potential advantage of hepcidin therapy for HCV patients is restoration of iron homeostasis.

Serum hepcidin correlated positively with serum ferritin in our study, which accords with other studies that found ferritin correlated positively with serum hepcidin and with mRNA hepcidin [8], however, some studies found no correlation between ferritin and serum hepcidin and between ferritin and pro-hepcidin [20]. Nagashima et al. [21], found that pro-hepcidin had negative correlation with serum ferritin in CHC patients.

There was positive correlation between serum hepcidin and serum iron levels which was in agreement with El Wakil et al. [16], and in disagreement with Fujita et al. and Tsochatzis et al. [8,20].

In the CHC group serum hepcidin levels were negatively correlated with their hemoglobin values which were in agreement with Lin et al. [22]. However El Wakil et al. [16], found no correlation between hepcidin level and hemoglobin level.

The hepcidin levels in rheumatoid arthritis group was higher than control group which was in agreement with Dallalio et al. [23], and Koca et al. [24], who showed that rheumatoid arthritis patients have higher serum concentration of hepcidin than healthy control.

When investigating serum pro-hepcidin concentration as a marker of endogenous hepcidin levels in rheumatoid arthritis patients Fleming et al. [25], found the same finding of an increased serum pro hepcidin in group of patients with rheumatoid arthritis and anemia of chronic disease (ACD).

Our results were in agreement with Susan et al. [26], who documented in their study that serum hepcidin level in rheumatoid arthritis group was higher than control group with higher serum ferritin and iron levels which were positively correlated with hepcidin level. The study concluded that serum hepcidin and ferritin level could be useful as marker of ACD in rheumatoid arthritis patients. On the other hand Roe et al. [27], stated in his study that levels of pro-hepcidin were unrelated with ferritin or iron parameters.

Thalassemia syndromes represent the major cause of iron overload in Mediterranean countries [13]. Based on the results in our study, the level of hepcidin in thalassemia group was higher than control group. This was in agreement with Origa et al. [28], who found increased urinary hepcidin level in thalassemia major patients more than in control and in disagreement with Casanovas et al. [29], and EL Beshlawy et al. [30], who found that serum hepcidin levels are decreased in all patients of chronic hemolytic anemia, they suggested that the diseased erythron dysregulates iron homeostasis by inhibiting hepcidin synthesis, even in the presence of iron overload.

In our study serum hepcidin levels were positively correlated with serum iron and ferritin levels, this was in agreement with Nemeth et al. [31].

Andrews [32], stated that patients of thalassemia major have decreased concentrations of hepcidin due to opposing influences of ineffective erythropoiesis and concomitant iron overload. They also reported that hepcidin expression was down regulated in a hepatocytic cell line after treatment with thalassemic sera, which suggests the presence of a humoral factor that down regulates hepcidin.

The current study agrees with Origa et al. [28], who reported that hepcidin levels were elevated in thalassemia major, due to transfusions that reduce erythropoietic drive and deliver a large iron load, resulting in relatively higher hepcidin levels. In the presence of higher hepcidin levels, dietary iron absorption is moderated and macrophages retain iron, contributing to higher serum ferritin. Brissot et al. [33], had reported that hepcidin deficiency in thalassemia major due to ineffective erythropoiesis which leads to over expression of growth differentiation factor-15 (GDF-15) by the erythroblasts, which inhibits hepcidin expression. This could explain why hepatic iron overload can develop in thalassemia in the absence of transfusions, and why hepcidin expression is relatively low in this disease despite transfusional iron excess (which should, by itself, lead to marked increased hepcidin expression).

Overall, these findings suggest that hepcidin assays reflect hepatic hepcidin production, but also indicate that this correlation is not ideal. This fact is possibly not only due to limits in the accuracy of hepcidin

measurements, but also to several events (protein degradation and secretion, hepcidin-ferroportin interaction, hepcidin internalization at its target sites and extra-hepatic production) which might have a role in determining the net amount of circulating and functional protein, although the effect of other genetic or environmental factors including the possibility of minor infections or other inflammatory stimuli, severity of the condition, compliance to therapy, circadian rhythm may also lead to important variation in outcome if sampling time is not standardized [34].

In conclusion, the discovery of hepcidin has increased the possibility of understanding the disturbances of iron homeostasis especially in anemia with iron overload and anemia of chronic disease. In the future, hepcidin measurement may be useful as a part of the diagnostic and prognostic evaluation of thalassemia syndromes. It may be possible to use exogenous hepcidin to restore normal iron homeostasis in patients with thalassemia and also in HCV patients complicated by anemia and iron overload.

We recommend that further studies should be carried out on role of iron depletion in the treatment of hepatitis C specially when there is impaired treatment response to interferon in patients with raised hepatic iron concentration.

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