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The Evaluation of the Role of (Pro)hepcidin in Anemia Encountered in Inflammatory Bowel Diseases

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Objective: Hepcidin is a peptide that acts as a hormone that provides iron homeostasis in the body and has antimicrobial activity. The synthesis of hepcidin is stimulated during inflammation and causes inflammation anemia. For this purpose, we aimed to determine the role of (pro)hepcidin in anemia in inflammatory bowel diseases (IBD) and its correlation with clinical and biochemical findings.

Materials and Methods: A total of 61 patients, 19 (31.1%) of whom were diagnosed with Crohn's disease (CD), 42 (68.9%) with ulcerative colitis (UC), and a control group of 23 were included. Hepcidin and biochemical parameters which are related to anemia were measured.

Results: There was a significant difference between the CD group and vs control group in terms of hepcidin level. As the disease activity increases, the hepcidin level decreases with a probability of 83%. Hepcidin levels were found to be significantly lower in the CD group. Although hepcidin levels were lower in the UC group than in the control group, the result was not statistically significant.

Conclusion: We didn't detect a statistically significant difference in the level of hepcidin between IBD and the control group.

Keywords: Crohn's disease, hepcidin, inflammatory bowel disease, ulcerative colitis

INTRODUCTION

There is no physiological mechanism that provides the elimination of iron from the body. Desquamated epithelial cells from the gastrointestinal tract and/or bleeding result in loss of iron. Therefore, the iron balance is controlled by the aging erythrocytes' recycling mechanism and other sources (1). In addition, the physiological stabilize of this element, which is toxic in large doses, is provided by controlling its absorption.

The hepatic bactericidal protein known today as 'Hepcidin' was named by Park et al. in 2001, and also Krause et al. named Hepcidin as "LEAP-1" (liver expressed antimicrobial peptide) at the same time (2–4). Hepcidin is an antimicrobial protein that works like a hormone in iron metabolism (2). It reduces the absorption of iron from the small intestine, prevents the return of iron from aged erythrocytes to plasma via macrophages, and prevents its mobilization from the stores in the liver (5, 6).

Iron deficiency secondary to bleeding is remarkable in the pathophysiology of anemia in patients with IBD. However, it is thought that possible changes in hepcidin metabolism and the existing chronic inflammation in these patients may contribute to this situation (7, 8). Several methods have been described to measure hepcidin levels, but there is no validated method to assay serum hepcidin reliably (9).

We aimed to assess of the role of hepcidin metabolism in anemia detected in patients with Crohn's Disease (CD) and Ulcerative Colitis (UC).

MATERIALS and METHODS

This study is cross-sectional and observational. 61 patients with chronic inflammation due to inflammatory bowel disease (IBD) and 23 healthy volunteers were included. The diagnosis was made through endoscopic procedures, histopathological examination, and radiological methods. The patients with comorbid diseases were excluded from the study.

The patient registration form questioned age, gender, disease duration, tobacco or alcohol use, location of disease involvement, activation status, and drug use history. Disease activation for UC patients was calculated using the Seo et al. (10) clinical activity index, and for CD patients, the Harvey and Bradshaw (11) clinical activity index was used.

Table 1. Characteristics of patients and control groups

	CD	UC	Control	p
Age (years)*	35 (25–65)	41 (16–89) ^a	27 (21–61) ^a	0.003 [†]
Gender (female)	11 (57.9%)	20 (47.6%)	14 (60.9%)	0.540 [‡]
Tobacco	10 (52.6%)	10 (23.8%)	6 (26.1%)	0.066 [‡]
Alcohol	1 (5.3%)	2 (4.8%)	5 (21.7%)	0.085 [¶]
Place of involvement			N/A	N/A
Pancolitis	6 (31.6%)	15 (35.7%)		
Terminal ileum+cecum	8 (42.1%)	–		
Distal	2 (10.5%)	19 (45.2%)		
Transvers	2 (10.5%)	–		
Terminal ileum+sigmoid	1 (5.3%)	–		
Extensive	–	1 (2.4%)		
Rectal	–	7 (16.7%)		
Duration of the disease (month)*	47 (1-300)	42 (3-236)	N/A	0.073 [¥]
Activation of the disease			N/A	<0.001 [¶]
Remission	15 (79.0%)	–		
Mild	2 (10.5%)	27 (64.3%)		
Moderate	2 (10.5%)	11 (26.2%)		
Severe	–	4 (9.5%)		

CD: Crohn's disease; UC: Ulcerative colitis; *: Data were displayed as median (min–max); †: Kruskal Wallis test; ‡: Pearson's χ^2 test; ¶: Fisher Freeman Halton test; ¥: Mann Whitney U test; N/A: Not applicable; a: UC vs Control (p=0.002)

This study was approved by Haseki Research and Training Hospital Clinical Research Ethics Committee, 2009/44 and it conforms to the Declaration of Helsinki. Written or verbal permission was obtained from each patient.

Biochemical Parameters

The parameters lactate dehydrogenase (LDH), iron, total iron-binding capacity (TBIC), ferritin, transferrin saturation, vitamin B12, folic acid, red blood cells (RBC), hemoglobin (Hb), Hematocrit (Hct), mean corpuscular values volume (MCV) and prohepcidin were evaluated from the blood sample taken from the patients. Biochemical parameters were studied on the Architect 16200 device, hematological parameters Hb, Hct, and MCV values were analyzed using the Advia 120 device, and the ferritin level was measured by the chemiluminescence method using Bio DPC's Immulite 2005 device.

The samples from the patients were centrifuged at 2500 xg and 4 degrees for 10 minutes, then their serums were stored at -20 degrees. In this study, prohepcidin kit (DRG Instruments GmbH, Germany), which was reported to be more reliable in previous studies, was used and studied competitively using the ELISA method. All serums were analyzed following the recommended usage instructions in the kit. Absorbances were read at 450 nm on an Elx800 automated plate reader.

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics version 25.0 software (IBM Corporation, Armonk, NY, US). Categorical data were expressed as numbers (n) and percentages (%) while quantitative data were given as mean±SD and median (min–max).

The mean differences among groups were evaluated by One-Way ANOVA. Depending on the number of independent groups the Mann Whitney U or the Kruskal-Wallis tests were applied for the comparisons of the not normally distributed data. Qualitative data were analyzed by Pearson's χ^2 or Fisher Freeman Halton test, where appropriate. A p-value less than 0.05 was considered statistically significant.

RESULTS

A total of sixty-one patients with IBD [(39.56±13.77/years, (50.8%) female)] constituted the study group. Forty-two of the patients were UC [(40.50±14.96 years, 20 (47.6%) female)], 19 were CD [(37.47±10.73 years 11 (57.8%) female)], and 23 were healthy volunteers [29.87±8.1 14 (60.8%) female)]. There was no difference between the distribution of gender and age (p>0.05). The mean duration of the disease in patients with IBD was 60.31±57.66 months. The demographic features of the groups are represented in Table 1.

There was no difference between the UC vs. CD group in terms of age. On the other hand, there was significant difference between CD vs. control group and UC vs. control group (p=0.560, p=0.002 and p=0.001, respectively), which was because the IBD group had a higher age than the control group (p=0.002). There was no statistically significant difference between the groups in terms of male-female distribution, smoking, alcohol and median disease duration (p>0.05). Disease activation was statistically significantly more severe in the UC group than in those with CD (p<0.001).

Table 2. Biochemistry results related to anemia

	CD	UC	Control	p
Hb (g/dl)*	12.8±2.0	12.8±1.7	13.1±1.4	0.753 [†]
Fe (ug/dl)**	42.0 (5.0–133.0)	49.0 (7.0–203.0)	68.0 (23.0–146.0)	0.570 [‡]
TIBC (ug/dl)**	322.0 (149.0–394.0)	327.0 (231.0–532.0)	335.0 (255.0–471.0)	0.136 [‡]
Ferritin (ug/L)**	46.3 (6.2–729.0) ^a	21.9 (1.6–164.0) ^a	30.1 (3.8–269.0)	0.024[‡]
Transferrin saturation**	14.6 (3.3–53.1)	15.9 (1.5–77.4)	21.8 (5.4–57.2)	0.124 [‡]
LDH (U/L)**	173.0 (101.0–468.0) ^a	200.0 (148.0–433.0) ^{a,b}	165.0 (125.0–508.0) ^b	<0.001[‡]
B12 (pg/ml)**	159.0 (79.0–759.0) ^c	239.0 (87.0–510.0)	279.0 (128.0–692.0) ^c	0.027[‡]
Folate (ng/ml)**	7.1 (3.0–18.8)	7.1 (2.7–20.0) ^b	5.4 (1.9–8.7) ^b	0.016[‡]
Prohepcidin (ng/ml)**	53.8 (27.6–130.8) ^c	64.7 (36.4–128.5)	67.9 (36.8–108.8) ^c	0.040[‡]

CD: Crohn's disease; UC: Ulcerative colitis; Hb: Hemoglobin; Hct: Hematocrit; Fe: Iron; TIBC: Total iron binding capacity; LDH: Lactate dehydrogenase; B12: Vitamin B12, descriptive statistics were shown as * mean±SD** median (min–max); where appropriate; †: One-Way ANOVA; ‡: Kruskal Wallis test; a: CD vs UC (p<0.05); b: UC vs Control (p<0.05); c: CD vs Control (p<0.05)

Biochemistry results was shown in Table 2. There was no statistically significant difference between the groups in terms of hemoglobin, iron, TBIC and transferrin saturation levels, respectively (p>0.05). There was a statistically significant difference between the groups in terms of ferritin levels (p=0.024), which was because the ferritin level of the UC group was lower than the CD group (p=0.020). There was a statistically significant difference between the groups in terms of LDH levels (p<0.001), and the reason for this difference was that the LDH level of the UC group was higher than the CD and control groups (p=0.030 and p<0.001). There was a statistically significant difference between the groups in terms of B12 levels (p=0.027), which was due to the lower B12 level of the CD group compared to the control group (p=0.029). There was a statistically significant difference between the groups in terms of folate levels (p=0.016), and the reason for this difference was the higher folate level of the UC group compared to the control group (p=0.012). Although the CD group had higher folate levels than the control group, the difference was not statistically significant (p=0.292).

There was a statistically significant difference between the groups in terms of hepcidin levels (p=0.040), and the reason for this difference was that the hepcidin level of the CD group was lower than the control group (p=0.037). There was no statistically significant difference in hepcidin levels between CD vs UC and between UC vs control group (p=0.176 and p=0.986) (Fig. 1).

No statistically significant correlation was found between age, disease duration and other biochemical measurements and hepcidin levels (p>0.05). According to gender, there was no significant difference between hepcidin level and activation of disease in both IBD groups.

Hepcidin level was higher in the control group than in the IBD group with anemia, but there was no significant difference in hepcidin levels between the groups divided according to the presence of anemia (p=0.556). There was no difference between anemia and gender and type of the disease. There was no significant difference between IBD and control groups in terms of Hb, iron, TBIC, transferrin saturation, Vit B12 and hepcidin levels (p>0.05). Only the folate level was significantly lower in the control group than in IBD patients (p=0.003).

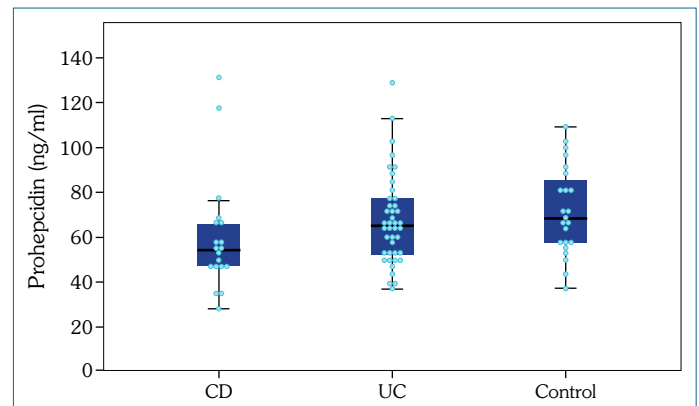


Figure 1. Comparison of pro-hepcidin levels among groups. The horizontal lines in the middle of each box indicates the median, while the top and bottom borders of the box mark the 25th and 75th percentiles, respectively. The whiskers above and below the box mark the maximum and minimum pro-hepcidin levels. Meanwhile, every single circle filled with green indicates that the pro hepcidin levels which belongs to each participant of the study

There wasn't a significant relationship between the severity of the IBD and hepcidin levels. As the disease activity increases, the hepcidin level decreases with a probability of 83%. On the other hand, there was a statistically significant difference in hepcidin levels due to disease activation (p=0.009), and the reason for this difference was that the hepcidin level was higher in the mild severe group than in the remission group (p=0.012). There was no difference in hepcidin levels according to the disease severity status in the IBD groups with and without anemia.

In terms of subgroups of the disease activation, there was no severe CD. The mean hepcidin levels in the mild (n=17) and moderate (n=2) subgroups were 56.59±19.65 and 83.16±67.43, respectively (p=0.842). In the UC group, hepcidin levels in mild (n=27), moderate (n=11) and severe (n=4) activation subgroups were 72.49±20.94 vs 57.01±14.13 vs 68.37±15.88, respectively. As a result of the comparison of disease activation and hepcidin, a difference was found only between mild and moderately activated

subgroups of the UC group ($p=0.046$). p value in the moderate vs severe subgroup is 1 and mild vs severe subgroup is 0.453.

When we excluded the patients with iron deficiency anemia from all 3 groups, we found that in the control group ($n=19$, mean age 30.21 ± 8.81 , mean hepcidin 75.80 ± 18.46); in the CD group ($n=17$, mean age 37.35 ± 11.26 , mean hepcidin 54.71 ± 20.08) and the UC group ($n=29$, mean age 41.69 ± 16.43 , mean hepcidin 66.60 ± 21.45). There was no significant difference between hepcidin and disease activation in the CD group ($p=0.302$). While there was a significant difference between the mild vs moderate subgroup ($p=0.021$) in the UC group, there was no difference with significantly between the mild vs severe ($p=0.401$) and moderate vs. severe ($p=1$) subgroups. In comparison with age, the p -value was found to be 0.176 for control vs CD, 1 for CD vs UC, and 0.012 for control vs UC. In the comparison with hepcidin, p values were determined as 0.002 for control vs CD, 0.121 for CD vs UC, and 0.218 for control vs UC. In the comparison between those with and without chronic disease anemia, no difference was found in both three groups in terms of hepcidin and age [control group ($p=0.655$ and 1, CD group ($p=0.859$ and 0.197), UC group ($p=0.896$ and 0.254)], respectively. Due to the small number of cases in the subgroups, it was thought that the present results should be confirmed with larger samples.

When adjusted for age and gender, hepcidin levels continued to be statistically significantly lower in patients diagnosed with Crohn's disease compared to the control group ($B=-0.264$, 95% CI: $-0.455 - -0.072$ and $p=0.008$). On the other hand, although hepcidin levels continued to be lower in patients diagnosed with ulcerative colitis when adjusted for age and gender, the result was not statistically significant ($B=-0.134$, 95% CI: $-0.301 - 0.034$ and $p=0.116$).

DISCUSSION

Hepcidin is a negative regulator of intestinal iron absorption, placental iron transport, and iron release from macrophages. In patients with IBD, anemia can occur for various reasons, as well as anemia of chronic disease due to chronic inflammation. Therefore, hepcidin levels are predicted to be lower in patients with IBD when other causes of anemia are excluded. But, there was no statistically significant difference between IBD patients and the control group in our study, similar to several previous studies (12).

Hepcidin is secreted locally from the bile or macrophages in the intestinal mucosa. It is produced by dendritic cells in response to microbial signals and subsequently to restrict iron release from intestinal phagocytes by the microbiota to prevent tissue infiltration and promote mucosal healing by *in-vitro* studies (13). It has been suggested that hepcidin expression may be affected by changes in erythropoietic activity and microbiota in a study conducted in rodents with IBD (14).

The relationship between hepcidin level and activation of the disease in IBD is not fully understood (15). Hepcidin synthesis increases significantly with infection and inflammation, and IL-6 is the stimulator responsible for this increase. Increased hepcidin levels during inflammation stimulate the uptake and degradation of ferroportin in macrophages, hepatocytes, and duodenal erythrocytes, and this is leading to iron retention in these cells and inhibition of iron flux into the plasma (2, 16).

It has been reported that serum hepcidin level is associated with disease activation (17, 18), and we also found a significant difference in hepcidin levels due to disease activation ($p=0.009$). No such difference was found in IBD groups with and without anemia. In the Swiss Inflammatory Bowel Disease Cohort Study (SIBDCS), it was stated that hepcidin wasn't an adequate marker for recognizing IBD activation and for distinguishing iron deficiency anemia from anemia of chronic disease (19). The decrease in hepcidin level attendant the decrease in disease activation with Anti-IL6 (siltuximab) and anti-IL6 receptor (tocilizumab) still makes the studies on this point interesting (20, 21).

The main limitations of our study are conducted in a single center and with a small number of patients. Discovering the role of Hepcidin in iron metabolism is thought to lead to new treatment opportunities for anemia of inflammation. Therefore, such studies may gain importance with more reliable methods and larger groups of patients with IBD in further studies.

CONCLUSION

In our study, no statistical difference was found between the presence of anemia and the severity of the disease in IBD patients. However, when evaluated closely with previous studies, there is a lack of larger studies to evaluate the relationship between hepcidin and IBD.

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Author Contributions: Concept – ŞP, ZK; Design – ZK; Supervision – ŞP, MA; Materials – ŞA; Data Collection and/or Processing – ŞA; Analysis and/or Interpretation – ŞA, ZK; Literature Search – ŞA, MA; Writing – ŞA, ŞP; Critical Reviews – MA.

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