

**Original Article**

## **Comparison of Serum Levels of Hepcidin and Pro-hepcidin in Hemodialysis Patients and Healthy Subjects**

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**ABSTRACT.** Hepcidin prevents absorption of iron from the intestine and inhibits release of iron from macrophages and hepatocytes. For this reason, it seems that high levels of hepcidin are a predisposing factor for anemia in chronic inflammatory conditions such as chronic kidney disease and dialysis patients. This study was designed to determine the role of changes in the level of serum hepcidin in the management of hemodialysis patients. This study included 44 dialysis patients and 44 controls. The hepcidin and pro-hepcidin levels were measured by the enzyme-linked immunosorbent assay method. The serum ferritin level was measured by the chemiluminescence method. The mean hepcidin level was  $999.3 \pm 996.7$  ng/mL in the case group and  $770.4 \pm 815.9$  ng/mL in the control group ( $P = 0.25$ ). The mean pro-hepcidin level was, respectively,  $186.1 \pm 220.3$  pg/mL and  $150.87 \pm 207.7$  pg/mL, in the case group and control groups ( $P = 0.45$ ). The mean (standard deviation) ferritin level was  $816.4 \pm 379.4$  ng/mL in the case group and  $193 \pm 171.8$  ng/mL in the control group ( $P < 0.001$ ). In the case group, the correlation between serum ferritin and hepcidin was not significant ( $r = 0.6$ ,  $P = 0.08$ ). Also, there was no significant correlation between serum ferritin and pro-hepcidin levels ( $r = 0.6$ ,  $P = 0.08$ ). A positive correlation was seen between pro-hepcidin and hepcidin levels ( $r = 0.92$ ,  $P < 0.01$ ). In this study, the results showed that the serum hepcidin levels are high in dialysis patients and that there was no correlation with the serum ferritin levels.

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### **Introduction**

Chronic renal failure (CRF) is a severe debilitating disease and is associated with many systemic complications, and anemia is one of them.<sup>1</sup> Renal anemia is considered as a form of

anemia of chronic diseases that is often of the normochromic normocytic type. Anemia of chronic diseases is seen in many diseases associated with inflammation, such as cancer, chronic infections and autoimmune diseases.<sup>2</sup> Anemia is a major factor in many of the symptoms associated with decreased renal function. These complications cause a significant increase in mortality due to cardiovascular disease in dialysis patients.<sup>3</sup> The kidneys are the primary site for the synthesis of erythropoietin (EPO) in adults. Interleukin-6 is the most important cytokine involved in the production of hepcidin and the inflammatory condition axis of hepcidin–interleukin (IL)-6 is responsible for iron deficiency during inflammation.<sup>4,5</sup>

Hepcidin is a low molecular weight protein that has recently been discovered and plays an important role in iron metabolism. It is a regulator of renal iron homeostasis.<sup>6</sup> Pre-pro-hepcidin, with 84 amino acids, is produced in the hepatocytes and is then broken down to 60 amino acid pro-hepcidin that appears in the plasma.<sup>7</sup> Hepcidin prevents the release of iron from the macrophages and decreases iron absorption in the intestine.<sup>8</sup> Various physiological and pathological conditions are effective in the synthesis of hepcidin. On the other hand, synthesis of hepcidin increases in infections and inflammatory processes.<sup>9</sup>

Hepcidin is considered as one of the acute phase proteins. It is believed that this effect can lead to anemia of chronic disease, which is caused by a decrease of circulating iron available for erythropoiesis, despite normal iron stores.<sup>10</sup> The hepcidin level increases in patients with CRF and hemodialysis (HD) patients.<sup>11,12</sup> Hepcidin is cleared only in some patients during dialysis. Exactly why this happens is not clear yet, but it may be due to differences in the membranes of various dialyzers, state of kidney function or induction of hepcidin during the process of HD.<sup>6</sup> Hepcidin may be associated with resistance to factors triggering the production of EPO and an imbalance in iron metabolism in CRF patients. In addition, hepcidin can also be discussed as a therapeutic agent as decreasing the levels of hepcidin can lead to improved iron uptake

from the intestine and macrophages; thus, anti-hepcidin therapy can be a substitute for intravenous iron therapy.<sup>13</sup> The aim of this study was to evaluate the level of pro-hepcidin and hepcidin in patients with CRF on HD.

## Materials and Methods

In this study, 44 HD patients and 44 healthy controls were sampled. Before dialysis, 6 mL of blood was taken from each patient and, after separation, the sera were stored in the freezer at  $-70^{\circ}\text{C}$ . Subjects with active infection (fever or other evidence of infection) were excluded. The control subjects were healthy individuals with normal serum urea and creatinine. Hepcidin (IBL) and pro-hepcidin (Glory Science) were measured by the enzyme-linked immunosorbent assay method. The serum ferritin was measured by the luminescence quantitative method (Monobind).

To investigate the correlation between different variables and eliminate confounding variables in the subjects of the study, we used multivariate analysis (logistic regression). For this purpose, variables that had a  $P$ -value  $<0.2$  on univariate analysis were entered into the multivariate analysis. For comparison of the mean of variables that was not normally distributed, the Mann–Whitney test was used and for variables that had normal distribution, the Student  $t$ -test was used. The chi-square test was used to evaluate the qualitative variables and the  $t$ -test was used to compare the quantitative variables between the two groups. A  $P$ -value  $<0.05$  was considered significant. This study was performed with the approval of the Research Ethics Committee of the Golestan University of Medical Sciences.

## Results

In each of the groups, 54.5% of the participants were male ( $P = 0.58$ ). 31.8% of the patient group and 27.3% of the control group had age below 50 years ( $P = 0.41$ ). The mean pro-hepcidin level in the case and control groups was  $186.1 \pm 220.3$  pg/mL and  $150.87 \pm 207.7$  pg/mL, respectively ( $P = 0.45$ ). The

mean hepcidin level in the CRF patients and in the normal controls was  $999.3 \pm 996.7$  ng/mL and  $770.4 \pm 815.9$  ng/mL, respectively ( $P = 0.25$ ). In CRF patients, the mean pro-hepcidin level was  $168.2 \pm 226$  pg/mL in males and  $207.7 \pm 216.9$  pg/mL in females ( $P = 0.54$ ) and the mean hepcidin was  $816.8 \pm 840.6$  ng/mL in males and  $1218.2 \pm 1140.4$  ng/mL in females ( $P = 0.77$ ). In the control group, the mean pro-hepcidin was  $123.6 \pm 161.5$  pg/mL in males and  $182.5 \pm 252$  pg/mL in females ( $P = 0.84$ ) and the mean hepcidin was  $702.3 \pm 757.2$  ng/mL in males and  $849.2 \pm 893.4$  ng/mL in females ( $P = 0.73$ ) (Table 1).

The mean serum ferritin was  $816.4 \pm 379.4$  ng/mL in the case group and  $193 \pm 171.8$  ng/ml in the control group ( $P < 0.001$ ). In the case group, no significant correlation was observed between serum ferritin and hepcidin and pro-hepcidin levels ( $r = 0.6$ ,  $P = 0.08$ ). Also, in this group, a significant positive correlation was observed between pro-hepcidin and hepcidin levels ( $r = 0.92$ ,  $P < 0.01$ ). In the control group, no significant correlation was seen between serum ferritin and hepcidin ( $r = 0.4$ ,  $P = 0.14$ ) and pro-hepcidin levels ( $r = 0.7$ ,  $P = 0.06$ ). Also, in this group, a significant correlation was seen between pro-hepcidin and hepcidin levels ( $r = 0.8$ ,  $P < 0.01$ ). The level of hepcidin had a direct correlation with the duration on dialysis in the case group, but it was not significant ( $r = 0.3$ ,  $P = 0.06$ ). Also, a direct correlation was observed between duration on dialysis and pro-hepcidin levels ( $r = 0.3$ ,  $P = 0.09$ ). On multivariate regression analysis of the variables such as age and ferritin, pro-hepcidin and hepcidin levels, the only variable that was significantly associated with the risk of anemia was ferritin levels ( $P < 0.001$ ).

### Discussion

This study was conducted to evaluate the

serum levels of hepcidin in HD patients and normal subjects. In our study, the mean pro-hepcidin level had no significant difference in the case and control groups. Tomosug et al, in a study in 2006, measured the hepcidin levels in patients with CRF and found that the hepcidin level was approximately two- to three-times higher than in the control group. In contrast, serum levels of 2-microglobulin, which is a low molecular weight protein that is controlled almost totally by glomerular filtration, there was a 20–30-times increase in the levels. Thus, glomerular filtration has little effect on hepcidin level that theoretically may explain the presence of an unknown carrier in the circulation for hepcidin, which can cause decreased renal clearance of this molecule.<sup>14</sup> Young et al in a study in 2009 showed that the pro-hepcidin level increases in dialysis patients and correlates inversely with the glomerular filtration rate (GFR). However, pro-hepcidin has minimal interaction with iron or inflammatory parameters in dialysis patients; also, it seems that pro-hepcidin is an intermediate metabolite and without physiological activity and its level is not associated with the active production of hepcidin.<sup>15</sup> In the study performed by Zaritsky et al in 2009, the serum ferritin level in children with CRF was higher than in adults with CRF.<sup>16</sup> In our study, there was no significant correlation between ferritin and hepcidin and pro-hepcidin levels.

In a study performed by Busbridge et al in patients with inflammatory bowel disease, a direct correlation was reported between ferritin and hepcidin levels.<sup>17</sup> The mismatch of the results may be due to successful HD or small sample size. It is recommended that further studies should be performed with a larger sample size and the blood levels of these variables should be compared with the erythrocyte indices. In our study, no significant correlation was seen between duration on dialysis and

Table 1. The mean hepcidin and pro-hepcidin levels in the two age-groups studied.

Group	Test	Under 50 years	Over 50 years	P-value
Case	Pro-hepcidin (pg/mL)	$186.2 \pm 228.7$	$186.1 \pm 220.2$	0.6
	Hepcidin (ng/mL)	$1021.6 \pm 148.9$	$988.9 \pm 989.7$	0.57
Control	Hepcidin (ng/mL)	$977.4 \pm 1026.9$	$684.7 \pm 714.6$	0.6
	Pro-hepcidin (pg/mL)	$215.9 \pm 294.4$	$124 \pm 158.1$	0.6

heparin and pro-heparin levels. In a study performed by Li et al, the heparin level was higher in patients with renal failure and those on HD.<sup>11</sup> Peters et al in their study in 2010 from Holland found that the heparin level was higher in patients on chronic HD than in the control group. They also showed that the ferritin level in CRF and HD groups was higher than in the control group and found a significant correlation between heparin and ferritin levels. They concluded that the ferritin level is the only predicting factor for heparin level.<sup>12</sup>

According to studies performed in 2008 and 2010, it seems that the heparin level has no correlation with duration of dialysis, but the GFR is an important factor that shows correlation with the heparin level.<sup>6,12</sup> Abbasi et al reported that the pro-heparin level in CRF patients was higher than in the control group.<sup>18</sup> According to a study that was performed in 2011, the heparin level did not show a correlation with sex, race and age.<sup>19</sup> In our study, the mean serum ferritin was significantly higher in the dialysis group than in the control group. In a study performed by Nakanishi et al in 2011, direct and significant correlation was observed between serum ferritin and heparin levels.<sup>20</sup>

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**Conflict of interest:** none

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