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The Role of Prohepcidin and Hepcidin in Anemia Associated with Systemic Lupus Erythematosus

[1]Mehmet Dagli, [2]Sema Yilmaz, [3]Abdullah Sivrikaya, [4]Gulperi Celık and [3]Husamettin Vatansev

[1]Department of Internal Medicine, Selcuk University,
Selcuklu Faculty of Medicine, Division of Hematology, Konya, Turkey
[2]Department of Internal Medicine, Selcuk University,
Selcuklu Faculty of Medicine, Division of Rheumatology, Konya, Turkey
[3]Department of Biochemistry, Selcuk University, Selcuklu Faculty of Medicine, Konya, Turkey
[4]Department of Internal Medicine, Selcuk University,
Selcuklu Faculty of Medicine, Division of Nephrology, Konya, Turkey

Abstract: The aim of the study is to investigate the role of circulating prohepcidin and hepcidin, a homeostatic regulator of iron metabolism and a mediator of inflammation, in anemia associated with systemic lupus erythematosus(SLE) and differential diagnosis of iron deficiency anemia (IDA). Fourty patients with SLE (12 with anemia and 28 without anemia), 30 patients with IDA and 20 healthy adults were studied. Serum prohepcidin and hepcidin levels were analyzed by using a commercially available kits (DRG prohepcidin and hepcidin ELISA kits). Serum prohepcidin and hepcidin levels in patients with SLE were significantly higher than those with IDA and healthy group. In the group with IDA and SLE positive correlation was determined between the values of serum prohepcidin, hepcidin, Hb, TIBC and ferritin. Serum prohepcidin and hepcidin levels are not closely associated with disease activity in patients with SLE, but might play a role in the pathobiology of chronic disease anemia associated with SLE.

Key words: Prohepcidin · Hepcidin · Anemia · Systemic lupus erythematosus

INTRODUCTION

The incidence rate of inflammatory anemia, known as chronic disease anemia, changes between 30 to 80% during SLE, an autoimmunal inflammatory disease [1]. In autoimmunal diseases, four different mechanisms have been determined to be effective in the development of anemia. These mechanisms were found in experimental trials as follows: 1) TNFα, IL_6 and INFx playing a key role in the development of autoimmunal diseases compromise erythropoesis in blood marrows and influence the differentation and apopytosis of erythroid progenitor cells negatively.2) Inflammatory cytokines inhibit the peripheral use of iron. 3) In autoimmunal diseases, the production and response of erythropoetin are destroyed. 4) The life expectancy of erythrocytes is shortened [1-3]. Inflammation anemia, considered as a part of immune system, is characterized with decreased iron and iron binding capacity, increased ferritin in conjuction with the level of transferrin and the existence of iron in the macrophages within the bone marrow. This situation indicates that the iron mobilization in the depots is delete1, 2].

Hepcidin, space hormone in peptide structure and its precursor prohepcidine, a cationic peptide which has been detected recently, work as a central regulator in iron metabolism with its 4 disulfide connections and 25 amino acid residues. Hepcidin is the regulator of iron absorbency, macrophage and iron cycling in hepatic stores [3, 4]. It was stated that hepcidin and prohepcidin increased 100 times during the inflammation, contributed to the decrease in iron absorption in bowels, held in the iron in macrophages, decreased in the plasma iron and in the formation of inflammation anemia [4, 5]. In studies

Corresponding Author: Mehmet Dagli, Department of Internal Medicine, Selcuk University,
Selcuklu Faculty of Medicine, Division of Hematology, Konya, Turkey.
Tel: +90-532-427-2365, Fax: +90-332-241-60-65, E-mail: drmdagli@hotmail.com.

performed on healthy individuals; 1)mRNA was found to be correlated with mRNA level, IL_6 in adipose tissue and CRP levels. 2) When IL_6 was infused to healthy individuals, the level of hepcidin was increased and serum iron and the saturation of transferin was decreased. The fact that these data were determined experimentally was accepted as a sign of a contribution of prohepcidin and hepcidin to inflammatory anemia [4-6]. Hepatocytes increase and decrease the secretion of hepcidin according to iron status of the body, this function of it was found to demolish and internalize ferroportine existing on the surface of enterocyte, macrophage, hepatocyte and placental trophoblasts and are described as the solely molecule to provide the excretion of iron from the cells [6-8]. The loss of ferroportine in the cell membrane stops the cellular iron exportation and decreases the plasma transferrin iron transition. In the iron deficiency, the increase of erythropoiesis; the secretion of hypoxide hepcidin was shown to decrease [8, 9]. However, in inflammation, the formation of prohepcidin and hepcidin is induced and as a result of the increase in hepcidin, iron is trapped in the macrophages, hepatocytes and enterocytes by sequestrating. In conclusion, the decrease in plasma iron contributes to the development of anemia accompanying with inflammation [10].

The objective of our study is to research the role and significance of prohepcidin and hepcidin on the development of inflammation anemia which is frequently seen among the patients with SLE and its distinguishing diagnosis of IDA.

MATERIALS AND METHODS

According to 1997 SLE criteria of ACR (American College of Rheumatology), 40 patients with SLE and applying to the rheumatology clinic in our hospital (39 females, 1 male and the age range between 18 and 67; the average age, 48), 30 patients with IDA (24 females, 6 males and the age range between 18-67; the average age, 32), 20 healthy controls (12 females, 8 males and their age range between 16 and 41; the average age, 28) were accepted into the study. In their blood samples, whole blood count, serum iron, total iron binding capacity (TIBC), C-reactive protein (CRP) and sedimentation levels were analyzed, as well as prohepcidin and hepcidin.

The patients with SLE were classified into two groups, such as the group with SLE anemia (SLE+a) of 12 patients (12 females) and the one without SLE anemia (SLE-a) of 28 patients (27 females and 1 male), under the criteria indicating the hemoglobin levels of 12 gr/dl for females and 14 gr/dl for males suggested by WHO. The reference values were accepted as follows; 192-282 mg/dl for transferrin, 50-170ng-/dl for serum iron, 120-420 ng/dl for TIBC and 15-150 ng/ml for ferritin. In order to exhibit the disease activity, the values of CRP and sedimentation were used. The CRP value of >3,2 and sedimentation value of > 20 mm/h were accepted as active values. In the active SLE group (20 females, 1 males, the average age 48) and inactive SLE group (19 females, the average age 42), prohepcidin and hepcidin levels were analyzed. The blood samples of 2 ml were taken from the patients in the morning after 12-14 hours of fasting to the tubes with EDTA without anticoagulance and sedimentation whole blood counts. The samples of blood without anticoagulance were centrifugated and their serum was separated right after clotting. Among the serum samples, transferrin, ferritin, iron, TIBC and CRP levels were immediately analyzed while prohepcidin and hepcidin were kept at -85 C until analyzed. The levels of serum transferine were measured using Beckman Coulter test kits (lot No: T911130), Iron,TIBC levels 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) and Dade Behring auto-analyzer. Ferritin levels were studied in E170 auto-analyzer using Roche test kits (cat No: 03737551). Whole blood count was measured with Sysmex XT-2000i original kits (lot No:A0115) and sedim levels were analyzed through Alifax device. The analysis of prohepcidin (No: 12K069-3) and hepcidin (No: 39K119) were carried out in Kayto RT-2100 brand ELIZA device through using DRG brand ELIZA test kits. The patients with other inflammatory diseases and those who were determined to have factors that might cause anemia were excluded. Written permissions were taken from the ethical committee of Selcuklu Medical Faculty and all the subjects.

Data Analysis: The data were transferred into computer environment and statistical analysis was performed using the program of ''SPSS 18,0 for Windows''. Arithmetic mean and standard errors of the parameters were measured. Since the measured parameters showed abnormal distribution, Mann-Whitney U test was used. It was carried out via Correlation Pearson and Spearman's correlation tests. The significance level was accepted as $p<0.05$.

Table 1: Results of Age, Hb, MCV, Sedimentation, Iron, TIBC, Ferritin, CRP, Prohepcidin and Hepcidin in Patient and Control Groups

	SLE(+a)	SLE(-a)	DEA	SE
Age (year)	45±10[a]	41±12[a]	30±11[b]	31±10[b]
Hb (gr/dl)	10±1[b]	13±1[a]	8.9±10[b]	14.1±2.1[a]
MCV(fl)	76±7[a]	86±4[a]	73±5[a]	88±9[a]
Sedimentation(mm/h)	45±22[a]	16±14[b]	14±8[b]	10±5[c]
Iron (ng/dl)	40±21[a]	43±27[b]	17±10[c]	89±15[d]
T.D.B.K.(ng/dl)	236±56[a]	233±62[a]	418±44[b]	315±21[a]
Ferritin (ng/ml)	18±15[a]	63±19[c]	7±9[b]	79±31[c]
Transferrin (mg/dl)	177±63[a]	181±49[b]	143±2[a]	205±7[b]
CRP (mg/dl)	5±7[a]	5±3[a]	2,9±1[b]	2,6±1[b]
Prohepcidin (mg/dl)	138±17[a]	129±16[a]	96±8[b]	91±18[b]
Hepcidin (mg/dl)	62±30[a]	58±20[a]	48±18[b]	45.3±10[b]

Statistical analysis is non significant between the values with the same letters on, $p>0.05$; however, those with different letters on are of significant statistical analysis, $p<0.05$

Table 2: Percentage of Anemia Frequency in Active and Inactive SLE Groups, Values of Hb, Prohepcidin and Hepcidin

	Active SLE (n;21)	Inactive SLE (n;19)
Anemia Frequency %	52%	48%
Hb (gr/dl)	10.4±1.6[a]	12.9±1.6[a]
Prohepcidin (ng/ml)	156±14[a]	116±19[b]
Hepcidin (ng/ml)	71±13[a]	59±11[b]

RESULTS

Both groups of SLE(+a) and SLE(-a) had higher mean age rates than those with IDA and control group. Of all patients, 39 were women and 1 was man. In literature, the prevalance rate of SLE was given to be 10/1 as W/M. In SLE(+a) group; Hb, iron and transferring levels were lower than in RA(-a) and control groups. In SLE(+a) group; sedimentation value was significantly higher, when compared to other groups. CRP level in both groups of SLE(+a) and SLE(-a) was significantly higher than IDA and control groups; however, no difference was found between them. The result was related to the fact that especially all SLE patients had taken up 3 or 4 different types of drugs for their treatment. TIBC was higher in the group with IDA, compared with the values of other groups; however, ferritin levels were significantly low. In SLE(+a) group, prohepcidin and hepcidin levels were significantly higher than those with IDA and healthy group; however, no significant difference was found between the groups of SLE(+a) and SLE(-a). In the group with IDA, while a positive correlation was found between prohepcidin and hepcidin and the values of hemoglobin (r:0.353, p=0.01; r:0.241, p=0.04, respectively) no correlation was found among other signs of anemia, such as serum iron, TIBC and ferritin. A positive and significant correlation was found between prohepcidin and hepcidin in both SLE(+a) and SLE(-a) groups (r:0.817, p=0.001). Between the levels of hepcidin and other parameters, no significant correlation was present. While a significant negative correlation between the level of hemoglobin and sedimentation (ESR) in SLE(+a) and SLE(-a) groups (r:-0.367, p=0.001), a significant negative correlation between iron value and CRP results (r:-0.344, p=0.018) and a significant correlation between transferine level and ferritin and level of iron (r:-0.360, p=0.016; r:-0.408, p=0.005, respectively) were found, a significant positive correlation was determined between TIBC values in both groups (r:0.545, p=0.0001) (Table 1). The levels of prohepcidin and hepcidin in active SLE group were observed to be significantly higher, compared to inactive SLE group, but no significant correlation was detected between the two groups (Table 2).

DISCUSSION AND CONCLUSION

IDA, investigated among hypochromia microcyter anemias, is often confused with chronic disease anemia. It was stated that 'Erythroid Burst-Forming Unit (E-BFU) and Erythroid Colonie Forming Unit (E-CFU)' providing the proliferation and the differentiation of erythroid precursors in the chronic disease anemia are dysfunctioned and cytokines, such as TNF-α, IFN-α, IL_1 and IL_6, determined to increase in autoimmunal events, lead to the condition [11, 12, 13, 16]. The increase in TNF-α and IL_6 increases hepatic secretion of hepcidine, represses the ferroportin expression and this situation stimulates iron retention in the macrophages and prevents the direct absorption of iron in the circulation [17, 18]. In studies performed, ferroportin-removed cells were observed not to absorb iron [18, 19]. In the decrease or absence of hepcidin, ferroportin was indicated to perform iron load by continuously absorbing iron. Lee *et al.*, confirmed the notion in their studies [21]. Transferrin was reported to be more useful from the point of iron than

serum iron and TIBC. The decrease in the value of transferrin was attributed to the decrease of net serum values in IDA and binding of iron by RES (reticular endothelial system) in chronic disease anemia [20, 21]. In our study, transferrin levels were found to be lower in the both SLE groups than controls. Kahgo *et al*., in their study, indicated that serum transferrin levels reflected the cellular iron shortage and could be used in differential diagnosis of inflammation anemia and IDA [22]. Ferritin level is the most frequently used parameter used to distinguish between chronic disease anemia and IDA as differential diagnosis [22, 23] In our study, SLE patients were classified in two groups by accepting ferritin of 50 mg/dl as cut off value. In SLE group with high ferritin, prohepcidin and hepcidin levels were higher than control and IDA groups. Significant correlation between the level of prohepcidin and ferritin confirmed this result. This situation corresponded to the study was executed by Chijiva *et al*. [24] S. Means *et al*. [19], found prohepcidin levels to be significantly higher in chronic disease anemia patients and stated that it reflected the underlying inflammatory event. In our study, high levels of prohepcidin and hepcidin in the SLE group with high ferritin levels may be reflecting the existence of inflammatory event. Hepcidin is an acute phase protein. This observation has been used as a base in the studies for inflammatory disease [11, 12, 25]. Nemeth *et al*., showed that urinary hepcidin increased in inflammation anemia, when compared to control group [25]. Malyszka *et al*. and Dallalio *et al*., indicated that prohepcidin levels increased in the chronic hemodialysis patients [26, 27]. In our study; espcially in active SLE group, prohepcidin and hepcidin levels were found to be significantly high, but no correlation could be determined between them Lee *et al*., indicated that IL_1 and IL_6 increasing in inflammation influentially stimulate the hepcidin [19, 26]. Demirag *et al*., indicated that hepcidin plays a role in chronic disease anemia seen in autoimmunal disease, that hepcidin levels are positively correlated with disease activity and negatively with Hb values and that hepcidine levels are markedly increased in active patients, compared to inactive patients, although anemia frequencies are similar [17]. Roe *et al*., executed their studies on healthy individuals, suggested that prohepcidin had no role in iron hemostasis [11]. However, other studies parallel to those performed by Kulaksýz et al., Theurl et al., Demirag et al., S.Jayaranee et al. and Hea-Kim et al, showed that prohepcidin and hepcidin could play a central regulatory role in the hemostasis of iron in inflammation [12-16]. All of these data highlight hepcidin to be significant in inflammatory events, especially anemias related to inflammation. In the present study, higher prohepcidin and hepcidin values were found in the patients with anemic SLE than those with IDA and healthy control groups. All the data are consistent with the findings determined in the literature. It is difficult to distinguish chronic disease anemia and IDA, especially when they exist together, because the iron deficiency is a commonly encountered event among autoimmunal diseases (AID). Vreugdenhil et al., in their study of painted bone marrow, showed that the iron deficiency was over 50% among the patients with anemic AID and researched the role of iron, vitamin B12 and folic acid in RA [29, 30, 31]. In order to enlighten that hepcidin and its precursor prohepcidin are closely related to the disease activity in SLE patients, studies on a large scale with untreated participants are needed.In the pathobiology of inflammation or chronic disease anemia frequently encountered in SLE and other AIDs, hepcidin, prohepcidin and and cytokines are proved to play an active role together. The treatment of chronic disease anemia are carried out palliatively at present. In the treatment of chronic disease anemia, it was concluded that controlling the release of hepcidin, prohepcidin and other cytokines, as well as the treatment of primary disease, contribute to the treatment.

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