The relationship between serum hepcidin level and hypoxemia in the COPD patients

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Abstract  Hepcidin has a regulatory role in inflammation, the immune system, and iron metabolism. It has been shown that proinflammatory cytokine interleukin 6 (IL-6) is an important inducer of hepcidin synthesis during infection and inflammation.

Aim of the work: To study the relationship between serum hepcidin level and hypoxemia in the COPD patients and its relation to COPD severity.

Patients and methods: A prospective case control study to compare serum hepcidin levels and other parameters in 70 COPD patients treated at the Pulmonology Department, King Fahad Hospital Dammam, with 34 age and sex matched healthy controls. All subjects participating in the study underwent a complete physical examination and detailed pulmonary function tests (PFTs). A sample from the radial artery for arterial blood gas analysis was done. As well as a panel of other tests including hemoglobin, hematocrit (hct), Iron, CRP, ferritin and total iron binding capacity. A hepcidin prohormone enzyme immunoassay kit (RE 54051, IBL) was used for serum hepcidin measurement.

Results: COPD patients had significantly lower serum hepcidin level compared to the control group (204.60 ± 53.12 and 280.81 ± 50.61, respectively). Furthermore there was a significantly greater reduction in serum hepcidin level in patients with severe COPD compared to patients with mild COPD. A positive correlation was found between serum hepcidin levels and arterial oxygen saturation (SaO2, %) and FEV1 level (P = 0.005). There was a negative correlation between serum hepcidin level and the ages of patients and packs of cigarettes consumed per year (P = 0.003).

Conclusion: Our study demonstrated a significant reduction in serum hepcidin levels in COPD patients, and the degree of reduction correlated with the severity of COPD and hypoxemia.

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Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a major public health problem. It is the fourth leading cause of chronic morbidity and mortality in the United States, and is projected...
to rank fifth in 2020 in burden of disease caused worldwide, according to a study published by the World Health Organization [1]. Local and systemic inflammation, together with arterial hypoxemia and oxygen delivery to tissue, are known to influence the prognosis of COPD [2]. In many chronic inflammatory diseases, changes occur in iron metabolism. Hepcidin, a recently discovered hormone responsible for regulating iron homeostasis, is becoming increasingly important as a possible inflammatory marker. Hepcidin is a peptide hormone produced primarily by the liver and secreted into the circulation. Its synthesis increases in response to iron and inflammation and decreases in response to erythropoiesis. Hepcidin regulates systemic iron metabolism by interacting with its receptor ferroportin, a transmembrane iron export protein. Ferroportin is abundantly expressed on the cell surface of reticuloendothelial [3] macrophages (i.e., resident macrophages of the liver, spleen, and bone marrow) and on the basolateral membrane of duodenal enterocytes. These 2 macrophages of the liver, spleen, and bone marrow and on the basolateral membrane of duodenal enterocytes. Hepcidin inhibits iron release at both of these sites by binding to cell surface ferroportin and causing its internalization and subsequent degradation. Hepcidin can therefore be considered as a negative regulator of iron absorption and recycling. Hepcidin (hepatic bacilcristal protein) discovered by Park et al. [4] is synthesized in the liver, has a role in iron metabolism, and is coded by human hepcidin gene (HAMP: OMIM 606464) located on chromosome 19q13.1 [5,6]. Nemeth et al. [7] have shown that the cytokine interleukin (IL)-6 is necessary and sufficient for the induction of hepcidin during inflammation, establishing that the iron regulatory peptide plays a key role in the anemia of chronic diseases. Induction by IL-6 involves signal transduction by JAK kinase regulation of Signal Activator of Transcription [8,9], adding to the complexity of transcriptional responses dictating hepcidin regulation [10,11]. Although production by the liver most likely accounts for the majority of hepcidin in the systemic circulation the regulatory peptide can be synthesized in other tissues including heart [12,13], kidney [14], adipose tissue [15], spinal cord [16], myeloid cells [17], splenic and alveolar macrophages [18], and monocytes [19].

Aim of the work

To study the relationship between serum hepcidin level and hypoxemia in the COPD patients and its relation to COPD severity.

Patients and methods

The study was approved by the King Fahad Hospital Ethics and Research committee. All patients and control subjects gave their written informed consents before participating in the study. The current study included 2 groups: Group I: 70 COPD patients aged (mean ± SD) 63.18 ± 7.19 years, between January 2013 and August 2014, and who were in stable condition were included in this study, which was seen at the Pulmonology Department of King Fahad Hospital Dammam, Kingdom of Saudi Arabia, and Group II: 34 age and sex matched randomly selected control subjects with no pulmonary disease aged (mean ± SD) 62.6 ± 6.1 years. The patients were divided according to the GOLD criteria as follows: Mild COPD (n:20), Moderate COPD (n:32), and Severe COPD (n:18). The severity of the COPD was categorized into mild (forced expiratory volume in one second/forced vital capacity [FEV1/FVC < 0.70, FEV1 ≥ 80% predicted), moderate (FEV1/FVC < 0.70, 50% ≤ FEV1 < 80% predicted), and severe (FEV1/FVC < 0.70, 30% ≤ FEV1 < 50% predicted). The treatment of our patient’s protocols was explained to GOLD criteria [20]. All the patient group and the control group included in the study had C-reactive protein (CRP) levels within the normal limits (upper normal level, 10 mg/l). Anemia was defined as Hb < 12 g/dl. Baseline arterial oxygen saturation was defined ≤88%. The study included patients with COPD over 40 years of age, having a diagnosis of COPD according to the GOLD 2009 [20], being at a stable phase of COPD.

Exclusion criteria

Exclusion criteria were acute COPD exacerbations in the last 3 months (increased cough, dyspnea, sputum production, and/or purulence) [20], other infections, a blood transfusion in the last 6 months, anti-inflammatory therapy (oral, parenteral systemic glucocorticosteroids) within the last 3 months, and anemia within the last 6 months. Demographic information and smoking history of patients were recorded. Body mass indexes (BMI; kg/m²) were calculated for all groups. Respiratory function tests were performed in all patients at the respiration laboratory of our clinic using a Jaeger spirometer according to Thoracic Society (ATS) guidelines [21] using Vmax29 Sensor Medics (VIASYS). FEV1, FVC, and FEV1/FVC (Tiffeneau index) parameters were measured at least three times and the values were recorded. The lung graphs of the patients were evaluated. The patient group and the control group also underwent physical examinations.

In all patient group and the control group included in the study, blood and serum samples for full blood testing were taken in the morning on an empty stomach. Serum iron, total iron binding capacity, ferritin, hemoglobin, and hematocrit (hct) levels were assessed using standard laboratory methods. Serum samples for hepcidin were centrifuged for 10 min at 3000 rpm and stored at −20 °C. A hepcidin prohormone enzyme immunoassay kit (RE 54051, IBL) was used for serum hepcidin measurement. Also, a sample from the radial artery for arterial blood gas analysis was obtained.

The statistical analysis

Comparison among the groups was conducted using Student’s t-test for normally distributed variables. The nonparametric Mann–Whitney U test was used for variables with a non-Gaussian distribution. Normally distributed variables were expressed as mean ± SD. For correlation studies, the Pearson correlation test was used. A P value of <0.05 was considered statistically significant. The commercial statistical software package used was SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The relationship of hepcidin levels with other variables was examined using the Spearman Correlation coefficient.

Results

Demographic information and laboratory results of the COPD patients group and the control group are shown in Table 1.
The serum hepcidin level in the healthy control group was higher than the COPD patient group with significant difference (280.81 ± 50.61 and 204.60 ± 53.12), respectively (P < 0.001). Among the COPD patient group, the hepcidin level in the mild COPD patient group had the highest mean level (259.72 ± 49.81 ng/ml), and the severe COPD patient group had the lowest mean hepcidin level (149.33 ± 31.74 ng/ml). The mean level in the moderate COPD patient group was found to be (201.25 ± 24.9 ng/ml). The mean level in the control group was 280.81 ng/ml. The serum hepcidin level in the mild COPD patient group was higher than the moderate and severe COPD patients. There was no difference in terms of hepcidin levels between the moderate and severe COPD patient groups (P = 0.624) and there was difference in hepcidin levels between mild and severe COPD patients group (P = 0.003) are shown in Table 2. Each COPD patient group was compared with the normal group. There was no difference between the healthy control group and the mild COPD patient group (P = 0.781), whereas there was a difference between the control group and the moderate (P = 0.004) and severe (P = 0.002) COPD patient groups. The hepcidin level of the control group was higher than the moderate and severe COPD patient groups. Fig. 1 shows the serum hepcidin levels in COPD patients and control group. Serum hepcidin levels were lower in COPD patients than the control group, and decreased by severity of COPD. There was a positive correlation between serum hepcidin level and hct (r = 0.53, P = 0.006), iron (r = 0.69, P = 0.000), ferritin (r = 0.48, P = 0.001), PaO2 (r = 0.67, P = 0.000), SaO2 (r = 0.51, P = 0.001) Fig. 2, and FEV1 level (r = 0.51, P = 0.009). There was a negative correlation between serum hepcidin level and the ages of patients (r = 0.63, P = 0.002), packs of cigarettes consumed per year (r = 0.45, P = 0.003).

Discussion

In our study, serum hepcidin levels were significantly different between the healthy control group and the COPD patient group (P = 0.004), serum hepcidin levels were significantly different between the healthy control group and the moderate COPD patients (P = 0.004), between the healthy control group and the severe (P = 0.002) COPD patients, between the mild and moderate COPD patients (P = 0.001), and between the mild and severe COPD (P = 0.003) patients group. No relationship was found between Serum hepcidin levels in the healthy control group and the mild COPD patients (P = 0.541). These findings suggest that hepcidin could be an important marker which could be used to evaluate the disease state. In COPD patients, tissue hypoxia occurs when the partial oxygen pressure (PaO2) falls below 60 mmHg which causes systemic effects. Serious complications may occur when vital organs cannot receive sufficient oxygen. Hypoxemia is seen more in the severe COPD patient group than in the mild and moderate COPD patient groups. Systemic hypoxia reduced hepcidin production in the liver. However, the molecular mechanisms in which hypoxemia plays a role to repress hepcidin production has not yet been fully understood [22].

In our severe COPD patient group, there was a positive correlation between serum hepcidin levels and PaO2 (P = 0.000). This result demonstrates that there is a correlation between the degree of hypoxemia and the serum hepcidin level in the severe COPD patient group. The serum level of hepcidin that increases with inflammation and decreases with hypoxia and anemia. Hepcidin is a liver produced peptide implicated in the anemia of inflammation [23]. Inflammation increases hepcidin expression. Also, serum hepcidin was abnormally increased in patients with inflammation (CRP > 10 mg/L), in patients with multiple myeloma [24,25] and in patients with chronic kidney disease [26] without associated inflammatory disorders. In a study, it was found that hepcidin was expressed by airway epithelial cells and was induced by both interferon gamma and IL-6 in a cell-specific pattern and may serve as a protective factor through its direct antimicrobial effects [27].

Our patient group and the control group had no anemia and high serum CRP levels. Hepcidin plays a crucial role in the anemia of chronic disease. We think that in our severe COPD patient group, the decrease in serum hepcidin levels is related to hypoxemia rather than inflammation. In another study it was shown that hepcidin is also produced in mouse macrophages infected with intracellular Mycobacteria [28]. However, there are conflicting findings about the serum hepcidin level related to acute and chronic inflammation in the literature. In a previous study, the pro-hepcidin concentration was significantly higher in the patients with active rheumatoid arthritis (RA) than those with inactive to moderate RA [29].

Our results showed that the serum hepcidin level increased with increasing hct (P = 0.009) and serum iron (P = 0.000) levels in the severe COPD patient group. Hepcidin production

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Table 1 Demographic and laboratory characteristics of the COPD patient groups and the control group.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=34)</th>
<th>COPD patients (n=70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>62.6 ± 6.1</td>
<td>63.18 ± 7.19</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.21 ± 3.41</td>
<td>23.81 ± 5.12</td>
</tr>
<tr>
<td>Cigarette (package/year)</td>
<td>–</td>
<td>36.20 ± 3.15</td>
</tr>
<tr>
<td>Hemoglobin (12–16 g/dl)</td>
<td>14.7 ± 2.09</td>
<td>15.4 ± 1.99</td>
</tr>
<tr>
<td>Iron (Fe) (50–170 g/dl)</td>
<td>83.2 ± 6.03</td>
<td>83.96 ± 4.92</td>
</tr>
<tr>
<td>Ferritin (18–250 ng/dl)</td>
<td>89.72 ± 13.6</td>
<td>90.25 ± 11.3</td>
</tr>
<tr>
<td>TIBC (228–428 g/dl)</td>
<td>301 ± 26.4</td>
<td>298.97 ± 30.12</td>
</tr>
<tr>
<td>SaO2 (%)</td>
<td>97.16 ± 2.56</td>
<td>93.54 ± 3.89</td>
</tr>
<tr>
<td>PaO2 (mmHg)</td>
<td>95.88 ± 1.5</td>
<td>69.8 ± 14.23</td>
</tr>
<tr>
<td>PaCO2 (mmHg)</td>
<td>41.24 ± 3.1</td>
<td>60.28 ± 10.32</td>
</tr>
<tr>
<td>FEV1% of predicted</td>
<td>90.85 ± 3.9</td>
<td>65.33 ± 12.8</td>
</tr>
<tr>
<td>Hepcidin (ng/ml)</td>
<td>280.81 ± 50.61</td>
<td>204.60 ± 53.12</td>
</tr>
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</table>

BMI = body mass index; TIBC = total iron binding capacity, PaO2 = partial arterial oxygen pressure; PaCO2 = partial arterial carbon dioxide pressure; SaO2 = arterial oxygen saturation.
also correlated with the serum ferritin level. It was found that the serum hepcidin level increased when the serum ferritin level increased in the severe COPD patient group. In our study, no relationship was found between BMI and serum hepcidin levels in the moderate and severe COPD patient groups. Only a positive correlation was found between hepcidin levels and BMI \((P = 0.01)\) in the mild COPD patient group. Impaired pulmonary function is a strong risk factor for the development of COPD and a marker of disease severity. BMI has a negative effect on the quality of life of patients with COPD, and previous studies have found a relationship between poor prognosis, mortality, and BMI \([30,31]\). A number of studies have found a relationship between low serum hepcidin levels and BMI \([32,33]\). Also, the correlation between serum hepcidin levels and BMI was shown in some studies on obesity \([34]\). All of our patients had normal or low BMI. In our study, serum hepcidin levels decreased with aging \((P = 0.002)\). However, this relation may come from multifactorial reasons and more likely to be related to hepatic function, a decrease in lung and chest wall compliance, an increase in prevalence of a ventilation–perfusion disorder, low arterial oxygen pressure, and an increase in physical dead space make hypoxemia more apparent. The hepatic synthesis of hepcidin increases when the serum iron concentration goes up. In contrast, hepcidin synthesis decreases when there is iron deficiency.

**Conclusion**

Our study demonstrated a significant reduction in serum hepcidin levels in COPD patients, and the degree of reduction correlated with the severity of COPD and hypoxemia.

**Conflict of interest**

The authors have disclosed no conflicts of interest.

**References**


**Table 2** Serum hepcidin level among COPD patients.

<table>
<thead>
<tr>
<th>COPD patients</th>
<th>Moderate (n:32)</th>
<th>Severe (n:18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepcidin (ng/ml)</td>
<td>201.25 ± 24.9</td>
<td>149.33 ± 31.74</td>
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**Figure 1** Serum hepcidin level among COPD patients and the control group.

**Figure 2** Positive correlation between the serum hepcidin level and SO2.


I. Aeberli, R. Hurell, Overweight children have higher circulating hepcidin concentrations and lower iron status but have dietary iron intakes and bioavailability comparable with normal weight children, Int. J. Obes. 33 (2009) 1111–1117.