Serum Prohepcidin and Hepcidin Levels in Patients with Rheumatoid Arthritis: A Prospective Study

Romatoid Artritli Hastalarda Serum Hepsidin ve Prohepsidin Düzeyleri: Prospektif Çalışma

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ABSTRACT Objective: Anemia of chronic disease is a common feature of active rheumatoid arthritis. Hepcidin is an acute-phase protein with specific iron regulatory properties, which along with the anemia seen with increased hepcidin expression, have led many to consider it the major mediator of anemia of chronic disease. The aim of this study was to evaluate the serum levels of hepcidin and its prohormone, prohepcidin, in patients with rheumatoid arthritis (RA) in comparison with healthy controls. **Material and Methods:** Fifty patients with RA and twenty healthy adults were prospectively enrolled. Complete blood count, erythrocyte sedimentation rate, serum hepcidin, prohepcidin, iron, total iron binding capacity, ferritin, transferrin and C-reactive protein levels were measured. **Results:** Serum prohepcidin and hepcidin levels were significantly higher in patients with RA compared to healthy controls (p=0.010). In patients with RA, a positive correlation was determined between the serum hepcidin and prohepcidin levels (r=0.725, p<0.001). **Conclusion:** Our findings suggest that hepcidin and prohepcidin are strongly associated with disease activity in RA patients and might play a significant role in the pathobiology of anemia of chronic disease associated with RA. Future studies to determine serum levels of hepcidin at different time points during the clinical course of RA patients will be needed to confirm our results.

Key Words: Prohepcidin; hepcidin; arthritis, rheumatoid

ÖZET Amaç: Kronik hastalık anemisi, aktif romatoid artritli hastalarda sık görülen bir özelliktir. Kronik hastalık anemisinde önemli düzenleyici olan ve anemili hastalarda artış gösteren hepsidin, özel demir düzenleyicisi ve akut faz proteinidir. Bu çalışmanın amacı, sağlıklı kontrol ile romatoid artritli (RA) hastaların karşılaştırılmasında hepsidin ve prohormon olan prohepsidinin serum düzeylerini değerlendirmektir. **Gereç ve Yöntemler:** Elli RA'lı hasta ve 20 sağlıklı yetişkin prospektif olarak çalışmaya alındı. Tam kan sayımı, eritrosit sedimantasyon hızı, serum transferin, hepsidin, prohepsidin, demir, total demir bağlama kapasitesi, ferritin ve C-reaktif protein düzeyleri ölçüldü. **Bulgular:** Serum prohepsidin ve hepsidin düzeyleri; sağlıklı kontrol grubu ile karşılaştırıldığında, RA hastalarında anlamlı derecede yüksekti (p=0,010). RA'lı hastalarda serum hepsidin ve prohepsidin seviyeleri arasında pozitif korelasyon saptandı (r=0,725, p<0,001). **Sonuç:** Bulgularımız, RA hastalarında hastalık aktivitesi ile hepsidin ve prohepsidin arasında güçlü bir ilişki olduğunu ve bunların RA ile ilişkili kronik hastalık anemisi patobiyolojisinde önemli bir rol oynayabileceğini göstermiştir. RA hastalarının klinik seyrinde, farklı zamanlarda hepsidinin serum düzeylerini belirlemek çalışmamızı doğrulayacaktır.

Anahtar Kelimeler: Prohepsidin; hepsidin; artrit, romatoid

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Rheumatoid arthritis (RA) is a systemic, autoimmune disease of unknown origin, characterized by chronic joint inflammation leading to destruction of bone and cartilage, reduction of functional capacity, and increased mortality.[1]

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The anemia of chronic disease (ACD) is defined as a mild anemia associated with a chronic inflammatory, infectious or neoplastic illness and with a characteristic disturbance of iron metabolism.[2] The most consistent features are low serum iron and normal or increased serum ferritin levels, reflecting normal or increased iron stores in patients with ACD.[3,4] Conventional laboratory indices of iron status include serum iron, transferrin, 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.[5,6]

Hepcidin is a liver-made cysteine-rich cationic peptide proposed to be a central regulator of intestinal iron absorption and iron recycling by macrophages.[7,8] The inducibility of hepcidin by inflammatory stimulus suggested that hepcidin, by limiting iron export from macrophages, could have a key role in anemia of inflammation.[9,10] Levels of hepcidin and its prohormone, prohepcidin were found to be increased during inflammation, which resulted in decrease in iron absorption and retention of iron in macrophages, decrease in serum iron, eventually causing ACD.[10,11] It was reported that the serum hepcidin level may distinguish anaemia due to chronic inflammation and/or iron deficiency in RA patients.[12]

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 RA, and the possible utilization of serum prohepcidin and hepcidin levels in the differential diagnosis of ACD.

MATERIAL AND METHODS

STUDY DESIGN

The study has been approved by the institutional review board and subjects have given their informed consents. The study was carried out in accordance with the World Medical Association Declaration of Helsinki. RA was defined according to the American College of Rheumatology criteria of 1987.

Fifty patients with RA (43 males and 7 females; mean age 41.30±8.12 years) and 20 healthy adults as a control group (12 males and 8 females; mean age 31.75±10.77 years) were prospectively enrolled. RA patients were also divided into two groups according to disease activity as "Active-RA group" consisting of 29 patients (22 males and 7 females; mean age 32.37±7.00 years) and the "Inactive-RA group" consisting of 21 patients (19 males and 2 females; mean age 33.00±9.20 years). Baseline characteristics of patients with RA and the healthy controls are shown in Table 1.

Complete blood count, erythrocyte sedimentation rate (ESR), serum hepcidin, prohepcidin, iron, total iron binding capacity (TIBC), ferritin, transferrin, and C-reactive protein (CRP) levels were measured. The normal ranges were 50 to 170 μg/dL for serum iron, 120-420 μg/dL for TIBC, 192 to 282 mg/dL for serum transferrin, and 15 to 150 ng/ml for serum ferritin. RA disease activity was measured with the CRP and ESR. A CRP >3.2 and an ESR >20 mm/h suggested active disease. Accordingly, the "Active-RA group" and the "Inactive-RA group" were identified.

Blood samples were divided into two tubes (a tube with an anticoagulant-EDTA and without an anticoagulant) from the patients and control groups in the morning at the end of 12-14 hours of fasting. The analysis of serum prohepcidin (Cat

TABLE 1: Baseline characteristics of patients.

	RA (n=50)	Control Group (n=20)	P value
Male/Female	43/7	12/8	0.024
Age, yr	41.30±8.12	31.75±10.77	0.562
Hemoglobin, gr/dL	10.20±3.05	14.12±2.10	0.043
MCV, fl	80.36±6.61	88.96±8.91	0.055
ESR, mm/h	35.37±11.93	10.75±5.50	0.013
Prohepcidin, mg/dL	169.1 (6.83-268.54)	103.1 (31.6-147.8)	0.010
Hepcidin, mg/dL	57.8 (12.49-75.27)	39.3 (11.1-68.6)	0.010
Fe, μg/dL	31.53±18.65	89.05±15.49	0.010
TIBC, μg/dL	210.92±54.16	215.80±21.08	0.657
Ferritin, ng/ml	54.13±18.07	69.00±31.78	0.048
Transferrin, mg/dL	167.78±63.39	205.30±7.55	0.032
CRP, mg/dL	26.1 (2.90-68.90)	3.3 (2.9-3.5)	0.010

MCV: Mean corpuscular volume; TIBC: Total iron binding capacity; CRP: C-rective protein.

No:12K069-3) and hepcidin (Cat No:39K119) were carried out at room temperature with an ELISA kit by using Rayto RT 2100 C microplate reader (Rayto Electronics, China). The levels of serum transferrin were measured using Beckman Coulter test kits (lot No: T911130), Iron, TIBC levels were measured with thermo brand test kits (lot No: V36305) in Synchron LX-20 auto analyzer, the CRP levels with Siemens branded test kits (lot No:167504A) in Dade Behring auto-analyzer. Ferritine levels were studied in E170 auto-analyzer using Roche test kits (Cat No: 03737551). Complete blood count was measured with original kits (lot No:A0115) in Cell Dyn 4000 analyzer device. ESR levels were analyzed through Alifax device.

DEFINITION OF ANEMIA

Anemia was defined by a hemoglobin (Hb) concentration <13.0 g/dL in males and <12.0 g/dL in females.[13] According to the World Health Organization (WHO), mild anemia corresponds to a Hb ≥9.5 g/dl, moderate anemia to a Hb ≥8 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).[14]

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 Statistical Package for the Social Sciences (SPSS, Version 18.0, Chicago, IL, USA). The results were expressed as mean± standard deviation (SD) and median (min-max) for non-parametric tests. The comparisons between two groups were done using Mann-Whitney U test. Parametrics were performed using the independent-samples t test. Correlation analysis was performed using Pearson correlation coefficient. p values of <0.05 were considered to indicate statistical significance.

RESULTS

Serum prohepcidin levels in RA group (265±44 mg/dL) were significantly higher than those healthy controls (123±18 mg/dL) (p=0.010). In patients with RA, a positive correlation was determined between the serum hepcidin and prohepcidin levels (r=0.725, p<0.001).

Serum prohepcidin levels in active-RA group (356±94 mg/dL) was significantly higher than inactive-RA group (276±89 mg/dL) (p=0.048) (Table 2).

Serum hepcidin levels in RA group (91±70 mg/dL) were significantly higher than healthy controls (45.3±10 mg/dL) (p=0.010) (Table 1). Serum hepcidin levels in active-RA group (109±53 mg/dL) was significantly higher than inactive-RA group (79±21 mg/dL) (p=0.039) (Table 2).

Serum hemoglobin levels in healthy controls (14.12±2.10 g/dl) were significantly higher than RA group (10.20±3.05 g/dl) (p=0.043). Serum iron levels in RA group (31.53±18.65 g/dl) were significantly lower than healthy controls (89.05±15.49 g/dl) (p=0.010). No significant difference in serum MCV levels was found between RA group (80.36±6.61 fl) and healthy controls (88.96±8.91 fl).

Serum ferritin levels in RA group (54.13±18.07 ng/ml) were significantly lower than healthy controls (69.00±31.78 ng/ml) (p=0.048). Serum transferrin level in RA group (167.78±63.39 mg/dL) was significantly lower than healthy controls (205.30±7.55 mg/dL) p=0.032). ESR rates in active RA group (35.35±11.86 mm/h) was significantly higher than inactive RA group (20.40±10.14 mm/h) (p<0.001). Serum CRP levels in active-RA group (34.99±2.68 mg/dL) was significantly higher than inactive-RA group (3.22±1.60 mg/dL) (p=0.012).

TABLE 2: The analysis of prohepcidin and hepcidin levels in active RA and inactive RA groups.

	Active RA (n=29)	Inactive RA (n=21)	P value
Number of patients	29 (68%)	21 (32%)	0.025
CRP, mg/dL	34.99±2.68	3.22±1.60	0.012
Prohepcidin, mg/dL	226.12±40.18	124.21±29.48	0.048
Hepcidin, mg/dL	61.51±13.02	49.61±15.41	0.039

CRP: C-rective protein.

DISCUSSION

Our data mainly suggest that serum hepcidin and prohepcidin levels are significantly higher in patients with RA compared to the healthy controls.

Hepcidin, a small cysteine-rich peptide,[14,15] can affect both inflammation and red blood cell kinetics in health and disease.[10,16] It is exclusively produced in the liver and it circulates in plasma, consistent with its postulated role as a hormone involved in iron homeostasis.[17,18] Further, hepcidin mRNA expression is increased in response to inflammatory stimulus such as lipopolysaccharides and infection.[19] 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.[20] Nemeth et al. indicated that in acute inflammation, urinary hepcidin excretion is increased when compared to the control group.[10] Małyszko et al. and Dallalio et al. reported increased prohepcidin levels in the chronic hemodialysis patients.[21,22] Demirag et al. and Abdel-Khalek et al. indicated that hepcidin levels were positively correlated with disease activity and negatively correlated with hemoglobin values in patients with RA.[23,24] Hepcidin levels increased in patients with active RA when compared to patients with inactive RA.[14] All of these data suggest that hepcidin plays an important role in inflammatory conditions. The role of hepcidin becomes particularly important in anemia of inflammation. In our study, the highest serum hepcidin and prohepcidin levels were observed in RA patients. In addition, patients with RA had higher serum hepcidin and prohepcidin levels compared to the healthy controls.

It was reported that hepcidin production increased in case of iron load,[7,10] and decreased in rats fed with low iron.[19] In clinical studies, urinary hepcidin[10] and serum prohepcidin[14] levels were shown to be high in ACD group in comparison to healthy control group. In our study, prohepcidin and hepcidin levels were higher in RA patients compared to healthy control 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. Kohgo 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 iron deficiency anemia (IDA).[25] In our study, serum transferrin levels in RA group were significantly lower than the healthy controls.

Serum ferritin levels are normal or increased in ACD. Serum ferritin level increases as acute phase reactant in RA. Hepcidin is known to be closely associated and positively correlated with ferritin,[10,22,26] but there are also reports of correlation between prohepcidin and ferritin levels.[21,27-29] A positive correlation was demonstrated between serum prohepcidin and ferritin levels in chronic renal failure.[21] 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.[27] On the other hand, in other studies, serum prohepcidin levels were reported as unrelated with ferritin or other iron parameters.[28-30] In our study, a negative correlation was demonstrated between serum prohepcidin and ferritin levels in the RA group. A positive correlation was demonstrated among serum ferritin, CRP levels and ESR rates in the active-RA group.

Literature data point to raised C-reactive protein (CRP) concentration as a marker of systemic inflammation in RA patients.[23] In our study, serum CRP levels in active RA group were significantly higher than the healthy controls.

Normally, most of iron used for erythropoiesis is recovered from the degradation of red blood cells by reticuloendothelial macrophages. 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. Classically, chronic disease anemia is associated with low serum iron and TIBC and high or normal serum ferritin levels.[2] In the present RA patients, serum iron status was consistent with these classical data, however TIBC was not significantly different when compared to

healthy controls. Iron dynamics during the complicated course of RA seems to be chaotic. Chronic disease anemia may not only be normochromic-normocytic it may also have hypochromic-microcytic or normocytic features.[31] Our active-RA group had significantly lower serum iron levels and MCV compared to inactive-RA group and healthy controls. Vreugdenhil et al. have shown that the anemia was normochromic-normocytic in 60% and hypochromic-normocytic in 30% of those with chronic disease anemia.[31] Differentiation between iron deficiency and chronic disease anemia can sometimes be difficult, especially when they coexist. Vreugdenhil et al. have also demonstrated that frequency of iron deficiency detected by stainable bone marrow were over 50% and 52% in anemic RA patients.[31,32] These data suggest that hepcidin is an important pathogenetic marker in pathobiology of anemia in RA patients. In our study, in addition to hepcidin, we found that the serum iron level was a significant predictor for hemoglobin level in all RA patients.

This study has some limitations. In inflammatory diseases, ACD is due to poor intake and/or absorption and increased loss of iron, and therefore, it may be difficult to differentiate between ACD and IDA.[3] The serum transferrin receptor may be a better indicator to distinguish between ACD and IDA. The limitation of our study was that we were unable to analyze the transferrin receptor in the participants. The hemochromatosis gene is an upstream regulator of hepcidin, and it could influence the prohepcidin levels in some individuals, and lack of determination of the hemochromatosis gene mutation status may be second limitation of this study. It should not be forgotten that ACD is a complex phenomenon and hepcidin and prohepcidin are not the only molecules playing role in this condition. Numerous cytokines, particularly TNF-α, which does not induce hepcidin mRNA, can play an important role in patients with ACD.[10,33]

CONCLUSIONS

In conclusion, our findings suggest that hepcidin and prohepcidin are strongly associated with disease activity in RA patients and might play significant roles in the pathobiology of ACD associated with RA. Future studies to determine serum levels of hepcidin at different time points during the clinical course of RA patients will be needed to confirm our results. Moreover, inflammatory cytokines which can induce hepcidin synthesis, such as IL-6, and serum erythropoietin levels should also be investigated concurrently to determine the exact mechanisms underlying the contribution of hepcidin in the crossroad of anemia and inflammation associated with RA.

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