Serum prohepcidin and hepcidin levels in patients with ankylosing spondylitis: a prospective study

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Abstract

Background: Anemia is a common complication in patients with inflammatory diseases such as ankylosing spondylitis. Recent data suggest that hepcidin is a major mediator of anemia with a central role in iron homeostasis and metabolism. The aim of this study was to evaluate the serum levels of hepcidin and its prohormone, prohepcidin, in patients with ankylosing spondylitis in comparison with healthy controls and patients with iron deficiency anemia.

Methods: Forty patients with ankylosing spondylitis (13 with anemia and 27 without anemia), 30 patients with iron deficiency anemia and 20 healthy adults were prospectively enrolled. Complete blood count, erythrocyte sedimentation rate serum hepcidin, prohepcidin, iron, total iron binding capacity, ferritin, transferring, and C-reactive protein levels were measured.

Results: Serum prohepcidin and hepcidin levels were significantly higher in patients with ankylosing spondylitis compared with both healthy controls and patients with iron deficiency anemia (p<0.005). In patients with ankylosing spondylitis, positive correlation was determined between the serum hepcidin and prohepcidin levels (respectively;185±44, 73±7, p<0.05).

Conclusions: To the best of our knowledge, this is the first report of serum prohepcidin and hepcidin levels in the patients with ankylosing spondylitis. Serum prohepcidin and hepcidin levels are closely associated with disease activity in patients with ankylosing spondylitis and might play a role in the pathogenesis of anemia of chronic disease associated with ankylosing spondylitis.
Background

Ankylosing spondylitis (AS) is a frequently occurring chronic inflammatory disease that causes arthritis of the spine, and other large joints. Its pathogenesis is incompletely understood [1]. It is a member of the group of the spondyloarthropathies with a strong genetic predisposition.

Although causes of anemia are multifactorial, the most common form of anemia in patients with AS is anemia of chronic disease (ACD) [2]. Shortened erythrocyte lifespan, impaired iron metabolism, and impaired erythropoietin response are suggested to be involved in the pathogenesis of ACD which is also called anemia of inflammation [3]. Systemic and/or local (bone marrow) production of cytokines directly or indirectly influence erythropoiesis [4]. Iron deficiency anemia (IDA) can be caused by dietary deprivation of iron or by iron malabsorption and may be the first clinical sign of increased blood loss. Quite often, IDA warrants extensive investigations of the gastrointestinal tract, given the relatively high probability that ulcers or malignant tumors are the cause of excessive blood loss. The distinction between IDA and ACD is not clear; the commonly used laboratory tests do not necessarily distinguish these common causes of anemia [5]. Conventional laboratory indices of iron status include serum iron, transferring, total iron binding capacity, transferrin saturation, and ferritin. Although each of these measurements has merit, no single determination gives a reliable index of iron status [6].

Hepcidin, a recently discovered anti-microbial, cysteine-rich cationic peptide, decreases intestinal iron absorption and on release from stores like macrophages, hepatocytes and enterocytes [7]. It is proposed that hepcidin may be playing a key role in the ACD pathogenesis, due to its effect on iron metabolism, and its close relation with cytokines/inflammation [8,9]. Hepcidin and its prohormone, prohepcidin levels were found to
be increased 100 times during inflammation, which resulted in decrease in iron absorption and retention of iron in macrophages, decrease in serum iron and eventually causing the ACD [9].

The aim of this study was to examine the role and significance of hepcidin and its prohormone, prohepcidin on the development of ACD which is frequently seen in patients with AS and the possible utilization of serum prohepcidin and hepcidin levels in the differential diagnosis of ACD and IDA.

Methods

Study Design: The study has been approved by an institutional review board and subjects have given informed consent. All study was carried out in accordance with the World Medical Association Declaration of Helsinki. AS was defined according to the American College of Rheumatology criteria of 1987.

Complete blood count, erythrocyte sedimentation rate (ESR), serum hepcidin, prohepcidin, iron, total iron binding capacity (TIBC), ferritin, transferring, and C-reactive protein (CRP) levels were measured. The normal ranges of values were 50 to 170 ng/dL for serum iron, 120-420 ng/dL for TIBC, 192 to 282 mg/dL for serum transferrin, and 15 to 150 ng/mL for serum ferritin. The blood samples of 2 ml were collected into the EDTA tubes from the patients in the morning at the end of 12-14 hours of fasting. The analysis of prohepcidin (No:12K069-3) and hepcidin (No:39K119) were carried out at room temperature with ELISA kit by using Kayto RT 2100 C microplate reader (Kayto Electronics, China).

Definition of anemia: Anemia was defined by a hemoglobin (Hb) concentration <13.0 g/dL in males and <12.0 g/dL in females [10]. According to the World Health Organization (WHO) mild anemia corresponds to a Hb ≥9.5 g/dL, moderate anemia to a Hb ≥8 g/dL but <9.5 g/dL, and severe anemia to a Hb <8.0 g/dL. Iron-deficiency anemia was characterized by the
presence of anemia associated with low serum ferritin (<10 ng/mL for females, <15 ng/mL for males) or with a transferrin saturation <16% together with serum ferritin levels <30 ng/mL. The diagnosis of ACD required the presence of reduced transferrin saturation (<16%), normal/reduced serum transferrin with normal/high serum ferritin (>100 ng/mL) [11].

Patients were not eligible for the study if other conditions which could cause anemia or interfere with erythropoiesis were present (malignancy, previous chemotherapy or radiotherapy, connective tissue diseases, infections, other inflammatory diseases).

Statistical Analysis: The statistical analysis was performed using the program of “SPSS 18.0 for Windows”. Arithmetic mean and standard deviations of the parameters were measured. Since the measured parameters don’t tests were carried out the significance level was accepted as p<0.05.

Results

Forty patients with ankylosing spondylitis, (25 male and 15 female; mean age, 38±9), 30 patients with iron deficiency anemia (24 male and 6 female; mean age, 31±5) and 20 healthy adults as a control group (12 male and 8 female; mean age, 29±8) were prospectively enrolled. Baseline characteristics of patients with AS, patients with IDA and healthy controls were shown in Table 1.

Serum prohepcidin levels in patients with AS (185±44) were significantly higher than those with IDA group (109±8) and healthy controls (123±18) (p<0.05). The difference between IDA group (109±8) and healthy controls (123±18) for serum prohepcidin level was statistically significant (p<0.05). In patients with AS, a positive correlation was demonstrated between serum prohepcidin and CRP levels.
Serum hepcidin levels in patients with AS (73±7) were significantly higher than those with IDA group (38±18) and healthy controls (45±10) (p<0.05). The difference between IDA group (28±18) and healthy controls (45±10) for serum hepcidin level was statistically significant (p<0.05).

Hemoglobin levels in IDA group (8.9±10) were significantly lower than both AS group (13.1±1) and healthy controls (14.1±2) (p<0.05). Serum iron levels in both AS (36±21) and IDA groups (17±10) were significantly lower than healthy controls (89±15) (p<0.05). Serum TIBC levels in patients with AS (227±62) were significantly lower than those with IDA group (418±44) and healthy controls (315±21) (p<0.05).

Serum ferritin levels in AS group (67±6) were significantly higher than IDA group (7±6) (p<0.05). No significant difference in serum ferritin levels was found between patients with AS (67±6) and healthy controls (69±13) (p>0.05). A positive correlation was demonstrated between serum prohepcidin and ferritin levels. Serum transferrin levels in AS group (143±8) were significantly lower than both IDA group (193±2) and healthy controls (205±7) (p<0.05).

ESR rates in patients with AS (28±16) were significantly higher than those with IDA group (14±8) and healthy controls (10±5) (p<0.05). Serum CRP levels patients with AS (19±16) were significantly higher than those with IDA group (2.9±1.2) and healthy controls (6±5) (p<0.05).

**Discussion**

Our data mainly suggest that serum hepcidin and prohepcidin levels are significantly higher in patients with ankylosing spondylitis compared with both healthy controls and patients with iron deficiency anemia. To our knowledge, this is the first reported study to measure serum hepcidin and prohepcidin levels in patients with AS.
Hepcidin production was shown to be increased in vivo and in vitro experimental and clinical inflammation models [9,12]. It is exclusively produced in the liver and it circulates in plasma, consistent with its postulated role as a hormone involved in iron homeostasis [13,14]. Further, hepcidin mRNA expression is increased in response to inflammatory stimuli such as lipopolysaccharide and infection [15]. Although it has not yet been shown to interact with proteins of iron transport, its apparent activity suggests that hepcidin directly regulates the iron transport machinery [16]. Nemeth et al. indicated that in acute inflammation, urinary hepcidin excretion is increased when compared to the control group [9]. Malyszka et al. and Dallalio et al. reported increased prohepcidin levels in the chronic hemodialysis patients [17,18]. Demirag et al. indicated that hepcidin levels were positively correlated with disease activity and negatively correlated with hemoglobin values [19]. Hepcidin levels in patients with active rheumatoid arthritis increased when compared to patients with inactive rheumatoid arthritis [11]. In our study, serum prohepcidin and hepcidin levels in AS group were significantly higher than healthy controls.

It was reported that hepcidin production increases in iron load [7,9], and decreases in rats fed with low iron [15]. In clinical studies urinary hepcidin [9] and serum pro-hepcidin [11] levels were shown to be high in ACD group in comparison to IDA group. In our study, prohepcidin and hepcidin levels were higher in the ACD group than IDA group.

Serum transferrin level was reported to be more useful than serum iron level and total iron binding capacity in measuring the body iron status. Kahgo et al., in their study, indicated that serum soluble transferrin receptor level reflected the cellular iron shortage and could be used in differential diagnosis of ACD and IDA [20]. In our study, serum transferrin levels in AS group were significantly lower than healthy controls. Serum transferrin levels in IDA group were significantly lower than both healthy controls and patients with AS.
Serum ferritin level is the most frequently used laboratory parameter to distinguish between ACD and IDA [21,22]. Serum ferritin levels are decreased in IDA while normal or increased in ACD. Serum ferritin level increases as acute phase reactant in AS. Hepcidin is known to be closely associated and positively correlated with ferritin [9,18] but there are also reports of correlation between prohepcidin and ferritin levels [17,23-25]. A positive correlation was demonstrated between serum prohepcidin and ferritin levels in chronic renal failure [17]. Furthermore, Nagashima et al. reported that serum prohepcidin levels negatively correlated with ferritin levels in patients with viral hepatitis C, while this correlation was positive in patients with viral hepatitis B and healthy controls [23]. On the other hand, in other studies serum prohepcidin levels were reported as unrelated with ferritin or other iron parameters [24,25]. In our study, serum ferritin levels in IDA group were significantly lower than both healthy controls and patients with AS. No significant difference in serum ferritin levels was found between healthy controls and patients with AS.

Literature data point to raised C-reactive protein (CRP) concentration as a marker of systemic inflammation in AS patients [26]. In our study, serum CRP levels in AS group were significantly higher than both healthy controls and patients with IDA. Positive correlation was determined between serum CRP levels and serum prohepcidin levels.

Erythropoiesis is highly dependent upon iron availability, and the most common nutritional cause of anemia is iron deficiency [4]. Normally, most iron used for erythropoiesis is recovered from the degradation of red blood cells by reticuloendothelial macrophages. When this recycling process is inefficient or macrophage iron release is inhibited, serum transferrin saturation falls and erythropoiesis is impaired [2]. Infection, malignancy, and chronic inflammation all may result in inefficient macrophage iron release and subnormal intestinal iron absorption, contributing to the anemia of chronic disease. These alterations have the effect of limiting the availability of iron to red blood cell precursors, even though total body
iron stores may be adequate early in the course of the anemia. Some investigators have hypothesized that elevated cytokine levels induce changes in normal transfer of iron between macrophages and developing red blood cells, and some cytokines have been shown to alter the expression of macrophage transferrin receptor and ferritin, but there is currently no direct evidence that any particular cytokine inhibits cellular iron egress [27]. In our study, IDA group had significantly higher TIBC than other groups. IDA group also had significantly lower serum ferritin levels than AS group and healthy controls. In addition, IDA group had significantly lower serum iron levels than AS group and healthy controls. Pure iron deficiency anemia is hypochromic-microcytic in character and, in this type of anemia, serum iron and ferritin levels are low, and TIBC is high [3]. Classically, chronic disease anemia is associated with low serum iron and TIBC and high or normal serum ferritin levels [2]. In the present AS patients, serum iron status was consistent with this classical data. Chronic disease anemia may not only be normochromic-normocytic it may also have hypochromic-microcytic or normocytic features [28]. Vreugdenhil et al have shown that the anemia was normochromic-normocytic in 60% and hypochromic-normocytic in 30% of those with chronic disease anemia [28]. Differentiation between iron deficiency and chronic disease anemia can sometimes be difficult, especially when they both coexist. Iron deficiency is not infrequent in anemic RA patients. These data suggest that hepcidin is an important pathogenetic marker in pathobiology of anemia in RA patients without iron deficiency. In our study, in addition to hepcidin, we found that the serum iron level was a significant predictor for Hb level in all AS patients.  

This study has some limitations. In inflammatory diseases, IDA can coexist with ACD due to poor intake and/or absorption and increased loss of iron, and so, to differentiate between ACD and IDA may be difficult [3]. Thus, our failure to use more pertinent indicators such as transferrin receptor to distinguish between ACD and IDA may be one of the limitations of the
present study. The hemochromatosis gene is an upstream regulator of hepcidin, and it could influence the prohepcidin levels in some individuals, so not to determine the hemochromatosis gene mutation status may be second limitation of this study.

Conclusions

Serum prohepcidin and hepcidin levels are closely associated with disease activity in patients with AS and might play a role in the pathogenesis of anemia of chronic disease associated with AS. To the best of our knowledge, this is the first report of serum prohepcidin and hepcidin levels in the patients with AS.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

MD designed the study, analyzed and interpreted the data, drafted and revised the article and approved for the last version to be published. SY analyzed and interpreted the data, revised the article and approved for the last version to be published. AS analyzed and interpreted the data, revised the article, and approved for the last version to be published. TD obtained the data, and approved for the last version to be published.

Acknowledgements
References


**TABLES**

Table 1. Baseline characteristics of patients with ankylosing spondylitis, patients with iron deficiency anemia and healthy controls.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>AS Group</th>
<th>IDA Group</th>
<th>Control Group</th>
</tr>
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<tbody>
<tr>
<td>Age, yr</td>
<td>38±9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31±5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29±8&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Complete blood count</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hemoglobin, gr/dL</td>
<td>13.1±1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.9±10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.1±2.1&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>MCV, fl</td>
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<td>73±5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88±9&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>ESR, mm/h</td>
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<td>14±8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10±5&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Prohepcidin, mg/dL</td>
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<td>109±8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>123±18&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Hepcidin, mg/dL</td>
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<td>28±18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.3±10&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Fe, ng/dL</td>
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<td>17±10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89±15&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Transferrin, ng/mL</td>
<td>143±8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>193±2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>205±7&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>CRP, mg/dL</td>
<td>19±16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9±1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.6±1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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The letters (<sup>a,b,c,d</sup>) indicate the level of significance (p<0.05) of differences between groups in measured parameters. AS = Ankylosing spondylitis; IDA = Iron deficiency anemia; MCV = Mean corpuscular volume; ESR = Erythrocyte sedimentation rate; TIBC = Total iron binding capacity; CRP = C-reactive protein.
Additional files provided with this submission:

Additional file 1: BMC_Cover_Letter.doc, 29K
http://www.biomedcentral.com/imedia/2135417504588336/supp1.doc