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Does iron-deficiency anemia affect M1 macrophage activation and inflammation?

Demir eksikliği anemisi M1 makrofaj aktivasyonu ve inflamasyonu etkiler mi?

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ABSTRACT

Aim: Iron deficiency anemia (IDA) is a prevalent disorder and may be a problem for various systems. Anemia of inflammation has been extensively investigated before, but there is still a lack of knowledge about macrophage activation in IDA. Hence, the aim of this study was to investigate the relationship between IDA and macrophage activation.

Patients and Methods: The present study included 88 female subjects. The participants were divided into two groups: 48 IDA patients in the patient group and 40 healthy subjects in the control group. M1 macrophage activation was measured with the triggering receptor expressed on myeloid cells-1 (TREM-1). TREM-1 levels and C-reactive protein were compared between patient and control groups. The relationship between TREM-1 levels and hemogram parameters and iron status was investigated.

Results: TREM-1 levels of the patient group were significantly higher than of the control group [124.5 (6.8-770.5) pg/ml vs 48.5 (0.66-401.1) pg/ml, p=0.02], while CRP levels remained similar between the groups. There was no correlation between TREM-1 levels and hemoglobin, mean erythrocyte volume, ferritin, transferrin saturation and serum iron (p =0.96, 0.14, 0.21, 0.16, and 0.26, respectively) in IDA patients.

Conclusion: The present study showed that IDA might increase TREM-1 levels and this condition might be a clue of macrophage activation. IDA patients should be considered in terms of pro-inflammatory conditions and further investigations are needed to clarify the association mentioned above.

Keywords: Anemia, iron deficiency, triggering receptor expressed on myeloid cells-1

ÖZ

Amaç: Demir eksikliği anemisi (DEA) farklı sistemler için sorun olabilen yaygın bir bozukluktur. İnflamasyonun oluşturduğu anemi çalışılmakla birlikte, DEA'nın yol açtığı makrofaj aktivasyonu hakkında bilgi eksikliği bulunmaktadır. Bu çalışmada DEA ile makrofaj aktivasyonu arasındaki ilişkinin araştırılması amaçlanmıştır.

Hastalar ve Yöntem: Çalışmaya 88 kadın katılımcı alındı. Katılımcılar; 48 DEA hastası ve 40 sağlıklı kontroller olmak üzere iki gruba ayrıldı. M1 makrofaj aktivasyonu, triggering receptor expressed on myeloid cells-1 (TREM-1) düzeyleri ölçülerek değerlendirildi. CRP ve TREM-1 düzeyleri iki grupta karşılaştırıldı. TREM-1 düzeyleri ile vücudun demir durumu ve hemogram parametreleri arasındaki ilişki araştırıldı.

Bulgular: TREM-1 düzeyi, hasta grubunda kontrol grubuna göre daha yüksek saptandı [124.5 (6.8-770.5) pg/ml vs 48.5 (0.66-401.1) pg/ml, p=0.02]. CRP düzeyi her iki grupta benzerdi. Hasta grubunda TREM düzeyi ile hemoglobin, ortalama eritrosit hacmi, ferritin, transferrin saturasyonu, serum demiri arasında korelasyon saptanmadı (sırasıyla p değerleri 0.96, 0.14, 0.21, 0.16, 0.26).

Sonuç: Bu çalışmada DEA'da TREM-1 düzeylerinin artışının gösterilmesi, makrofaj aktivasyonunun göstergesi olabilir. DEA hastaları proinflamatuvar durumlar açısından göz önünde bulundurulabilir. Bahsedilen ilişki için daha kapsamlı çalışmalara ihtiyaç bulunmaktadır.

Anahtar Kelimeler: Anemi, demir eksikliği, triggering receptor expressed on myeloid

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Introduction

Iron deficiency anemia (IDA) is a frequent and global healthcare problem, in particular for young women in developed countries. Iron is necessary for biologic functions, such as cell cycles, energy production, respiration and DNA synthesis [1]. Impairing oxygenation at tissue level, IDA has adverse effects on the cardiovascular system [2] and while anemia is well-known in inflammatory conditions, the effects of anemia on immunity, especially on macrophages, require clarification. Macrophages are the main elements of the innate immune system and they play a critical role in the stimulation of the adaptive immune system. They can polarize to M1 and M2 macrophages according to the type of stimulation, wherein M1 macrophages produce pro-inflammatory proteins while M2 macrophages produce anti-inflammatory factors [3]. The triggering receptor expressed on myeloid cells-1 (TREM-1) is a glycoprotein that weighs 30 kDa and owns an extracellular V-type Ig-like domain from the immunoglobulin superfamily. It is expressed from cell membranes of neutrophil, monocyte, and macrophages. TREM-1 induces various pro-inflammatory chemokines and cytokines and also reflects M1 macrophage activation [4]. It has a crucial role in initiating the inflammatory process by cross-talking with Toll-like receptors and/or nucleotide-binding oligomerization domain-like receptors (NLRs). It was defined in infectious diseases and considered as a response to infections. There are soluble and membrane-bounded TREM-1 forms [5] and although TREM-1 is a main potential biomarker of infectious diseases [6], the negative results of high TREM-1 levels were studied in inflammatory but non-infectious diseases, such as irritable bowel syndrome [7], inflammatory bowel diseases [8], obstructive sleep apnea [9], and coronary artery disease [10].

It is still not known whether IDA may direct macrophages to M1 polarization and cause pro-inflammatory status. Therefore, the present study aimed to investigate the effects of IDA on M1 macrophage activation, via TREM-1 levels.

Materials and methods

Participants

The present study included 88 female subjects who were between 18 and 45 years of age and in the reproductive period. The subjects were selected among people who admitted to Kırıkkale University, Department of Internal Medicine Outpatient Clinic between August and October 2019. The participants were divided into two groups: while the patient group was composed of patients with IDA, healthy participants constituted the control group. For this purpose, hemoglobin (Hb) level <12 g/dl, transferrin saturation <16%, and ferritin <10 µg/l were accepted as the criteria of the diagnosis of IDA [1]. TREM-1 levels were compared between the two groups and the relationship between these, iron parameters and hemogram parameters of the patients were sought. The alanine aminotransferase (ALT), as a liver function test, and serum creatinine, as a kidney function test, were noted for the evaluation of organ failures. C-reactive protein (CRP) levels were compared between the two groups regarding inflammation. The patients with inflammatory conditions, such as polycystic ovary syndrome, anemia resulting from secondary causes, menopause, smoking, any organ failure, chronic illnesses as well as any infectious disease, were excluded from the study. Informed consent was obtained from all the participants.

Biochemical analysis

All blood samples were collected from the antecubital vein after a 12-hour fasting period. The samples were then centrifuged at 3500 rpm for 10 minutes and their sera were separated. The serum was divided into groups and stored at -80°C until the analysis. Serum human TREM-1 levels were measured using the micro ELISA method with the help of the HUMAN TREM-1 (Cusabio® code: CSB-E04836h, uniprot: Q9NP99). Blood counts, iron parameters, and liver and kidney function tests were studied at Kırıkkale University, Biochemistry Laboratory. The results were exported as pg/ml.

Statistical analyses

The IBM SPSS version 25.0 was used for all the statistical analysis. Normally distributed data was summarized as mean and standard deviation, while the median (minimum-maximum) was given for non-normally distributed values. The normality was checked with the Kolmogorov-

Smirnov test. The two-group comparisons were performed using the Mann-Whitney U test or the T-test. Spearman's Correlation Coefficient was calculated to determine the correlation between TREM-1 levels and Hb, ferritin, and transferrin saturation. The significance level was taken as $p< 0.05$ in all statistical analysis.

Ethical consideration: Kırıkkale University, Institutional Review Board granted ethical approval for the study (Date: 07.08.2019, Number: 17/04).

Results

All the participants were divided into two groups: 48 IDA patients in the patient group and 40 healthy participants in the control group. The median age of the patient group was 35 years while it was 32 years in the control group. Hemoglobin, transferrin saturation, ferritin, and serum iron levels were significantly lower in the IDA group, as expected ($p<0.001$ for each parameter). Thrombocyte counts were higher in the patient group, which was evaluated as reactive thrombocytosis. CRP levels were similar between the groups, while TREM- 1 levels were significantly higher in the patient group than in the control group [124.5 (6.8-770.5) pg/ml vs 48.5 (0.66-401.1) pg/ml, p=0.02] (Table 1). There was no correlation between TREM levels and Hb, ferritin, transferrin saturation and serum iron ($p = 0.96, 0.21, 0.16, 0.26$, respectively)

Discussion

The present study concluded that IDA might raise plasma TREM-1 levels and this result may be a clue for the direction of pro-inflammatory macrophages by IDA. TREM-1 levels may also not be related to the levels of iron parameters. The difference of platelet counts in the two groups can be explained by reactive thrombocytosis of IDA. TREM-1 elevation may be about anemia but iron status.

A recent study showed that cell iron status might influence macrophage polarization. Low iron diet exacerbated pro-inflammatory response via M1 macrophages, while intracellular iron increase reduced inflammation and M2 macrophage polarization in vivo and in vitro [11].

One study on an animal model concluded that neonatal piglets with dietary iron deficiency had impaired peripheral immunity [12]. Another animal study showed that iron supplementation in mice induced innate cellular defenses and made the organism stronger to malaria infection [13].

Table 1. Two-group comparisons about subject and laboratory values.

	Patients with IDA (n=48)	Control group (n=40)	Significance
Age, years	35 (19-45)	32 (20-45)	p=0.14
BMI, kg/m2	21.9 (19.3-24.8)	23.6 (19.4-24.8)	p=0.37
Hemoglobin (g/dl)	9.6(5.5-11.6)	13.1(12.0-15.5)	p<0.001
Platelet counts ($x10^3$/µl)	304 (176-556)	256 (156-347)	p<0.001
Leukocyte counts ($x10^3$/µl)	6.5(4.2-9.4)	7.4(4-11.2)	p=0.18
Ferritin (µg/L)	4.6 (2.1-8.8)	46 (16-203)	p<0.001
Transferrin saturation (%)	5 (0.8-17)	26 (16-128)	p<0.001
Serum iron (µg/dl)	21.5 (2-58)	66 (28-186)	p<0.001
Creatinine (mg/dl)	0.6 (0.4-0.8)	0.7(0.5-0.8)	p=0.11
ALT	12.7(5.6-20.7)	12 (5-21)	p=0.78
C-reactive protein (mg/l)	2.43 (0.3-4.6)	2.26 (0.8-3.6)	p=0.61
TREM-1 (pg/ml)	124.5 (6.8-770.5)	48.5 (0.66-401.1)	p=0.02

Macrophages have a critical role in iron homeostasis by recycling iron through phagocytosis of old erythrocytes and making it suitable for erythropoiesis. Macrophage polarization is closely related to differential regulation of iron metabolism due to the molecules involved in iron uptake, storage, and release [14,15]. Low intracellular iron in macrophages may prevent the expression of pro-inflammatory cytokines, such as IL-6 and TNF-α, and it may also decrease the expression of inducible nitric oxide synthase [16,17]. Iron deficiency in M2 macrophages also prevent the formation of functional iron-containing enzymes in arachidonic acid metabolism. In addition, Iron may affect the biosynthesis of the iron-containing enzyme, tyrosine hydroxylase, which catalyzes the rate-limiting step in catecholamine biosynthesis and, consequently, the inflammatory response [18]. Nevertheless, the present study showed that TREM-1 levels were higher in the patient group, which is not consistent with the findings above.

Iron retention in macrophages stimulates the expression of pro-inflammatory cytokines and the innate immune response in vivo [19]. The iron accumulation and the induction of the M1 phenotype are characterized by the production of reactive oxygen and nitrogen species, which may result in impaired capacity for tissue repair [20]. Therefore, it can be asserted that iron has diverse effects on macrophage function via multiple pathways.

Askar et al. [21] demonstrated that pro-inflammatory cytokines, such as TNF-α and interleukin-6, antimicrobial proteins, such as hepcidin, defensin, and chemerin, acute phase reactants, such as C-reactive protein, were elevated in older patients with IDA. The investigators noted that both aging and IDA might affect pro-inflammatory cytokines. Their findings support ours and may be a clue for the inflammation occurring in IDA.

On the other hand, the present study had several limitations. It was a case-control study with a relatively small sample size. Moreover, only female patients were included in the study: male participants were excluded from the study because IDA in male patients was a result of other conditions, which could have affected the standardization among patients with IDA. Additionally, TREM-1 levels were evaluated only in the plasma, while the other parameters about macrophage polarization and inflammatory cytokines were not assessed. Moreover, we did not evaluate other types of anemia in this study. To the best of our knowledge, ours is the first research study about TREM-1 levels of IDA patients, hence more extensive and comprehensive studies are required to explain macrophage polarization and IDA.

The present study concluded that iron deficiency anemia, which is a prevalent disorder, may cause elevations in TREM-1 levels. This condition may imply that IDA induces macrophage polarization to M1 macrophages which are pro-inflammatory cells. IDA patients should be considered for both inflammation and response to inflammation, and further investigations are needed to clarify the mechanism of inflammatory processes in IDA patients.

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The authors declare that there is no financial support and conflict of interest regarding the publication of this article.

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