Abstract

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.

Methods

It is an observational and cross-sectional study. Totally 61 patients without any other inflammatory condition other than inflammatory bowel disease were included in the study. Hepcidin and biochemical parameters which are related to anemia were measured. All statistical analyses were performed by SPSS version 22.

Results

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. There was no significant difference between groups in terms of hepcidin level. There was no significant difference between IBD and control groups regarding hepcidin levels. As the disease activity increases, the hepcidin level decreases with a probability of 83%.

Conclusion

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

Key words: crohn 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 bleeding result in iron loss.
Therefore, the iron balance is controlled by the aging erythrocytes' recycling mechanism and other sources (1). In addition, the physiological stability 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 (3, 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).

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

**Material and Methods**

This cross-sectional and observational study aimed to examine the relationship between inflammation in inflammatory bowel diseases and hepcidin levels. Sixty-one 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. Patients with a definite diagnosis of inflammatory bowel disease, who were followed up regularly in our center and had no comorbidity that caused an inflammatory condition, were included in the study. The patients who didn’t meet the inclusion criteria 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 clinical activity index, and for CD patients, the Harvey Bradshaw clinical activity index was used (10, 11).

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 (pro)hepcidin 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. The ferritin level was measured using Bio DPC's Immulite 2005 device using the chemiluminescence method.

The samples from the patients were centrifuged at 2500 cycles and 4 degrees for 10 minutes, and 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:**
All statistical analyses were performed by SPSS version 22. Results were expressed as mean ± standard deviation and simple proportions to present relevant patient and procedure characteristics. The Kolmogorov–Smirnov test was applied to determine whether data were normally distributed. Student's t-test compared quantitative data if the parameters were normally distributed and Kruskal-Wallis test in case of non-normally distributed parameters. Categorical variables were found to be used by the chi-square test or the Fisher exact test. Statistical significance was accepted that p-value;< 0.05.

**Results**

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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. Biochemistry results was shown in Table 2.

There was no difference between UC vs. CD group regarding age. On the other hand there was a significant difference between CD vs. control group and UC vs. control group. (p=0.560, p=0.002 and p=0.001, respectively).

According to gender, there was no significant difference between hepcidin level and disease activation in both IBD groups (Table 3).

Hepcidin level was higher in the control group than the IBD group with anemia. Still, 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). The folate level was significantly lower in the control group than in IBD patients (p = 0.003).

There wasn’t had 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% (Figure 1). According to the disease severity status, there was no difference in hepcidin levels 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 the UC group’s mild and moderately activated subgroups (p=0.046).

P value in 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 three groups, we found that in the control group (n=19, median age 27, IQR: 6, median hepcidin 72.07, IQR: 28.20); in the CD group (n=17, median age 35, IQR:16, median hepcidin 52.87, IQR: 15.18) and in the UC group (n=29, median age 43, IQR: 22, median hepcidin 62.58, IQR:27.77). 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 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 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.

**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, and anemia of chronic disease is 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. Subsequently, it restricts 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, 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). Still, we found just a significant relationship between mild to moderate activation of disease in UC group. Also, in the Swiss Inflammatory Bowel Disease Cohort Study (SIBDCS), it was stated that hepcidin wasn’t a good marker for recognizing IBD activation and distinguishing iron deficiency anemia from anemia of chronic anemia disease (19). The decrease in hepcidin level attendant the reduction 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 were 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, more extensive studies are needed to assess the relationship between hepcidin and IBD when evaluated closely with previous studies.

References


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Table 1. Characteristics of patients and control groups.

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Table 2. Biochemistry results related to anemia.

Table 3. Comparison hepcidin level, disease activity and hemoglobin levels between genders with IBD.

Figure 1. Correlation between Hepcidin and activation of disease.

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Table 1. Characteristics of patients and control groups

<table>
<thead>
<tr>
<th></th>
<th>CD</th>
<th>UC</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>37.47 ± 10.73</td>
<td>40.50 ± 14.96</td>
<td>29.87 ± 8.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Gender (Female)</td>
<td>11 (58 %)</td>
<td>20 (47 %)</td>
<td>14 (60 %)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Tobacco (n / %)</td>
<td>10 (52 %)</td>
<td>10 (23 %)</td>
<td>6 (26 %)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Alcohol (n / %)</td>
<td>1 (5.3 %)</td>
<td>2 (4.8 %)</td>
<td>5 (21 %)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Place of involvement n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.951</td>
</tr>
<tr>
<td>- Pancolitis</td>
<td>6 (31.6 %)</td>
<td>15 (35.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Terminal ileum-cecum</td>
<td>8 (42.1%)</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Distal</td>
<td>2 (10 %)</td>
<td>9 (45 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Transvers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Terminal ileum+sigmoid</td>
<td>2 (10 %)</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Extensive</td>
<td>1 (5.3 %)</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Rectal</td>
<td>None</td>
<td>1 (2.4 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>7 (16 %)</td>
<td></td>
<td></td>
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</table>
Table 2. Biochemistry results related to anemia

<table>
<thead>
<tr>
<th></th>
<th>CD</th>
<th>UC</th>
<th>Control</th>
<th>p (IBD vs control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>12.82 ± 2.04</td>
<td>12.79 ± 1.71</td>
<td>13.12 ± 1.4</td>
<td>0.337</td>
</tr>
<tr>
<td>Fe (ug/dl)</td>
<td>52 ± 38.21</td>
<td>57.40 ± 39.52</td>
<td>77.17 ± 37.32</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>TIBC (ug/dl)</td>
<td>296.78 ± 67.53</td>
<td>340.45 ± 71.68</td>
<td>41.73 ± 58.28</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Ferritin (ug/L)</td>
<td>122.89 ± 185.67</td>
<td>31.73 ± 33.32</td>
<td>44.87 ± 56.44</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>17.50 ± 13.38</td>
<td>18.01 ± 14.03</td>
<td>23.4 ± 13.53</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>191.78 ± 83.04</td>
<td>212.83 ± 58.47</td>
<td>179.17 ± 75.27</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>B12 (pg/ml)</td>
<td>217.21 ± 165.41</td>
<td>253.42 ± 106.17</td>
<td>286.82 ± 131.52</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Folt (ng/ml)</td>
<td>8.27 ± 5.04</td>
<td>8.73 ± 4.32</td>
<td>5.63 ± 1.66</td>
<td>0.003</td>
</tr>
</tbody>
</table>

CD: Crohn's disease, UC: ulcerative colitis
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<table>
<thead>
<tr>
<th>Prohepcidin (ng/ml)</th>
<th>59.39 ± 25.81</th>
<th>67.09 ± 19.98</th>
<th>71.47 ± 19.62</th>
<th>0.118</th>
</tr>
</thead>
</table>

Table 3. Comparison hepcidin level, disease activity and hemoglobin levels between genders with IBD.