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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. *Objective;* 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. Methods: Forty patients with ankylosing spondylitis (13 with anemia and 27 without anemia), 20 healthy adults (HA) 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. ($p<0.005$). 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.

Key word: Ankylosing Spondylitis · Prohepcidin · Hepcidin · Anemia

INTRODUCTION

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 morrow) production of cytokines directly or indirectly influence erythropoiesis [4]. The distinction between HA 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, in addition, inhibits the release of iron from iron storage sites located in macrophages, hepatocytes

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and enterocytes [7]. It is proposed that hepcidin may be p laying 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 [9,10] .

MATERIALS AND METHODS

Study Design: Prospective cross-sectionnal cohort study of patients with AS and with HG. The study has been approved by an institutional review board (Selçuklu Medicene Faculty Etic 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 (C R P) 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. 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, other spondylarthropathies like Psoriasis, Crohn's disease and ulcerative colitis).

Statistical Analysis: Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Version 18.0, Chicago, IL, USA). The results were expressed as mean ± SD. The comparisons between two groups were assessed using Mann-Whitney U test.Parametrics were performed using the ındependent-samples T test. Correlation analysis was performed using Pearson correlation tests. Multivariate analysis were performed with linear regression analysis. P values of < 0.05 were considered to indicate statistical significance.

RESULTS

Forty patients with ankylosing spondylitis (13 with anemia and 27 without anemia) (25 male and 15 female; mean age, 38±9) and 20 healthy adults(HA) as a control group (12 male and 8 female; mean age, 29±8) were prospectively enrolled. Baseline characteristics of patients with AS and healthy controls were shown in Table 1. Serum prohepcidin levels in patients with AS (185±44) were significantly higher than healthy controls (123±18) ($p<0.05$). In patients with AS, a positive correlation was demonstrated between serum prohepcidin and CRP levels. ($p>0.005$)

Serum hepcidin levels in patients with AS (73±7) were significantly higher than healthy controls (45±10) ($p<0.05$). Hemoglobin levels in AS group (13.1±1) were not significantly healthy controls (14.1±2). Serum iron levels in AS (36±21) were significantly lower than healthy controls (89±15) ($p<0.05$). Serum TIBC levels in patients with AS (227±62) were significantly lower than healthy controls (315±21) ($p<0.05$).

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 healthy controls (205±7) ($p<0.05$).

ESR rates in patients with AS (28±16) were significantly higher than healthy controls (10±5) ($p<0.05$). Serum CRP levels patients with AS (19±16) were significantly higher than healthy controls (6±5) ($p<0.05$).

Table 1: Baseline characteristics of patients with ankylosing spondylitis, patients with iron deficiency anemia and healthy controls.

	AS Group	Control Group	P Values
Age, yr	39±10	31±10	NS
Hemoglobin, gr/dL	13.1±1	14.1±2.1	NS
MCV, fl	83±7	88±9	NS
Prohepcidin, mg/dL	185±44	123±18	0.05
Hepcidin, mg/dL	73±7	45.3±10	0.01
Fe, ng/dL	36±21	89±15	0.03
TIBC, ng/dL	227±62	315±21	0.04
Ferritin, ng/mL	67±60	69±31	0.45
Transferrin, ng/mL	143±8	205±7	0.05
CRP, mg/dL	19±16	2,6±1	0.05
ESR, mm/h	28±16	10±5	0.05

AS = Ankylosing spondylitis; IDA = Iron deficiency anemia; MCV = Mean corpuscular volume; ESR = Erythrocyte sedimentation rate; TIBC = Total iron binding capacity; CRP = C-reactive protein

DISCUSSION

Our data mainly suggest that serum hepcidin and prohepcidin levels are significantly higher in patients with ankylosing spondylitis compared with healthy controls(HC).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 in rheumatoid arthritis.[19]. Hepcidin levels in patients with active ankylosing spondylitis increased when compared to patients with inactive ankylosing spondylitis [11]. In our study, serum prohepcidin and hepcidin levels in AS group were significantly higher than healthy controls(HC). 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 prohepcidin [11] levels were shown to be high in ACD group in comparison to HC group. In our study, prohepcidin and hepcidin levels were higher in the ACD group than HC 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 [20]. In our study, serum transferrin levels in AS group were significantly lower than healthy controls. Serum ferritin level is the most frequently used laboratory parameter to distinguish between ACD and HG [21,22]. 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, 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. 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]. 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 hypochromicnormocytic in 30% of those with chronic disease anemia [28]. These data suggest that hepcidin is an important pathogenetic marker in pathobiology of anemia in AS 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, 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. BÖ obtained the data and approved for the last version to be published.

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