

Serum prohepcidin concentrations in rheumatoid arthritis and its relation to disease activity

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Objectives

The aim of this study was to assess the possible relations between serum level of prohepcidin in patients with rheumatoid arthritis (RA) and their rheumatoid anemia profiles and disease activity.

Patients and methods

A total of 80 patients with RA (34 male and 46 female) were enrolled. Their mean age was 43.3 ± 11.5 years, and the mean duration of the disease was 7.7 ± 7.0 years. RA disease activities were measured using Disease Activity Score 28 (DAS28). Anemia profiles were measured. Serum concentration of prohepcidin, the prohormone of hepcidin, was measured using enzyme-linked immunosorbent assay.

Results

The patients' mean concentration of serum prohepcidin was 211.4 ± 5.88 ng/ml, which was significantly higher than in the control group (167 ± 5.2 ng/ml). Serum level of interleukin-6 and tumor necrosis factor- α were significantly higher in RA patients than in the healthy control group (21.11 ± 5.88 vs. 3.36 ± 1.3 pg/ml and 17.8 ± 3.7 vs. 3.7 ± 1.1 pg/ml, respectively). The prohepcidin concentration was correlated with rheumatoid factor, C-reactive protein, erythrocyte sedimentation rate, and DAS28. There was a significant correlation between prohepcidin with tumor necrosis factor- α and interleukin-6. The prohepcidin concentration was significantly higher in the patients with active RA (DAS28 > 5.1) than those with inactive-to-moderate RA (DAS28 \leq 5.1). Serum prohepcidin concentration in patients negatively correlated with serum iron ($r = -0.23$, $P = 0.04$). However, the prohepcidin concentration did not correlate with other anemia profiles. There was no difference of prohepcidin concentration between the patients with anemia of chronic disease and those without.

Conclusion

Serum concentration of prohepcidin reflects the disease activity, regardless of the anemia states in RA patients, and thus prohepcidin could be used as another useful marker for RA disease activity.

Keywords:

anemia of chronic disease, hepcidin, prohepcidin, rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) is an autoimmune-inflammatory disease that may lead to joint destruction and disability. Autoimmune processes are highly involved in the pathogenesis of RA. Early diagnosis and immediate, effective therapy are crucial to prevent joint deterioration, functional disability, and unfavorable disease outcome [1].

Anemia is one of the common extra-articular manifestations of RA. The prevalence of anemia is 26.9–77.6% in patients with RA with the pattern of normochromic and normocytic anemia [2].

It is associated with reduced serum iron and transferrin saturation but with normal or elevated ferritin levels. Anemia of chronic disease (ACD) responds poorly to iron supplementation in RA, as it relates to the inflammatory process. It is thought to result from cytokine-mediated inhibition of iron

utilization, erythroid progenitor differentiation and erythropoietin production, and cytokine-driven apoptosis of erythroid progenitors. These inflammatory cytokines include tumor necrosis factor- α (TNF- α), interleukin-1b (IL-1 β), IL-6, and interferon- γ . There is a correlation between more severe RA and greater severity of anemia [3].

Nikolaisen *et al.* [4] concluded that IL-6-mediated bone marrow suppression is the main mechanism for development of ACD in RA.

Hepcidin, a recently identified peptide, acts as a central regulator of iron metabolism. It is regarded as a factor regulating the uptake of dietary iron and its mobilization from macrophages and hepatic stores. It is considered as a mediator of anemia of inflammation [5].

The inflammatory signal, chiefly IL-6, induces hepcidin synthesis, and then the upregulated hepcidin decreases

duodenal iron absorption and increases sequestration of iron by macrophage, resulting in hypoferrremia [6]. This is supported by following two evidences. First, hepcidin mRNA level in adipose tissue correlates with IL-6 and C-reactive protein (CRP) [7]. Second, when IL-6 is infused to healthy individuals, the hepcidin level increases, and serum iron and transferrin saturation decrease [6]. Hepcidin mRNA is induced by IL-6 [8].

Prohepcidin is an easily measurable precursor of hepcidin, which is a key regulator of iron homeostasis [9].

Aim of the work

The aim of this study was to assess the serum level of prohepcidin, a prohormone of hepcidin, analyze the relationship with the anemia profiles of RA, and to determine its possible relationship with rheumatoid disease activity.

Patients and methods

This study was carried out on 90 individuals. They were divided into patients and healthy control (HC) groups.

Patients

A total of 80 patients with RA were enrolled in this study and all the patients met the American College of Rheumatology 1987 revised criteria for the classification of RA [10]. They were 34 male and 46 female, their ages ranged from 22 to 65 years, with a mean of 43.3 ± 11.5 years, and the duration of the disease ranged from 2 months to 30 years, with a mean of 7.7 ± 7.0 years. Informed consent was obtained from the patients before the study, and this study was conducted in the Rheumatology & Rehabilitation Department, Faculty of Medicine, Zagazig University.

Healthy control group

The control group included 10 apparently healthy volunteers with similar demographics: they were four male and six female, with a mean age of 44.1 ± 8.2 years.

Methods

All patients were subjected to the following:

Complete history taking

Complete history taking including onset, course, duration of disease, pain, swelling,

limitation of movement, duration of morning stiffness, subcutaneous nodules, fever, skin rash, dysphagia, palpitation, easy fatigability, Raynaud's phenomenon, eye affection, medications, and other system affection (including chest pain, dyspnea, cough, tingling and numbness in hands and feet, and urinary symptoms).

Disease activity evaluation in rheumatoid arthritis patients

Clinical assessments such as tender joint counts, swollen joint counts (SJC), and 100-mm visual analogue scale were performed. Erythrocyte sedimentation rate (ESR), CRP, the titer of IgM rheumatoid factors (RFs), and the titer of antibodies to cyclic citrullinated peptide (anti-CCP antibody) were measured. From these clinical and laboratory data, Disease Activity Score 28 (DAS28) [11] was calculated. According to DAS28, we divided patients into two groups: patients with active disease (DAS28 > 5.1, $n = 33$), and those with inactive-to-moderate disease (DAS28 ≤ 5.1, $n = 57$).

We excluded patients with other concomitant hematological diseases (thalassemia and sickle cell anemia), heart, lung, kidney, liver diseases, acute or chronic infections, malignancy, and current pregnancy or delivery within 6 months.

Laboratory study for anemia

Laboratory analyses for anemia, such as complete blood count, hemoglobin (Hb), hematocrit, serum iron, total iron binding capacity (TIBC), ferritin, and transferrin levels, were measured using standard laboratory methods. Anemia was defined as Hb less than 13 g/dl in male patients, and less than 12 g/dl in female patients. The patients were divided into two subgroups, according to the presence or absence of anemia: anemic ($n = 43$) and nonanemic ($n = 47$). ACD was defined as normocytic to microcytic anemia with decreased serum iron concentrations, normal to decreased transferrin levels, and normal or increased serum ferritin levels. The patients were divided into two subgroups: anemic ($n = 43$) and nonanemic ($n = 47$). Patients whose anemia did not result from ACD were excluded in this study.

Serum concentration of prohepcidin and proinflammatory cytokines (interleukin-6 and tumor necrosis factor- α)

Serum prohepcidin concentration was measured using the DRG Hepcidin Prohormone Enzyme Immunoassay Kit (DRG Instruments, Marburg, Germany), according to the manufacturer's instructions. Prohepcidin is the 84 amino-acid precursor of the active hepcidin peptide.

Serum TNF- α and IL-6 were measured by sandwich enzyme-linked immunosorbent assay using appropriate commercial kits (Medgenix, Biosource International, Camarillo, California, USA).

Statistical analysis

Data are expressed as the mean \pm SD. Statistical analysis was performed using Student's *t*-test for independent samples. Correlation coefficients were determined by Pearson's correlation test. *P*-values less than 0.05 were considered significant. *P*-values less than 0.001 were considered highly significant.

Results

The demographic, clinical, and laboratory data of the RA patients and the HC groups are summarized in Table 1. In the RA group, Hb, serum iron, and transferrin levels were lower than in the HC group. Both serum ferritin level and TIBC were higher in the RA group.

In the RA patients, the mean concentration of serum prohepcidin was 211.4 ± 5.88 ng/ml, which was significantly higher than in the HC group (167 ± 5.2 ng/ml). Serum level of IL-6 and TNF- α were significantly higher in the RA patients than in the HC group (21.11 ± 5.88 vs. 3.36 ± 1.3 pg/ml and 17.8 ± 3.7 vs. 3.7 ± 1.1 pg/ml, respectively).

We divided the patients into two subgroups: anemic ($n = 43$) and nonanemic ($n = 47$) (Table 2). In patients with anemia, serum iron was lower (53.2 ± 12.5 vs. 64.4 ± 9.1 μ g/dl, $P < 0.001$). However, ESR (32 ± 7.2 vs. 26 ± 7.6 mm/h, $P = 0.001$) and CRP (51.1 ± 27 vs. 32.4 ± 19.1 mg/dl, $P < 0.001$) were higher in patients with anemia than in the patients without anemia. Other clinical parameters (disease duration, RF, and anti-CCP antibodies) were not different between the anemic and nonanemic groups.

The patients were divided according to disease activity into two groups: patients with active disease (DAS28 > 5.1 , $n = 33$) and those with inactive-to-moderate disease (DAS28 ≤ 5.1 , $n = 57$) (Table 3); the serum prohepcidin level was significantly higher (220.1 ± 24.2 ng/ml) in patients with active disease than in those with inactive-to-moderate disease (206.4 ± 18 ng/ml).

With regard to the anemia profiles, serum iron, Hb, TIBC, and transferrin levels were all significantly lower in the active RA patients compared with inactive-to-moderate patients.

Table 1 Demographics and clinical laboratory parameters in the rheumatoid arthritis and healthy control groups

Parameters	Control	RA	<i>P</i> -value
Age (years)	41.6 \pm 11.7	42.9 \pm 11.4	0.44
Sex (male/female)	4/6	34/46	
Disease duration (years)		7.7 \pm 7.0	
DAS28		4.49 \pm 1.27	
ESR (mm/h)	9.4 \pm 3.8	41.37 \pm 24.9	<0.001
CRP (mg/l)	2.88 \pm 1.26	27.26 \pm 8.1	
Hb (g/dl)	13.3 \pm 1.06	12.3 \pm 1.4	<0.001
Serum iron (μ g/dl)	92.5 \pm 16.8	59.1 \pm 12.2	<0.001
Ferritin (ng/ml)	75.8 \pm 20.3	115.37 \pm 30.6	<0.001
TIBC (μ mol/l)	223.7 \pm 45.5	245.2 \pm 36.65	0.001
Transferrin (ng/ml)	236.69 \pm 32.49	225.8 \pm 29.03	0.019
Tender joint counts		14.57 \pm 7.7	
Swollen joint counts		5.97 \pm 4.5	
C-reactive protein (mg/dl)	2.88 \pm 1.26	27.26 \pm 8.1	<0.001
Rheumatoid factor (IU/ml)	–	121.7 \pm 22.9	
Anti-CCP antibody (U/ml)	–	122.3 \pm 20.3	
Prohepcidin (ng/ml)	167 \pm 52	211.4 \pm 5.88	<0.001
IL-6 (pg/ml)	3.36 \pm 1.3	21.11 \pm 5.88	<0.001
TNF (pg/ml)	3.7 \pm 1.1	17.8 \pm 3.7	<0.001

CCP, cyclic citrullinated peptide; CRP, C-reactive protein; DAS28, Disease Activity Score 28; ESR, erythrocyte sedimentation rate; IL-6, interleukin-6; Hb, hemoglobin; RA, rheumatoid arthritis; TNF, tumor necrosis factor.

Table 2 Relation between anemia profiles, prohepcidin, tumor necrosis factor, and interleukin-6 in rheumatoid arthritis patients according to Disease Activity Score 28

Parameters	DAS28 ≤ 5.1 ($n = 47$)	DAS28 > 5.1 ($n = 33$)	<i>P</i> -value
Hemoglobin (g/dl)	12.66 \pm 1.3	11.6 \pm 1.3	<0.001
Iron (mg/dl)	61.6 \pm 9.9	54.6 \pm 14.5	0.007
TIBC (mg/dl)	252.9 \pm 32.2	231.9 \pm 40.4	0.008
Ferritin (ng/ml)	109.3 \pm 27	125.9 \pm 33.8	0.012
Transferrin (ng/ml)	232.2 \pm 25.2	214.73 \pm 32.15	0.005
IL-6 (pg/ml)	19.9 \pm 5.3	23.2 \pm 6.2	0.009
Prohepcidin (ng/ml)	206.4 \pm 18	220.1 \pm 24.2	0.003
TNF (pg/ml)	16.6 \pm 4.8	19.9 \pm 5.1	0.003

IL-6, interleukin-6; TNF, tumor necrosis factor.

Table 3 Statistically significant correlations between prohepcidin, laboratory parameters, and clinical findings

Parameters	RA	
	<i>r</i>	<i>P</i> -value
Prohepcidin–IL-6	0.5	<0.001
Prohepcidin–TNF	0.5	<0.001
Prohepcidin–TJC	0.3	0.004
Prohepcidin–SJC	0.42	<0.001
Prohepcidin–DAS28	0.31	0.003
Prohepcidin–ESR	0.24	0.03
Prohepcidin–RF	0.24	0.024
Prohepcidin–anti-CCP	0.28	0.008
Prohepcidin–CRP	0.24	0.023

CCP, cyclic citrullinated peptide; CRP, C-reactive protein; DAS28, Disease Activity Score 28; ESR, erythrocyte sedimentation rate; IL-6, interleukin-6; RA, rheumatoid arthritis; RF, rheumatoid factor; SJC, swollen joint count; TJC, tender joint count; TNF, tumor necrosis factor.

In active patients, both TNF- α and IL-6 levels were significantly higher than in patients with inactive-to-moderate disease (19.9 ± 5.1 vs. 16.6 ± 4.8 pg/ml and 23.2 ± 6.2 vs. 19.9 ± 5.3 pg/ml, respectively) (Table 3).

In the RA patients, serum prohepcidin concentration negatively correlated with serum iron ($r = -0.23$, $P = 0.04$), but it did not correlate with TIBC, ferritin, and transferrin. There was no difference of prohepcidin concentration between the patients with anemic and nonanemic groups.

We determined the relationship between serum prohepcidin concentration, laboratory, and clinical parameters of the RA group (Table 4). We found that there was a highly significant correlation between serum prohepcidin level and IL-6 ($r = 0.5$, $P < 0.001$), TNF- α ($r = 0.5$, $P < 0.001$), and SJC ($r = 0.42$, $P < 0.001$). In addition, there was a significant correlation between the serum prohepcidin level and tender joint count ($r = 0.3$, $P = 0.004$), DAS28 ($r = 0.31$, $P = 0.003$), ESR ($r = 0.24$, $P = 0.03$), RF ($r = 0.24$, $P = 0.024$), anti-CCP ($r = 0.28$, $P = 0.008$), and CRP ($r = 0.24$, $P = 0.023$).

Discussion

Hepcidin is known as an acute-phase reactant. Hepcidin synthesis is regulated by extrinsic or intrinsic iron loading, anemia, and hypoxemia. Moreover, the hepcidin production is induced by a particular cytokine, IL-6, during inflammation [6]. A recent study has shown alteration in the hepcidin gene expression by IL-6 [12].

IL-6 is one of the proinflammatory cytokines, which has a central role in the pathogenesis of RA, a representative inflammatory disease. IL-6 induces acute-phase response, differentiates B and T cells, and promotes joint destruction in RA [13]. Because anemia is a common extra-articular manifestation of RA, we hypothesized that IL-6-hepcidin could be the possible link in RA to anemia.

Table 4 Clinical and laboratory parameters in the anemia subgroups of rheumatoid arthritis patients

Parameters	Anemic ($n = 33$)	Nonanemic ($n = 47$)	<i>P</i> -value
Serum iron ($\mu\text{g/dl}$)	53.2 ± 12.5	64.4 ± 9.1	<0.001
ESR (mm/h)	32 ± 7.2	26 ± 7.6	0.001
CRP (mg/l)	51.1 ± 27	32.4 ± 19.1	<0.001
Disease duration (years)	11.3 ± 9.6	7.7 ± 6.2	0.04
Rheumatoid factor (IU/ml)	120.9 ± 20.9	122.4 ± 24.7	0.8
Anti-CCP antibody (U/ml)	123.6 ± 19.6	121.1 ± 20.9	0.6
Prohepcidin (ng/ml)	211.1	211.7	0.9

CCP, cyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

In our study, the mean concentration of serum prohepcidin was significantly higher in the RA patients than in the HC group ($P < 0.001$). IL-6 and TNF- α levels were also significantly higher in the RA patients than in the HC group ($P < 0.001$). This is consistent with the studies conducted by Kim and colleagues [14,15].

No difference in serum prohepcidin levels between the RA patients with or without anemia in contrast to the study conducted by Koca *et al.* [15], who found that serum prohepcidin levels were lower in the anemic subgroup than in the nonanemic subgroup. This may be explained that serum prohepcidin could not reflect the iron state exactly in human disease setting. Another explanation may be in the study conducted by Nicolas *et al.* (2002) [16] who found that when anemia develops, hepcidin production decreases in response to anemia and hypoxia. It was postulated that serum prohepcidin could not represent the bioactive hepcidin [17], and thus serum prohepcidin concentration did not reflect actual anemic parameters, as supported by previous studies [18].

In the study conducted by Koca *et al.* [15], the prohepcidin concentration does not correlate with RA disease activity scores, TNF- α , or IL-6. However, in our study, serum prohepcidin concentration correlated with RA disease activity parameters such as DAS28, SJC, tender joint count, ESR, and CRP. The serum concentration of prohepcidin also correlated with proinflammatory cytokines, such as TNF- α and IL-6. This is consistent with the study conducted by Kim *et al.* [14]. Correlation of prohepcidin with proinflammatory cytokines suggests that prohepcidin was induced by the proinflammatory cytokines, especially IL-6. Moreover, when the RA patients were divided into two groups by disease activity, the RA patients with active disease had higher concentration of serum prohepcidin than those with inactive-to-moderate disease. This result suggested that serum prohepcidin concentration could be another useful marker for RA disease activity. It is known that high RF and anti-CCP titers in RA are associated with more severe clinical disease course. Our results showed that the prohepcidin levels in the RA group positively correlated with RF and anti-CCP titers. Cytokines, including IL-6 which can affect RF, anti-CCP, and prohepcidin production may explain this correlation [19].

In our study, levels of prohepcidin did not correlate with TIBC, ferritin, and transferrin, this is consistent with the results of the study conducted by Roe and colleagues [20,21].

Kim *et al.* [14] postulated that there is a different clinical significance between hepcidin and prohepcidin: the former might regulate iron metabolism, and the latter might regulate inflammatory process. Therefore, the available method for measurement of hepcidin is needed to reveal the accurate relationship between inflammatory diseases and anemia.

Conclusion

Hepcidin or prohepcidin might play an important role in RA as the mediator linking anemia and the inflammatory cytokines. Prohepcidin could be another useful marker for RA disease activity.

Acknowledgements

Conflicts of interest

None declared.

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