Interaction between Serum Prohepcidin, Anemia and Chronic Hepatitis in Hemodialyzed Patients

NABILA F. AMIN, M.D. 1; HANAN M. AHMED, M.D. 1; EFFAT A. TONY, M.D. 1; GHADA M. GALAL, M.D. 2; OMNIA A. MOHAMMED, M.D. 3; AMAL M. ABDEL-AL, M.D. 3 and AMAL A. MAHMOUD 3, M.D.

The Departments of Internal Medicine 1, Assiut University, Tropical Medicine, Gastroenterology 2, Sohag University and Clinical Pathology, Assiut University 3

Abstract

Background and Aim: Anemia is a major clinical multifactorial problem in hemodialysis (HD) patients. Iron metabolism is frequently impaired and may complicate the management of anemia. Hepcidin is synthesized in the liver. It is thought to be a key regulator of iron homeostasis. Therefore, we thought to assess prohepcidin level and iron indices in patients on regular HD with and without associated viral hepatitis.

Patients and Methods: Sixty patients (38 males and 22 females) with chronic renal failure (CRF) undergoing regular HD and twenty age and sex matched healthy controls were included. Peripheral hemogram, liver function tests, kidney function tests, lipogram, C-reactive protein, iron indices, serum prohepcidin, HBsAg, HBcIgM and anti-HCV antibody were tested for both patients and controls.

Results: 22 Patients were positive for anti-HCV, 16 were HBsAg positive and 22 were negative for both markers. Mean serum prohepcidin and ferritin levels in HD patients were significantly higher than controls. There was significant increase in the mean serum prohepcidin level in HD patients with hepatitis B compared to those with hepatitis C. Prohepcidin had significant negative correlations with both hemoglobin level and total iron binding capacity in HD patients with hepatitis C. However, in multiple regression analysis, serum prohepcidin level was independently related to hepatitis, and triglycerides level in the whole group of HD patients.

Conclusion: CRF patients on HD have elevated serum prohepcidin and hepatitis C patients have higher levels than those with hepatitis B. Prohepcidin was independently related to the presence of viral hepatitis and triglycerides suggesting the important role of the liver in hepcidin synthesis.

Key Words: Anemia – Hemodialysis (HD) – Prohepcidin – Chronic hepatitis.

Introduction

INFECTIONS are one of the important causes of morbidity and mortality in patients with end stage renal failure [1]. Chronic hepatitis is a major complication of chronic HD. Initially; hepatitis B virus (HBV) infection was the most common etiologic agent of chronic hepatitis in patients on chronic HD. Later; after HBV vaccines became available and measures for screening and exclusion of HBsAg positive blood were routinely used, HBV infection dropped significantly. Subsequently; however, HCV emerged as a new problem. In USA; rates of positive anti HCV reached up to 36% in HD patients in 1990s [2]. In renal transplant unit in Mansoura, there is a high prevalence (62%) of hepatitis C virus in end-stage renal disease patients awaiting renal transplant [3].

Anemia is a major clinical problem in patients on dialysis therapy and has substantial impact on morbidity and mortality. In addition to the reduced production of erythropoietin in the kidney, iron metabolism is also impaired in chronic kidney disease (CKD) and frequently may complicate the management of anemia [4].

Maintaining the correct iron balance is crucial for health. Current understanding of the regulation of iron metabolism is based on the biology of a number of critical proteins including transferrin, transferrin receptor, ferritin, iron regulatory protein, divalent metal transporter 1, ferroportin, hemoglobin and hepcidin [5]. Hepcidin is expressed as an 84-amino acid (AA) pre-propeptide in hepatocytes, renal epithelial cells, and other several tissues. Cleavage of the 24-AA peptide produces a 60 AA residue prohormone, prohepcidin (molecular weight 1 0kDa). Additional processing of the 34-AA pro-region, results in 25-, 22- and 20-AA peptides that are detectable in urine and serum. Circulating hepcidin appears to bind to ferroportin, the ferrous iron trans-membrane transporter, on the cell surface,
inducing receptor-ligand internalization and lysosomal degradation. Lysosomal degradation of ferroportin, leads to the trapping of iron at absorptive enterocytes, macrophages and hepatocytes, thus causing a decrease in available serum iron [6].

In addition to its role in iron homeostasis, prohepcidin is induced by inflammation, an effect believed to be dependent on cytokine production [7]. Fujita, et al. [8] reported that patients with chronic hepatitis C frequently have serum and hepatic iron overload but the mechanism is unknown. However, there is little data on hepcidin level in patient with CRF and associated viral hepatitis.

Therefore, we aimed to determine the level of prohepcidin in patients with CRF on HD with and without associated viral hepatitis and the possible relations between prohepcidin, anemia and iron status in these groups of patients.

Patients and Methods

This cross sectional study included 60 patients (38 males and 22 females) with CRF undergoing regular HD in Dialysis Unit, Department of Internal Medicine, Assiut University Hospitals. Their ages ranged from 30-50 years (mean 44.36 ±8.49 years). The mean duration of HD was 30 ±23 months. Patients were eligible for enrollment if they had a stable clinical state. Patients were excluded if they had evidence of infection (other than viral hepatitis), anemia due to non renal cause and patients with underlying malignancy.

None of our patients had received blood transfusion for at least 6 weeks before dialysis. The underlying causes of renal failure among HD patients were as follows: Diabetic nephropathy (n=4), lupus nephritis (n=4), polycystic kidney disease (n=4), chronic glomerulonephritis (n=6), chronic pyelonephritis (n=8), obstructive uropathy (n=10) and idiopathic (n=24). A Randomly selected 20 healthy volunteers matched for age and sex were included as a control group. All subjects gave an informed consent and the protocol was approved by Faculty of Medicine ethics committee. All patients were on regular HD (three sessions/week). The type of dialyzer membrane was haemophane and bicarbonate dialysate. Patients and controls were subjected to full medical history and thorough clinical examination.

Blood samples were drawn before dialysis and samples were centrifuged at 3000g for 10min. and serum aliquots were stored at -20ºC until analysis. Serum urea and creatinine, liver function tests and lipogram were done using Hitachi 911 autoanalyser. Creatinine clearance was estimated by Cockcroft and Gault formula [9].

Complete blood count assessment was performed on whole blood samples on EDTA using Beckman Coulter. Conventional C-reactive protein (CRP) in serum was assayed by latex serology test using AVITEX kits. Serum iron and TIBC were determined by colorimetric assay using Stanbio Iron and TIBC kit (procedure No. 0370). Serum ferritin was measured by immuno-enzymometric assay using Accu-Bind ELISA kits (Monobind Inc., USA code 2825-300). Serum levels of hepicon prohormone were determined also by a hepicon prohormone solid phase enzyme-linked immunosorbant assay kit (IBL International, Hamburg, and Germany RE54051).

HBsAg and HCV antibody were routinely tested before dialysis using AXSYM system based on microparticle enzyme immunoassay technology. Diagnosis of HCV was confirmed by PCR. Accordingly patients were classified into: 16 HBsAg positive, 22 anti-HCV positive patients and 22 patients negative for both markers.

Statistical analysis:

Data were analyzed with Statistical package for social sciences (SPSS) version 17. Statistical differences were calculated with student unpaired t-test. Pearson correlation co-efficient (r) between different variables were done. Values of p<0.05 were considered statistically significant. Multiple logistic regression analysis was performed to determine independent factors affecting dependent variable (prohepcidin).

Results

Demographic and laboratory parameters in HD patients and control groups are summarized in Table (1). Mean serum prohepcidin and ferritin levels in the total studied patients group were statistically significantly higher than the control group (310.65±126.88ng/ml Vs. 71.0±32.4ng/ml, and 549.96±250.25ng/ml Vs. 123.15±68mg/ml; p=0.02 and <0.0001 respectively). Serum iron level and TIBC were statistically significantly lower in the studied patients than controls (68.0±40.63µg/dl Vs. 98.9±39.4µg/dl and 340.0±12.5µg/dl Vs. 420.4±20.4µg/dl respectively; p=0.02 for each). Lipogram, showed a statistically significant elevation of TG in the patients group than the control group (133.06±2.3mg/dl Vs. 80.6±4.12mg/dl; p<0.0001). While HDL-C was statistically significantly lower in the study group than in the control group (36.72±8.48mg/dl Vs. 46.0±1.7mg/dl; p=0.02).
Table (2) shows no statistically significant difference in the mean levels of serum prohepcidin in HD patients with hepatitis B or C versus those patients without hepatitis. However, there was a statistically significant increase in the mean serum prohepcidin level in HD patients with hepatitis B compared to those with hepatitis C. This was further clarified in Fig. (1). No significant difference was detected in the mean levels of serum ferritin, iron and TIBC in HD patients with and without hepatitis, nor between HD patients with hepatitis B compared to those with hepatitis C.

Table (1): Demographic and laboratory data of HD patients and Controls (mean ± SD).

<table>
<thead>
<tr>
<th>Test</th>
<th>Group</th>
<th>HD patients (n=60)</th>
<th>Control group (n=20)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td>44.36±8.49</td>
<td>40.00±10.00</td>
<td>N.S</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td></td>
<td>8.77±0.33</td>
<td>14.12±2.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hematocrite ratio</td>
<td></td>
<td>24.54±6.1</td>
<td>44.8±5.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td></td>
<td>29.67±13.33</td>
<td>4.5±0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td></td>
<td>923.23±278.14</td>
<td>89.0±4.00</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td></td>
<td>13.2±7.1</td>
<td>105±5.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td></td>
<td>3.5±0.61</td>
<td>4.5±0.38</td>
<td>N.S</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td></td>
<td>235.30±12.14</td>
<td>84.2±5.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td></td>
<td>22.43±5.29</td>
<td>20.93±8.62</td>
<td>N.S</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td></td>
<td>48.21±10.97</td>
<td>22.75±12.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td></td>
<td>147.98±38.88</td>
<td>154.00±22.7</td>
<td>N.S</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td></td>
<td>133.06±2.3</td>
<td>80.6±4.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td></td>
<td>86.25±30.82</td>
<td>89.7±1.4</td>
<td>N.S</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td></td>
<td>36.72±8.48</td>
<td>46±1.7</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Serum ferritin (ng/ml)</td>
<td></td>
<td>549.96±257.25</td>
<td>123.15±68.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum prohepcidin (ng/ml)</td>
<td></td>
<td>310.65±126.88</td>
<td>71.0±32.4</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Serum iron (µg/ml)</td>
<td></td>
<td>68.0±40.63</td>
<td>98.9±39.4</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>TIBC (µg/ml)</td>
<td></td>
<td>340.0±12.5</td>
<td>420.4±20.4</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

Table (2): Comparison between some markers of iron status in HD patients without hepatitis and with hepatitis B and C (mean ± SD).

<table>
<thead>
<tr>
<th>Test</th>
<th>Group</th>
<th>(1) HD patients without hepatitis (n=22)</th>
<th>(2) HD patients with Hepatitis B (n=16)</th>
<th>(3) HD patients with Hepatitis C (n=22)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prohepcidin (ng/ml)</td>
<td></td>
<td>374.25±376.6</td>
<td>410.00±390.34</td>
<td>150.50±92.86</td>
<td>Between groups (1) versus (2) N.S (1) versus (3) N.S (2) versus (3) &lt;0.05</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td></td>
<td>655.9±288.7</td>
<td>447.59±3.8.09</td>
<td>577.82±206.96</td>
<td>Between groups (1) versus (2) N.S (1) versus (3) N.S (2) versus (3) N.S</td>
</tr>
<tr>
<td>Serum iron (µg/ml)</td>
<td></td>
<td>66.66±46.41</td>
<td>86.18±45.28</td>
<td>79.72±28.65</td>
<td>Between groups (1) versus (2) N.S (1) versus (3) N.S (2) versus (3) N.S</td>
</tr>
<tr>
<td>TIBC (µg/ml)</td>
<td></td>
<td>359.25±84.62</td>
<td>329.81±65.18</td>
<td>337.55±53.65</td>
<td>Between groups (1) versus (2) N.S (1) versus (3) N.S (2) versus (3) N.S</td>
</tr>
</tbody>
</table>

p<0.05 = Significant.    p<0.0001 = Highly significant.  NS = Non significant
Hepcidin, a peptide hormone produced by the liver, constitutes an important link between iron metabolism, host defense and inflammation [10,11]. It has a role as a negative regulator of iron release by duodenal enterocytes and reticulo-endothelial macrophages [8,12].

In the current study, we found that serum pro-hepcidin, and ferritin levels were significantly higher in HD patients in contrast to the serum iron and TIBC where they were significantly lower compared with controls. This was in agreement with Kulaksiz, et al., Malyszko, et al., Costa, et al. and Turgut [4,13-15].

Kulaksiz, et al. [13] suggested that the kidneys may participate in the synthesis of hepcidin and also in its elimination and proved that hepcidin was produced as an intrinsic peptide in the epithelial tubule and duct cells in the mammalian kidney and might be released luminally into the urine. Moreover, Nicolas, et al. [16] noticed that the erythropoietin deficiency in CRF patients may have a role in the higher level of hepcidin noticed in HD patients since erythropoietin is a down regulator of the liver hepcidin gene expression. They found that the gene encoding the hepcidin is regulated by anemia, hypoxia and inflammation. Anemia and hypoxia decrease the liver hepcidin expression while the inflammation dramatically increases its expression and reduces the serum iron. Robb and Wessling-Resnick [17] suggested the induction of hepcidin by dialysis and/or its insufficient removal by the dialysis.

There was significant higher level of hepcidin in hepatitis B patients than those with hepatitis C. These results are consistent with [8]. Who demonstrated lower hepatic mRNA hepcidin expression levels in patients with hepatitis C than those with
hepcidin B and assumed this to down regulation of hepcidin mRNA in liver by direct influence of hepatitis C on hepatic iron accumulation. Also, experimental studies showed that hepcidin transcription was down-regulated through specific inhibition of the promoter by HCV-induced reactive oxygen species [18]. On the other hand Malyszko, et al. [14], noticed that HD patients with and without hepatitis B or C did not differ in hepcidin level.

Nemeth, et al. [7] suggested that inflamatory status in the liver may influence hepatic hepcidin expression. They showed that the inflammatory cytokine IL-6 is the key inducer of hepcidin synthesis during inflammation and they reported that IL-6 infusion causes hypoferremia with increase in urinary hepcidin level. The lack of observation of high level of serum iron in our patients with hepatitis C could be explained by the fact that HD patients are in state of continuous iron loss from gastrointestinal bleeding or most important during hemodialysis during which patients can lose 2g of iron/year [14].

Kulaksiz, et al. [13] noticed that the interaction of a number of specific proteins as well as the interplay between iron absorption and loss are involved in the regulation of iron metabolism [19]. Ferritin is the cellular storage protein for iron and an acute phase reactant. Along with transferrin and transferrin receptors, it is a member of the protein family that orchestrates the cellular defense against stress and inflammation [20]. Our study revealed the highly significant level of serum ferritin in all studied patients in contrast to controls. This finding is in agreement with Eleftheriadis, et al., Malyszko, et al., Malyszko, et al and Turgut [4,14,19,21] who found a higher level of serum ferritin in HD patients. Schindler, et al. [22] suggested that HD patients are in an inflamatory state and this may be due to enhanced incidence of infections and the uremic milieus.

Malyszko and Mysliwiec [11] studied the role of hepcidin in anemia of chronic disease and inflammation. They noticed that iron bound to transferrin is the primary source for hemoglobin synthesis in erythroid precursors and the major iron recycling pathway consists of degradation of senescent erythrocytes by reticuloendothelial macrophages located in the bone marrow, hepatic Kupffer cells and spleen. The exit of iron from macrophages is controlled by ferroportin, which is regulated by hepcidin. During inflammation, hepcidin inhibits iron release from macrophages and enterocytes. Since most iron bound to transferrin is destined to born marrow, hypferremia results in a decrease in iron available for erythropoiesis.

Malyszko, et al. [19] concluded that the elevated hepcidin levels in hemodialyzed patients could be due to functional iron deficiency, anemia and low grade inflammation. Weinstein, et al. [23] suggested that anemia and hypoxia overrides the effect of iron and inflammation and decrease hepcidin mRNA. However, controversy about hepcidin level in HD patients was reported, where [12,21] noticed similar levels of hepcidin in HD and healthy controls despite the presence of hepcidin decreasing factors.

Our study as well as others [4,24] didn’t show significant correlation between hepcidin and different iron parameters in HD patients. On the other hand, Kato, et al. [6] noticed a significant correlation between hepcidin levels and serum ferritin levels.

However, there were significant negative correlations between prohepcidin and hemoglobin level and TIBC in hepatitis C patients. In multiple regression analysis, the only predictors of hepcidin level were hepatitis and TG level. Previous reports on the relationship between hepcidin and hemoglobin levels show inconsistent results [4,21,25].

Ganz [24], Eleftheriadis, et al. [21] and Turgut, et al. [4] found a non significant correlation between hepcidin on one hand and serum iron, ferritin and CRP on the other hand. Turgut, et al. [4] failed to notice any significant correlation between hepcidin and inflammatory parameters such as CRP and albumin in HD patients. Malyszko and Mysliwiec [11] suggested that the lack of finding of significant correlation between prohepcidin and CRP may be due to lack of CRP sensitivity for expressing inflammatory status and presence of other additional confounding regulatory factors.

The finding of significant correlation between hepcidin levels and serum TG levels in multiple regression analysis may stress the role of liver in hepcidin synthesis, as these parameters may reflect the hepatic synthetic function [14,26]. Moreover, [19] showed in their study in HD patients that hepcidin was independently related to TG and creatinine levels.

In conclusion the present study revealed that chronic renal failure patients on hemodialysis have elevated serum prohepcidin. This may be due to low grade inflammation in HD patients and the important role of the kidney in elimination of this hormone. Lower levels of prohepcidin found in
HD patients with hepatitis C than those with hepatitis B may be attributed to hepatic iron overload frequently found in these patients which may down-regulate prohepcidin secretion by the liver. There was significant negative correlation between prohepcidin and hemoglobin level and TIBC suggesting the role of hepcidin in anemia in these patients. Multiple regression analysis revealed that prohepcidin was independently related to the presence of viral hepatitis and triglyceride, suggesting the important role of the liver in hepcidin synthesis. The present study revealed high level of serum prohepcidin in chronic renal failure patients on haemodialysis and its role in anemia. So, in Further, it is possible that a hepcidin antagonist could be developed as a therapeutic tool in these patients and also evaluation of the hepcidin role in patients with hepatitis is needed.

References


