The relationship between anemia, liver disease, and hepcidin levels in hemodialysis patients with hepatitis

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ABSTRACT

We investigated the role of hepcidin in ameliorating anemia in hemodialysis patients with hepatitis. A total of 72 hemodialysis patients with hepatitis were classified according to their requirement of erythropoietin (EPO). Anemia parameters, C-reactive protein (CRP), and biochemical measurements were recorded along with the hepcidin. The number of patients receiving no EPO was higher among patients with liver disease when compared with those without liver disease (P = 0.002). The mean hepcidin levels of the patients who did not receive EPO did not differ statistically from those of the patients who received the maximum dose (P = 0.5). The hepcidin levels of patients with liver disease who received no EPO were lower compared to those patients without liver disease who received the maximum dose (P = 0.04). There was a positive correlation between hepcidin and mean platelet levels (r = 0.26, P = 0.027) and annual intravenous iron dose (r = 0.31, P = 0.007). In hemodialysis patients with hepatitis, liver disease may be one of the factors affecting erythropiesis, related with decreased hepcidin levels and iron hemostasis. Further studies are needed to verify these associations.

Key words: Anemia, erythropoietin, hemodialysis, hepatitis B, hepatitis C, hepcidin

Introduction

Anemia is a common complication of chronic kidney disease and erythropoietin (EPO) – stimulating agents are used to correct anemia in the majority of hemodialysis patients. However, some chronic hemodialysis patients can maintain high hemoglobin levels without the need of EPO. Hepatitis infection is commonly seen in hemodialysis patients and its long-term effects in these patients are unknown. In recent studies, the effects of hepatitis infection on anemia in hemodialysis patients were investigated and the patients with hepatitis were found to have higher hemoglobin levels and were less anemic, which demanded lower EPO doses than in the hepatitis-free hemodialysis patients.[1-4] No explanation is immediately obvious for the role chronic hepatitis plays in ameliorating anemia in hemodialysis patients.

Hepcidin is produced in the liver and it is a key regulator of iron homeostasis by blocking iron absorption from the gut and iron release from macrophage and hepatocyte stores. Given that it is filtered and degraded by the kidney, 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.[5] On the other hand, lower serum hepcidin levels were shown to be related to the severity of liver fibrosis in chronic hepatitis patients without renal failure.[6,7] Combining these data, we hypothesize that the probable lower serum hepcidin levels related with liver disease may have contributed to the improvement of anemia in hemodialysis patients. The associations between anemia, liver disease, and hepcidin levels in hemodialysis patients with hepatitis are investigated in this study.

Materials and Methods

Among 603 patients, a total of 72 hemodialysis patients – 29 with hepatitis B (HBV) and 43 with hepatitis C (HCV) – from the three hemodialysis units of Baskent University Hospital were included in this study. All patients were

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undergoing 4-hour dialysis sessions, 3 times weekly, with bicarbonate dialysate. Patients with known malignancies, active infectious, inflammatory, or hematological diseases, and cirrhosis with a history of gastro-intestinal bleeding were excluded. A total of 72 hemodialysis patients with hepatitis were classified according to their annual requirement of EPO. Patients who were able to maintain nearly normal hemoglobin levels (≥12 g/dL) without the administration of recombinant human EPO for at least a year were recorded as Group 1. The patients who maintained their hemoglobin levels above 10 g/dl with the lowest EPO dose requirements (Epoetin Beta 1 × 40-75 IU/kg/once a week/sc), and the EPO hyporesponsive patients whose hemoglobin levels were under 10 g/dl with the maximum EPO dose (Epoetin Beta 3 × 80 IU/kg/week/sc) were recorded as Groups 2 and 3, respectively. The maximum dose was the upper end of the dosage range allowed for dialysis patients according to instructions. Iron needs (1-year use of intravenous iron sucrose therapy) were noted from the patients' medical recordings. BUN, creatinine, calcium, phosphorus, parathormone, albumin, hemoglobin, LDL and total cholesterol, C-reactive protein, serum iron indices, and hepcidin measurements were recorded before a routine hemodialysis session. The patients were assessed for evidence of liver disease (persistently elevated liver enzymes, HCV RNA, HBV DNA positivity, hepatosplenomegaly and/or histologically active or advanced liver disease proven by liver biopsy). The original Histology Activity Index was used for scoring liver biopsies by using a grading and staging system. The grade gives an indication of the activity or amount of inflammation, and the stage represents the amount of fibrosis or scarring. The fibrosis score is assigned a number from 0 to 4, with 0 being no activity; 1, minimal scarring; 2, periportal fibrosis; 3, septal fibrosis; and 4, cirrhosis or advanced scarring of the liver. Ultrasonographic diagnosis of patients with acquired cystic kidney disease (ACKD) (defined as more than five cysts per kidney) was noted. The relationship between hepcidin levels and laboratory characteristics of the groups was assessed.

The levels of hepcidin-25 were measured by enzyme-linked immunosorbent assay (ELISA), using a commercial kit (DRG Instruments GmbH, Germany). This study was approved by the Baskent University Institutional Review Board (Project No.: KA10/127) and supported by the Baskent University Research Fund.

Statistics

Data were expressed as means ± standard deviation. Comparisons between the groups were made by Chi-Squared and Kruskall-Wallis tests. The non-parametric Mann-Whitney-*U* Test was used to test for significant differences in the mean tendency, and correlations were calculated using Spearman's rank correlation tests. $P < 0.05$ was considered significant.

Results

The mean age of these 72 patients was 49.6 ± 14.1 years. There were 24 female, 48 male patients (male 66.6%). Chronic renal failure was due to hypertension (n = 19, 26.4%), diabetes (n = 16, 22.2%), chronic glomerulonephritis (n = 12, 16.7%), polycystic kidney disease (n = 7, 9.7%), nephrolithiasis (n = 2, 2.7%), and unknown causes (n = 16, 22.2%). Adequacy of dialysis was not statistically different between the groups. Urea reduction rate was 66.3 ± 5.8 for Group 1, 68.1 ± 6.3 for Group 2, and 68.7 ± 6.9 for Group 3. Kt/*V* value was 1.40 ± 0.2 for Group 1, 1.45 ± 0.2 for Group 2, and 1.49 ± 0.3 for Group 3 ($P > 0.05$ for all). Twenty patients (n = 6 Group 1, n = 7 Group 2, n = 7 Group 3) were receiving folic acid and B12 vitamin supplements (27.7%). The number of patients with ACKD was 35 (48.6%) with the exclusion of polycystic kidney disease (n = 7), and between the groups there was no statistical difference related to ACKD ($P > 0.05$). Totally, 15 patients of Group 1 (83.3%), 19 patients of Group 2 (65.5%), and 8 patients of Group 3 (32%) had evidence of various degrees of liver disease. Twenty-four of those patients were diagnosed by liver biopsy. Five of the patients had fibrosis stages 0 and 1, and all five patients maintained their hemoglobin levels above 10 g/dl with the lowest EPO dose requirements (Group 2). Rest of the 19 patients had advanced fibrosis stages 2-4 (only one patient from Group 2 had cirrhosis); nine of them were EPO-free patients (Group 1), seven required the lowest EPO dose (Group 2), and the remaining three were EPO hyporesponsive patients (Group 3). Another 30 patients (41.6%) with hepatitis did not have any findings related with active and/or chronic liver disease.

Erythropoietin need

EPO-free patients (Group 1) were 25% (n = 18, 7 HBV and 11 HCV positive) and did not differ between HBV and HCV patients. In both Group 1 and Group 2, the ratios of male patients were higher than female patients ($P = 0.03$). Duration of hemodialysis, body mass index, and creatinine levels were all found to be higher in Group 1 than those in Group 3 ($P = 0.001$, $P = 0.024$, and $P = 0.04$, respectively). Among those patients in Group 1, transferin saturation indices and ferritin levels were low when compared with those of patients in Group 3 (for all $P < 0.05$). The number of patients receiving no EPO was significantly higher among patients with liver disease when compared with those without ($P = 0.002$).

Hepcidin

The groups of patients did not differ significantly in terms of hepcidin levels and presence of hepatitis B and C. The difference in numbers of patients with liver disease was not statistically significant ($P > 0.05$). The mean hepcidin levels (28.5 ± 25.8 ng/ml) of the patients who did not receive EPO ($n = 18$, 25%), although low, did not differ statistically from those patients who received the maximum dose (30.9 ± 29.9 ng/ml) ($P = 0.5$). The hepcidin levels of patients with liver disease who received no EPO ($n = 15$, 20.8%) were low (27.9 ± 7.6 ng/ml) when compared with those patients without liver disease and who received the maximum dose ($n = 17$, 23.6%) (33.1 ± 10.2 ng/ml, $P = 0.04$). As there was a positive correlation between hepcidin and mean platelet levels ($r = 0.26$, $P = 0.027$) and annual intravenous iron dose ($r = 0.31$, $P = 0.007$), no significant relationship was observed between CRP, ferritin, and other biochemical values [Table 1].

Discussion

Factors contributing to higher hematocrit levels in hemodialysis patients not receiving recombinant human EPO are still unknown. In recent years, few studies have demonstrated the ameliorating effects of chronic hepatitis infection on anemia in hemodialysis patients. In our previous study, we found that 8% of hemodialysis patients without hepatitis were able to maintain nearly normal hemoglobin levels (≥12 g/dL) without the administration of recombinant human EPO whereas the corresponding ratio in hemodialysis patients with chronic hepatitis was greater than 3-fold (25.3%).[8] However, no explanation is immediately obvious for the role chronic hepatitis plays in ameliorating anemia in hemodialysis patients. In addition to chronic hepatitis, other factors found to be possible contributors to normal and/or better hemoglobin levels in hemodialysis patients were male gender, higher body mass index, and more years on hemodialysis therapy.[9] However, serum EPO level was not found to be a factor in the lack of requirement of recombinant EPO, with the exception of some case reports with spontaneous erythrocytosis. Higher leptin levels related to greater body mass index and ACKD associated with more years on hemodialysis therapy were argued to stimulate erythropoiesis in hemodialysis patients. However, the number of relevant studies is very limited and the results are conflicting.

Table 1: Demographic and laboratory characteristics of hemodialysis patients with hepatitis

	Group 1 (*n*=18)	Group 2 (*n*=29)	Group 3 (*n*=25)	*P*
Gender (%)				
Male	31.2	43.7	25.0	0.03
Female	12.5	33.3	54.2	
BMI (kg/m²)	24.8±3.0	22.5±4.2	22.4±5.4	0.02
Hemodialysis duration (months)	124.3±42	89.7±64	56.0±54	0.001
Hemoglobin (g/dl)	12.8±1.0	10.9±0.9	9.9±1.3	<0.001
Transferin saturation (%)	22.8±11.5	34.3±14.3	36.5±20.1	0.008
Ferritin (ng/ml)	415±462	612±349	644±418	0.01
Creatinine (mg/dl)	10.6±1.8	9.9±2.7	8.8±2.4	0.04
C-reactive protein (mg/l)	7.4±4.7	10.1±11	12.8±14	0.96
Parathormone (pg/ml)	406.6±420	449.5±517	424.1±309	0.8
Hepcidin (ng/ml)	28.5±9.5	32.6±12.7	30.9±9.9	0.5
Iron need (i.v., *n* of 100 mg/ampule)	23±13.2	22.4±12.3	22.7±16	0.9

BMI: body mass index

To the best of our knowledge, this study is the first one focusing on a subgroup of hemodialysis patients with hepatitis according to their EPO requirements, liver disease, and hepcidin levels. In recent studies, an association was shown between lower serum prohepcidin, hepcidin, and the degree of liver function impairment in liver cirrhosis. The levels of the hepcidin were supposed to represent a biochemical correlate of fibrosis in chronic hepatitis infection.[10] In this study, the biopsy reports were noted retrospectively, and we did not consider performing liver biopsies simultaneously on our patients who were stable, as such procedures were invasive. However, fibrosis cannot run a remarkably variable course within a certain period of time, so our results may be reliable and support that liver disease and advanced fibrosis stages do not negatively affect EPO requirements in hemodialysis patients. In contrast, most of the patients with advanced fibrosis stages two to four were either EPO-free patients or patients who maintained their hemoglobin levels above 10 gr/dl with the lowest EPO dose requirements.

In this study, the mean hepcidin levels of the patients with hepatitis who did not receive EPO, although low, did not differ statistically from those of the patients who received the maximum dose. Although the *P* value did not show a strong association, the hepcidin levels of patients with liver disease who received no EPO were low when compared with those patients without liver disease and who received the maximum dose. These findings suggest that lower hepcidin levels related with the degree of liver fibrosis may be one of the contributors to the pathogenesis of EPO independence in hemodialysis patients with chronic hepatitis, but this still needs to be supported by further studies. Although the mean level of serum ferritin was above 400 pmol/l and the transferrin saturation was above 20% in all patient groups (both of which values were high due to target-driven treatment with intravenous iron), Group 1 and 2 patients had lower saturation indices and ferritin levels and higher serum iron binding capacities than Group 3. One-year use of intravenous iron sucrose therapy was not different between the groups. All those results may reflect better availability and utilization

of iron for erythropoiesis in Groups 1 and 2. The decrease in hepcidin expression in the liver leads to increased iron absorption through the duodenum and the mobilization of iron from reticuloendothelial stores to meet the demands of erythrocyte production. The level of serum hepcidin has been postulated to correlate with that of serum ferritin levels, however there was less variation in levels, the expected relationship with ferritin and hepcidin was not observed in this study. However, the positive correlation between hepcidin and mean platelet levels and annual intravenous iron dose may also support a relationship between liver disease, hepcidin, and iron metabolism in hemodialysis patients with hepatitis. Hepcidin synthesis is regulated by a number of factors, including iron status, inflammation, erythropoiesis, oxidative stress, hypoxia, hepatitis, and obesity. Sex and genetic background may also modulate hepcidin expression; additionally, an intra-individual variability was noted in short term measurements for serum hepcidin levels.[11,12] So, although our findings may suggest a possible role for hepcidin in better hemoglobin levels, further studies are needed to verify these associations.

The response to EPO varies among individual patients. According to the literature, 90-95% of renal anemia responded in a dose-dependent manner to EPO, whereas rest of the patients tested had a blunted or no response to EPO. In a large number of studies, several factors have previously been reported to contribute to EPO hyporesponsiveness, such as inflammation, oxidative stress, nutritional status, dialysis adequacy, iron deficiency, blood loss, infections, malignancies, secondary hyperparathyroidism, marrow fibrosis, aluminum toxicity, and hemoglobinopathies.[13,14] In this study, EPO hyporesponsive patients with higher ferritin and transferring saturation may reflect the poor utilization of existing iron for erythropoiesis. Factors contributing to this may be complex and multiple.

In contrast to EPO hypo-responsiveness, EPO independence and the factors contributing to it have been investigated only in a limited number of studies. Interestingly, the common findings in those studies have consistently been an increased number of years on hemodialysis therapy and a correlation between hemoglobin and hemodialysis duration in EPO-independent hemodialysis patients.[8,9,15] It is not immediately obvious which mechanisms contributed to the EPO independence in this subgroup of patients who have been on hemodialysis therapy for longer periods of time. So, the question "What stimulates erythropoiesis in these hemodialysis patients even in the absence of sufficient EPO production?" still needs to be answered for such patients with further studies both with and without hepatitis.

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