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Serum hepcidin and interleukin-6 in systemic lupus erythematosus patients: crucial factors for correction of anemia

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Abstract

Background: The incidence rate of anemia of chronic disease (ACD) in systemic lupus erythematosus (SLE) ranges between 30 and 80%. Serum iron is the main regulator of hepatic hepcidin production. Interleukin-6 (IL-6) upregulates hepcidin expression. The aim of this study is to compare between serum hepcidin and IL-6 in SLE patients and control subjects, and to find out if they are correlated with each other and with disease activity in order to find their role in treatment of anemia in SLE patients.

The study was carried out on 50 SLE patients, suffering from anemia, diagnosed according to SLICC revision of the ACR classification criteria for SLE, and 50 healthy individuals, taken as control. Disease activity was assessed using the SLE disease activity index (SLEDAI-2 K). Serum hepcidin and IL-6 were measured by ELISA kit.

Results: There was a highly statistically significant difference in serum hepcidin and IL-6 levels between patients and control subjects. There was a statistically significant correlation between serum hepcidin and IL-6 in SLE patients. Moreover, both of them were correlated with SLEDAI and ESR and negatively correlated with hemoglobin. The mean value of serum hepcidin in SLE patients with normocytic normochromic anemia was higher than that in patients with microcytic hypochromic anemia. However, this difference did not reach a statistically significant level.

Conclusion: High serum IL-6 and hepcidin levels are associated with anemia in SLE. They are correlated with each other and with disease activity. Although our study revealed serum hepcidin to be correlated with disease activity, it should not be used as a marker of disease activity in SLE patients as our patient's group was SLE patients suffering from ACD. However, IL-6 inhibition should be considered in patients with SLE with anemia to guide the control of anemia of chronic diseases resulting from cytokine production as a result of high disease activity in SLE patients. It should be noted that the occurrence of ACD associated with IL-6 flare up could be a player in other systemic rheumatic diseases and is not specific to SLE patients.

Keywords: Systemic lupus erythematosus, Hepcidin, IL-6, Anemia of chronic disease, Iron-deficiency anemia, Normochromic normocytic anemias

Background

Systemic lupus erythematosus is a systemic autoimmune disease that is characterized by multisystem involvement. Clinical features in SLE patients vary between mild joint and skin involvement to severe, life-threatening internal

organ disease. Anemia of chronic disease (ACD) is the commonest anemia in SLE [1], with an incidence rate 30–80% [2].

Hepcidin regulates iron homeostasis. It prevents mobilization of iron stored in macrophages and prevents absorption of iron from the gut [3]. It contributes to anemia by limiting iron availability for erythropoiesis. Hepcidin is, mainly, produced by the liver, but it is made elsewhere, including the kidney and macrophages [4].

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Hepcidin regulates iron absorption and iron cycling in the hepatic stores [5]. Serum iron is the main regulator of hepatic hepcidin production. Interleukin-6 (IL-6) upregulates hepcidin expression, which may explain the ACD [6].

It has been stated that hepcidin increased 100 times during inflammation. It decreases iron absorption in the bowels, holds iron in macrophages, and decreases plasma iron level [7].

Infusion of IL-6 to healthy individuals increased hepcidin level, increased serum iron levels, and decreased transferrin saturation [8].

The aim of this study is to assess serum hepcidin and IL-6 in anemic SLE patients, and to find out if they are correlated with each other and with disease activity, in order to find out their role in treatment of anemia in SLE patients.

Methods

Study population

This is a case control study, which was carried out on 50 Egyptian SLE patients, suffering from anemia, and 50 healthy individuals, age and sex matched with patients, taken as control.

SLE patients were diagnosed according to SLICC revision of the ACR classification criteria for SLE [9]. Healthy individuals were selected if they have no history of any disease, with normal hemoglobin level, ESR, CRP, LFTs, and KFTs.

Patients were recruited from the inpatient sections and outpatient clinics of rheumatology and rehabilitation department, during the period of January 2018 to July 2018.

Informed consent was obtained from all individual participants included in the study. The study was approved by the Institutional Review Board (IRB) of the faculty.

Patients were subjected to full history taking and general examination for the presenting clinical manifestations. Disease activity was assessed using the SLE disease activity index (SLEDAI-2K) [10].

Patients were considered anemic if their Hb was < 12 g/dl in females or < 15 g/dl in males, according to WHO threshold used to define anemia [11]. Anemia was defined as microcytic if mean corpuscular volume (MCV) is below 83 fl and was defined as hypochromic if mean corpuscular hemoglobin (MCH) was below 27 pg [12].

Patients with hemolytic anemia associated anti-phospholipid antibody syndrome (APS) and patients with established nephropathy were excluded from the study.

Laboratory investigations

Routine laboratory investigations were performed for diagnosis and follow-up of SLE patients, such as ESR,

CRP, liver and kidney function tests, 24 h urinary proteins, C3, and C4 levels. Anti-double stranded DNA (anti-ds-DNA) antibodies were detected and specified using immunofluorescence technique.

Five milliliters of blood were collected, from all the study participants, about 2 ml on EDTA tube for CBC and 3 ml on plain tube which was left for 30 min then centrifuge to separate serum with immediate measurement of serum iron, then part of serum was stored at -20 for further analysis of serum hepcidin by ELISA kit supplied by Elabscience and IL-6 by ELISA kit supplied by affymetrix eBioscience.

Statistical analysis

All statistical analyses were performed with SPSS, version 21.0 (IBM Corp, NY, USA). Quantitative data were presented by mean and standard deviation (SD). *t* test was used for comparison between two groups. Spearman's correlation (*r*) was done to show the closeness of association between two variables. Results were considered significant if *P* value is < 0.05 and highly significant if *P* value is < 0.001.

Results

Patient characteristics

This study was carried out on 50 SLE patients and 50 healthy control subjects. SLE patients were 45 females (90%) and 5 males (10%). Their ages ranged from 25 to 37 years old, with a mean of 30.7 ± 3.3 . Their disease duration ranged from 1 to 7 years, with a mean of 4 ± 1.45 years. The control subjects were 43 females (86%) and 7 males (14%). Their ages ranged from 20 to 40 years old, with a mean of 29.9 ± 5.3 . There was no statistically significant difference between patients and control as regard age or sex (*P* value 0.17 and 0.24, respectively).

Serum hepcidin and IL-6 levels

There was a highly statistically significant difference in serum hepcidin levels between patients and control subjects. The mean value of serum hepcidin in SLE patients was (78.7 ng/ml) more than double that of the mean value in the control group (35.5 ng/ml) (Table 1).

There was, also, a highly statistically significant difference in serum IL-6 levels between patients and control subjects. The mean value of serum IL-6 in SLE patients was 6 folds higher than that in the control group (Table 1).

Correlation with disease activity and Hb level

There was a statistically significant correlation between serum hepcidin and IL-6 in SLE patients. Moreover, both of them were correlated with SLEDAI and ESR. Besides, serum hepcidin showed statistically significant negative correlation with Hb level and IL-6 showed

Table 1 Serum hepcidin and IL-6 in SLE patients and control

	SLE patients, N (50)	Control, N (50)	t	P
Age (years)				
Mean ± SD	30.7 ± 3.3	29.9 ± 5.3		0.17
Sex	N (%)	N (%)		
Male	5 (10%)	7 (14%)		0.24 ethics
Female	45 (90%)	43 (86%)		
Hepcidin (ng/ml)				
Mean ± SD	78.7 ± 13.6	35.5 ± 5.5	16.5	< 0.001**
IL-6 (pg/ml)				
Mean ± SD	12.9 ± 4.2	2.6 ± 0.8	7.49	< 0.001**

SD standard deviation

**Significance at P value < 0.001

highly statistically significant negative correlation with Hb level (Table 2).

Hepcidin and IL-6 levels in different grades and different types of anemia

Our results showed that, the mean level of serum hepcidin in SLE patients with normocytic normochromic anemia was higher than that in patients with microcytic hypochromic anemia. However, this difference did not reach a statistically significant level (Table 3).

Discussion

Systemic lupus erythematosus is a potentially severe autoimmune disease with characteristic production of inflammatory cytokines and autoreactive antibodies due to the lack of immune tolerance leading to diverse clinical manifestations and damage to various organs, including skin, joints, kidneys, and the central nervous system [13]. SLE course is variable, mainly characterized by remission and relapse periods [14].

Hematologic involvement is common in SLE; all three blood cell lines could be affected. ACD is a normochromic, normocytic anemia, in which there is low serum iron, low transferrin, and normal or increased serum ferritin. It can coexist with anemia resulting from other

Table 2 Correlations of serum hepcidin and IL-6 with each other, and with hemoglobin level and markers of inflammation in SLE patients

	Hepcidin		IL-6	
	r	P	r	P
SLEDAI	0.32	< 0.05*	0.21	0.046*
ESR	0.48	< 0.05*	0.33	0.040*
Hb	- 0.55	0.04*	- 0.67	0.001*
IL-6	0.48	0.002*	-	-
Hepcidin	-	-	0.48	0.002*

*Significance at P value < 0.05

processes [1]. The incidence rate of ACD in SLE ranges between 30 and 80% [2].

It has been stated that ACD is characterized by iron retention inside the macrophage, induced by cytokines and hepcidin, which controls cellular iron efflux by binding to the iron export protein, ferroportin [15].

This study was designed to assess serum hepcidin and IL-6 levels in SLE patients suffering from anemia, and to study their correlation with each other and with SLEDAI.

In our study, both serum hepcidin and IL-6 levels were statistically higher in SLE patients when compared to control subjects (Table 1).

High serum hepcidin and IL-6 were correlated with each other and with SLEDAI and ESR (Table 2).

High serum hepcidin levels showed a statistically significant negative correlation with Hb level and IL-6 showed a highly statistically significant negative correlation with Hb level (Table 2). These results confirm that high levels of serum hepcidin and IL-6 are contributing to the severity of anemia.

Surprisingly, in this work, the mean level of serum hepcidin in SLE patients with normocytic normochromic anemia was higher than that in patients with microcytic hypochromic anemia. However, this difference did not reach a statistically significant level (Table 3). This finding pays the attention to elevated hepcidin level as a causative factor for ACD as well as IDA.

Previous studies had reported significant increase in serum hepcidin in SLE patients, when compared with control subjects [16]. Other numerous studies had stated that IL-6 in SLE patients was significantly higher than in control subjects [17–21].

It has been stated that urinary hepcidin is increased in anemic SLE patients, when compared with the control group [22]. Moreover, serum hepcidin was significantly higher in SLE patients with anemia than in patients with only IDA [16].

Shao et al. [19] and Chen et al. [23] found significant correlation between IL-6 and SLEDAI in their separate studies. Moreover, urinary IL-6 was correlated with SLEDAI and was reported as a useful parameter to assess disease activity in SLE patients [24].

It has been reported that hepcidin plays a role in ACD in autoimmune disease, and it was correlated with disease activity [25].

Mok et al. reported that serum hepcidin and IL-6 are correlated with disease activity in SLE patients, and are associated with ACD [26].

Demirag and colleagues found that hepcidin was negatively correlated with Hb in ACD [25]. Another study reported that treatment with IL-6 antagonists in RA has resulted in fall of hepcidin levels and increase in hemoglobin levels [27].

Table 3 Serum hepcidin in normocytic normochromic and microcytic hypochromic anemia in SLE patients

	Normocytic normochromic	Microcytic hypochromic	<i>t</i>	<i>P</i>
Serum hepcidin (ng/ml)				
Mean \pm SD	81.3 \pm 13.8	71.5 \pm 14.8	1.55	0.13
Range	65–100	55–89		

SD standard deviation

It has been stated that hepcidin determination may aid to differentiate between ACD and IDA and in selecting appropriate therapy for patients [15]. Oral treatment of IDA will be ineffective if hepcidin is blocking gut absorption. In this case, parenteral iron treatment would be more appropriate [28]. This finding makes hepcidin assessment essential prior to starting iron therapy.

Old reports had stated that prohepcidin was significantly higher in ACD [29].

The previous results may be explained by that IL-1 and IL-6, which are increasing in inflammation, stimulate hepcidin production by the liver [30, 31]. Hepcidin represses the ferroportin expression, leading to iron retention in the macrophages and preventing direct iron absorption in the circulation, leading to IDA [25].

Moreover, cytokines such as TNF- α , IFN- α , IL-1, and IL-6 promote dysfunctions in the differentiation and proliferation of erythroid precursors, leading to ACD [32].

Dagli et al. concluded that controlling the release of hepcidin, prohepcidin, and other cytokines contribute to the treatment of ACD [16].

Ganeb et al. concluded that prohepcidin may play a role in ACD occurring in autoimmune diseases, such as RA and SLE [33].

Conclusion

From our data and others', we conclude that hepcidin has a potential role in ACD, as well as IDA in SLE patients. Serum IL-6 and hepcidin levels are associated with anemia in these patients. In view of the finding that serum hepcidin is correlated with IL-6 as well as disease activity, IL-6 inhibitors should be considered in active SLE with anemia to control their disease activity, as well as correcting their anemia.

Abbreviations

ACD: Anemia of chronic disease; ACR: American College of Rheumatology; APS: Anti-phospholipid syndrome; IDA: Iron-deficiency anemia; INF- α : Interferon alpha; IL-6: Interleukin-6; MCH: Mean corpuscular hemoglobin; MCV: Mean corpuscular volume; RA: Rheumatoid arthritis; SLE: Systemic lupus erythematosus; SLEDAI: Systemic lupus erythematosus disease activity index; TNF- α : Tumor necrosis factor alpha

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Authors' contributions

All authors have read and approved the manuscript. AE had a major contribution in writing the manuscript, has formulated and revised the manuscript. MN has collected patients' data and participated in statistical analysis of patients' data. LK and AF have performed laboratory assessment of serum hepcidin and other laboratory investigations. SA performed clinical assessment of patients and has graded and assessed SLEDAI.

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Availability of data and materials

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Ethics approval and consent to participate

The study was approved by the Institutional Review Board (IRB) of Faculty of Medicine, Zagazig University (reference number ZU-IRB#4570/20-11-2017). Informed consents were obtained from each participant.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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