Iron Regulatory Hormone Hepcidin Decreases in Chronic Heart Failure Patients With Anemia

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Background: The etiology of anemia is still unclear in patients with chronic heart failure (CHF). Hepcidin is an iron regulatory peptide that is synthesized in the liver to suppress iron absorption and utilization. Hepcidin synthesis is suppressed by anemia, hypoxia and erythropoiesis, and induced by inflammation. Inflammatory cytokines, such as interleukin-6 (IL-6), increase the synthesis of hepcidin, resulting in anemia of inflammation (AI). The serum hepcidin concentration in CHF patients with anemia was measured in order to better understand anemia in CHF.

Methods and Results: Serum hepcidin-25, erythropoietin (EPO), ferritin and IL-6 concentrations were measured in 61 CHF patients. Among these patients, 36 patients had anemia. A group of 16 patients without cardiac disease or anemia were recruited as controls. Serum IL-6 and EPO were higher and hepcidin-25 was lower in CHF patients with anemia than in controls. Hepcidin-25 correlated with EPO and ferritin but not with IL-6. Results of multivariable regression analysis showed that independent predictors of serum hepcidin-25 included EPO and ferritin but not IL-6.

Conclusions: Serum hepcidin-25 concentrations were regulated by iron storage and erythropoiesis but not by IL-6 in CHF patients with anemia. These findings might indicate that AI is a minor cause of anemia in CHF. (Circ J 2010; 74: 301–306)

Key Words: Anemia of inflammation; Chronic heart failure; Hepcidin-25; Interleukin-6

Hepcidin was first discovered as an antimicrobial peptide.1 It is synthesized in the liver to regulate iron homeostasis.2 Hepcidin suppresses iron uptake in the duodenum and blocks iron release from the macrophages by binding to ferroportin, a cellular iron exporter, and inducing its internalization.3 Synthesis of hepcidin is activated by iron loading4 and decreased by anemia, hypoxia and erythropoiesis.5 Hepcidin synthesis is also induced by inflammatory cytokines, such as interleukin-6 (IL-6), during infections and inflammation, resulting in suppressed iron utilization and absorption and hence anemia of inflammation (AI) or anemia of chronic disease.6

Anemia has been demonstrated to be independently associated with increased risks of hospitalization and all-cause mortality in patients with chronic heart failure (CHF).7–12 The mechanisms underlying anemia in CHF are not fully elucidated and probably multifactorial. Proposed mechanisms include plasma volume expansion,13,14 iron deficiency,15 suppressed bone marrow activity and inflammation.16 Inflammatory immune activation has been shown in CHF, indicating that anemia in CHF might be a kind of AI. If so, serum hepcidin concentrations should be elevated in CHF patients. However, Nanas et al have shown that approximately 70% of CHF patients with anemia have iron deficiency anemia and AI was observed in only 19% of patients in end-stage CHF by examining the iron stores with bone marrow biopsy.17 If this is the case, serum hepcidin concentrations would be decreased in CHF patients. Thus, we developed the following 2 hypothesis:

(1) Hepcidin concentrations are upregulated by inflammatory cytokines to cause AI in patients with CHF.
(2) Hepcidin concentrations are downregulated by iron deficiency in patients with CHF.

In order to assess the above 2 hypotheses, we measured serum hepcidin-25 (the bioactive form of hepcidin) concentrations in CHF patients with anemia. We analyzed the association between serum hepcidin-25 concentrations and IL-6 or iron- and erythropoiesis-related parameters.
**Table 1. Characteristics of the Subjects**

<table>
<thead>
<tr>
<th></th>
<th>CHF patients</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With anemia</td>
<td>Without anemia</td>
</tr>
<tr>
<td>Sample size</td>
<td>36</td>
<td>25</td>
</tr>
<tr>
<td>Age (years)</td>
<td>72±14*</td>
<td>67±15</td>
</tr>
<tr>
<td>Gender (male %)</td>
<td>53*</td>
<td>68*</td>
</tr>
<tr>
<td>NYHA class (I/II %)</td>
<td>42/58</td>
<td>36/64</td>
</tr>
<tr>
<td>Etiology of heart failure (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>38</td>
<td>40</td>
</tr>
<tr>
<td>Idiopathic dilated cardiomyopathy</td>
<td>11†</td>
<td>32</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>Others</td>
<td>37</td>
<td>20</td>
</tr>
<tr>
<td>MCV (μm³)</td>
<td>93±7</td>
<td>94±6</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>11.2±1.1*</td>
<td>14.5±1.5</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>109±106</td>
<td>125±138</td>
</tr>
<tr>
<td>Serum iron (μg/dl)</td>
<td>72±28*</td>
<td>98±53</td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>22±9*</td>
<td>30±13</td>
</tr>
<tr>
<td>EPO (mU/ml)</td>
<td>32±26</td>
<td>26±25</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>548±498*</td>
<td>368±358*</td>
</tr>
<tr>
<td>eGFR (ml · min⁻¹ · 1.73 m⁻²)</td>
<td>57±28*</td>
<td>65±21*</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.4±0.5*</td>
<td>0.4±0.4*</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>42±16*</td>
<td>37±10*</td>
</tr>
</tbody>
</table>

Values are mean±SD.

CHF, chronic heart failure; NYHA, New York Heart Association; MCV, mean corpuscular volume; Hb, hemoglobin; TSAT, transferrin saturation; EPO, erythropoietin; BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; LVEF, left ventricular ejection fraction.

*P<0.05 vs control, †P<0.05 vs without anemia.

**Methods**

Participants

We prospectively studied consecutive 122 CHF patients who were admitted to our hospital with acute worsening of heart failure and recovered after standard medical treatment from April 2005 to September 2007. We used the data at discharge. Exclusion criteria were as follows: acute coronary syndrome, infections (C-reactive protein >2.0 mg/dl), severe renal failure (serum creatinine >2.0 mg/dl), hypothyroidism, bleeding, a history of acquired iron overload or hemochromatosis, chronic liver disease (alanine transaminase >2 times the upper limit of the normal range), autoimmune disease, or use of erythropoietin (EPO) and/or iron for treatment of anemia. We also excluded patients with radiographic findings of pulmonary congestion at discharge to minimize the effect of hemodilution on the hemoglobin concentration. After 61 patients were excluded, 61 HF patients were enrolled. Among these patients, 36 patients were diagnosed as anemia based on hemoglobin concentration (Hb <13 g/dl in men, Hb <12 g/dl in women according to the World Health Organization criteria) at the time of discharge. Glomerular filtration rate (GFR) was estimated by using the following equation from a simplified version of the Modification of Diet in Renal Disease (MDRD): eGFR (ml · min⁻¹ · 1.73 m⁻²) = 186.3 × serum creatinine⁻¹.154 × age⁻⁰.₂⁰³ × 0.88¹ (×0.742 if female). In this MDRD formula, 0.88¹ is a coefficient for eGFR specific to the Japanese population. They were not administered EPO and/or iron for treatment of anemia. A group of 16 individuals without any history of cardiac disease and anemia were recruited as the control group. Serum iron, ferritin, total iron binding capacity (TIBC) and complete blood count were evaluated on discharge. Transferrin saturation (TSAT) (%) was calculated as serum iron concentration/serum TIBC concentration ×100.

All patients gave informed consent in advance for their participation, and the ethics committee at our institution approved the protocol.

**Measurement of Hepcidin-25, IL-6 and EPO**

Peripheral venous blood was obtained at 7–9 am and transferred to non-heparinized tubes and centrifuged at 1,750 g for 15 min at 4°C. The obtained serum samples were stored at –80°C until they were assayed. Serum hepcidin-25, IL-6 and EPO concentrations were measured at discharge. Serum hepcidin-25 concentrations were determined by liquid chromatography tandem mass spectrometry. Serum IL-6 concentrations were determined by enzyme linked immunosorbent assays according to manufacturer manuals (Quantikine, R&D Systems, MN, USA). Serum EPO concentrations were determined by radioimmunoassay according to manufacturer manuals (Mitsubishi Chemical Medience Corporation, Tokyo, Japan).

**Statistical Analysis**

Values are expressed as means±SD. All statistical analyses were performed using a commercially available statistical software (STATVIEW version 5.0, SAS Institute Inc, North CA, USA). Differences between groups were assessed with the χ² test for categorical data. Hepcidin-25, IL-6, EPO and ferritin data were not normally distributed; therefore, these data were subjected to logarithmic transformation (log). Differences in continuous variables between groups were assessed with unpaired Student’s t-tests. The correlation between serum hepcidin concentrations and variables of interest was tested by simple linear regression analyses. Multivariable linear regression analyses was used to identify variables that might predict serum log hepcidin concentrations. P-values of <0.05 were statistically significant.
Results

Table 1 summarizes the demographics for the CHF patients with anemia (n=36), without anemia (n=25) and the control group (n=16). There was no difference in gender and severity of heart failure between the CHF patients with anemia and those without anemia. The CHF patients with anemia were older than those without anemia and the control group. The CHF patients with anemia had a lower prevalence of idiopathic dilated cardiomyopathy than those without anemia. Mean Hb concentrations were 11.1 g/dl in CHF patients with anemia. Serum iron and TSAT concentrations were lower in the CHF patients with anemia than in those without anemia. There was no difference in mean corpuscular volume and ferritin concentrations between the CHF patients with anemia and those without anemia.

Figure 1. (A) Serum hepcidin-25 and (B) interleukin-6 concentrations in chronic heart failure (CHF) patients with or without anemia, and in controls. Data in box plots are given as median, 25th–75th percentiles and range. NS, not significantly different.

Figure 2. Correlation between log serum hepcidin-25 and (A) erythropoietin, (B) ferritin concentrations in chronic heart failure patients with anemia.
The Concentration of Serum Hepcidin-25, IL-6 and EPO in CHF Patients

Serum hepcidin-25 concentrations were lower in CHF patients with anemia than in controls (14.1±14.0 vs 19.0±11.4 ng/ml, P<0.05). There were no differences between CHF patients without anemia and controls (21.9±18.3 vs 19.0±11.4 ng/ml). Serum IL-6 concentrations were higher (5.9±5.5 vs 1.4±0.4 pg/ml, P<0.05) in CHF patients with anemia than in controls. There were no differences in IL-6 levels between CHF patients with and without anemia (5.9±5.5 vs 5.4±7.6 ng/ml) (Figures 1A, B). There was a trend for higher serum EPO concentrations in CHF patients with anemia than in controls, but it was not statistically significant (Table 1).

Correlation Between Hepcidin-25 and Possible Correlates of Hepcidin-25

We analyzed the correlation between serum hepcidin-25 concentrations and the parameters that might be correlated with hepcidin-25 in CHF patients with anemia. Log serum hepcidin-25 concentrations correlated negatively with log serum EPO concentrations (r= -0.59, P<0.01) and positively with log serum ferritin concentrations (r= 0.67, P<0.01) (Figure 2). However, log serum IL-6 concentrations did not correlate with log serum hepcidin-25 concentrations (r=0.23, P=0.19) (Figure 3). Multivariable regression analysis showed that log serum EPO (P<0.01) and log ferritin (P<0.01) concentrations were independent predictors of log serum hepcidin-25 concentrations. However, serum IL-6 concentrations were not associated with serum hepcidin-25 concentrations (Table 2). Thus, serum hepcidin-25 concentrations are likely regulated by EPO and by iron storage, but not by IL-6 in CHF patients with anemia.

Discussion

This is the first study to examine serum hepcidin-25 concentrations in CHF patients with anemia. We found that serum hepcidin-25 concentrations decreased in CHF patients with anemia. Some regulatory pathways are thought to control hepcidin synthesis: this is upregulated by iron overload and inflammation, and downregulated by anemia, erythropoiesis and hypoxia. We showed that serum hepcidin-25 concentrations are regulated by EPO and iron storage but not by IL-6 in CHF patients with anemia.

Regulation of Serum Hepcidin Concentration in CHF

Serum hepcidin-25 concentrations in CHF patients with anemia were lower than those in control participants and negatively correlated with serum EPO concentrations. EPO is shown to suppress hepatic hepcidin synthesis. This effect is not due to the direct effect of EPO on the liver because blocking erythropoiesis in bone marrow by irradiation prevented downregulation of hepcidin synthesis by exogenous EPO. It is suggested that EPO increases erythropoiesis in the bone marrow and stimulates the secretion of some circulating factors that suppress hepcidin synthesis in the liver. Growth and differentiation factor 15 is one of the candidates for suppression, but it is proven only in patients with thalassemia. Increased serum EPO is thought to suppress hepcidin synthesis by stimulating erythropoiesis in the bone marrow in CHF patients with anemia.

Serum hepcidin-25 concentrations were also associated with serum ferritin concentrations, indicating that serum hepcidin concentrations is reasonably regulated by the storaged iron, even in CHF patients. Shortage in stored iron decreases mRNA expression of hepcidin in the liver by 2 mechanisms. One is the suppression of the synthesis of bone morphogenic protein 6 (BMP 6). BMP 6 is known to be induced by iron overload and also to increase hepatic hepcidin synthesis. The other is the induction of the hypoxia-inducible factor-1 (HIF-1). Promoter activity of hepcidin gene is suppressed by HIF-1. BMP-6 is a dimer of HIF-1α and HIF-1β. Iron-dependent enzyme prolyl hydroxylases hydroxylates HIF-1α and promotes the binding of HIF-1α to a von Hippel Lindau ubiquitin ligase, resulting in the degradation of HIF-1α by proteasome. Iron deficiency decreases prolyl hydroxylase activity, stabilizes HIF-1, and suppresses hepcidin synthesis. Serum iron and TSAT were lower in the CHF patients with anemia than in the controls even though they were within normal range. In a previous study, renal blood flow was decreased and inversely correlated to the serum EPO concentration in CHF patients. Decreased renal blood flow might contribute to EPO hyperproduction in CHF. EPO has been reported to activated erythropoiesis and stimulate the secretion of some circulating factors that decrease the hepcidin synthesis in the liver. This might have caused a decrease in serum hepcidin concentration in CHF patients. Otherwise, decreased dietary intake or poor absorption from the intestine might have deteriorated iron supply. Iron deficiency state might cause anemia and suppress hepcidi-
The present study shows for the first time that hepcidin-25 concentrations are lower in CHF patients with anemia than in control participants. Serum hepcidin-25 concentrations are related to iron storage and erythropoiesis but not to IL-6 in our CHF patients with anemia. These findings might indicate that AI is a minor mechanism of anemia in CHF.

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**References**


13. Andreone AS, Katz SD, Lund L, LaManca J, Hudaifah A, Hryniec K, et al. Hemodilution is known common in CHF, and there might have been some hemodilution in our participants with anemia, but we did not measure red cell volume. Third, the reticulocyte count and serum soluble transferrin receptor concentration reflecting erythropoietic activity were not measured in the current study. Finally, Bayele et al provided a perspective that there were heritable differences in hepcidin expression that might determine phenotypic variation in iron metabolism between individuals. We did not examine the genetic analysis in our participants. The genetic analysis might provide additional information with the serum hepcidin concentrations in CHF patients.

**Conclusions**

The present study shows for the first time that hepcidin-25 synthesis in CHF patients.


