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Original Article

Iron Deficiency Anaemia is Associated with Decreased Levels of Macrophage Migration Inhibitory Factor and Monocyte Chemoattractant Protein-1

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ABSTRACT

Many bodily systems are affected by iron deficiency anaemia (IDA), including the immune system. However, the pathophysiological mechanisms whereby this anaemia promotes deterioration in immunity remain largely unexplained. In order to enlighten this pathophysiological link , serum levels of macrophage migration inhibitory factor (MIF) and monocyte chemoattractant protein-1 (MCP-1), which play an important roles in a healthy functioning immune system, were examined in patients with IDA and healthy volunteers. A total of 30 patients with IDA (mean age 33.6±7.8 years, 30% male) and 30 healthy individuals (mean age 30.1 ± 8.8 years, 27 % male) were included. Serum MIF and MCP-1 levels were measured in a sandwich-assay format, using commercial enzyme-linked immunosorbent assay kits. There was no statistically significant difference in age and gender distribution between participants with IDA and controls (p>0.05). Serum MIF and MCP-1 concentrations were lower in the IDA group than in the control group (p < 0.005, and p < 0.01, respectively). When considering the role of MIF and MCP-1 in maintaining the normal immune response of the organism, a decrease in production in patients with IDA may contribute to immune dysfunction in these individuals.

Key words: *Iron deficiency anaemia, macrophage migration inhibitory factor, monocyte chemoattractant protein-1*

Demir eksikliği anemisinin Makrofaj Migrasyon İnhibitor Faktör ve Monosit Kemoatraktan Protein-1 düzeyleri ile ilişkisi

ÖZET

İmmün sistemi de içeren pekçok vücud sisteminin demir eksikliği anemisinden etkilendiği bilinmektedir. Bununla beraber, demir eksikliği anemisinin immün sistemde meydana getirdiği değişiklikleri hangi mekanizmalarla yaptığı tam olarak aydınlatılamamıştır. Bu çalışmada, bu patofizyolojik ilişki aydınlatmak amacıyla demir eksikliği anemisi olan hasta ve sağlıkı gönüllülerde, sağlıklı işleyen bir immün sistemde önemli rolleri bulunan monosit, makrofaj migrasyon inhibitor faktör (MIF) ve monosit kemoatraktan protein-1(MCP-1)'nin serum düzeyleri incelenmiştir. Demir eksikliği anemisi olan 30 hasta (ortalama yaş 33.6±7.8 yıl, % 30 erkek) ve sağlıklı gönüllü (ortalama yaş 30.1 ± 8.8 yıl, % 27erkek) çalışmaya dahil edildi.Serum MIF ve MCP-1 ticari kitler kullanılarak sandviç ELISA yöntemiyle ölçüldü. Hasta ve kontrol grubu arasında yaş, cinsiyet dağılımı açısından fark saptanmadı (p>0.05). Serum MIF ve MCP-1 düzeyleri demir eksikliği anemisi olan hastalarda düşük saptandı (sırasıyla , p < 0.005 ve p < 0.01). MIF ve MCP 'nin normal immün yanıttaki rolleri dikkate alındığında, demir eksikliği anemisi olan hastalarda bu iki sitokinin azalmış düzeyleri bu hasta grubunda saptanan immün disfonksyona katkıda bulunabilir.

Anahtar kelimeler: *Demir eksikliği anemisi, makrofaj migrasyon inhibitör faktör, monosit kemoatraktan protein-1*

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INTRODUCTION

Iron deficiency anaemia (IDA) is known to be a major public health problem in children and in women of childbearing age (1). Textbook descriptions of iron deficiency usually list various signs and symptoms; however, the most important clinical clue is the presence of chronic fatigue (2). Many bodily systems are affected by anaemia, including the immune system (3), and deterioration of cell-mediated immune functions, as well as decreased levels of circulating peripheral blood T lymphocytes as a consequence of iron deficiency, has been shown in some studies (4). Although human immunoglobulin levels are not decreased in iron deficiency (5), animal studies have shown that the humoral response is quite altered (6). Macrophages play an important role in nonspecific immunity and, with the contribution of polymorphonuclear leukocytes, represent a primary defence before the activation of both cell-mediated and humoral immunity. Data regarding in vivo macrophage function in iron deficiency are scarce; however, animal studies have demonstrated that such functions are impaired (7,8).

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine that is primarily produced by activated macrophages, and has been revealed as an important player in the systemic inflammatory response (9). MIF was discovered as a lymphokine that is involved in delayed hypersensitivity and various macrophage functions, including proinflammatory cytokine production, adherence and phagocytosis of macrophages, as well as induction of metalloproteinase and endogenous counterregulation of glucocorticoid activity (10).

Monocyte chemoattractant protein-1 (MCP-1), also known as CC chemokine ligand 2, is one of the key chemokines regulating migration and infiltration of monocytes/macrophages (11), which are major sources of MCP-1 (12). Mononuclear cells are mobilised from bone marrow and recruited to sites of inflammation by MCP-1.

The pathophysiological mechanisms whereby IDA promotes deterioration in immunity remain largely unexplained. Nevertheless, few in vivo studies have evaluated MIF and MCP-1 levels in IDA. The present study was designed to evaluate the influences of iron deficiency on serum MIF and MCP-1 levels.

MATERIALS AND METHODS

Study participants

The study was conducted in patients who were referred to the Internal Medicine Department of Namık Kemal University Medical Faculty, Tekirdag, Turkey, between April and June 2013. These individuals voluntarily participated in this study, the medical history of each was obtained and recent laboratory results were reviewed. A total of 30 patients (mean age 33.6±7.8 years, 30% male) presenting with IDA as the solitary pathology were included in the experimental group. The inclusion criteria were a haemoglobin (HGB) level of under 12.5 g/dl, a red blood cell (RBC) count of under 4x1012/l, a mean corpuscular volume (MCV) of under 80 fl, a mean corpuscular haemoglobin (MCH) of under 27 pg, a serum iron level of under 50 µg/dl, a total iron binding capacity (TIBC) of over 400 µg/dl and a serum ferritin level of under 20 µg/dl. The exclusion criteria were history of acute or chronic infection, a familial history of immunodeficiency, a history of cancer, a history of endocrinopathy, especially hypo- or hyperthyroidism, pregnancy and possible thalassaemia, according to the laboratory results

A total of 30 healthy individuals (mean age 30.1±8.8 years, 27% male), based on recent routine laboratory results, were enrolled as the control group during the same period. Inclusion criteria were absence of anaemia and iron deficiency with a HGB level of 13–16 g/dl, an RBC count of 4–6x1012/l, an MCV of 80–96 fl, an MCH of 27–33 pg, a serum iron level of 50–150 µg/dl, a TIBC of 250–400 µg/dl, and a serum ferritin level of 20–200 µg/dl. Participants in the control group had no history of chronic disease or drug consumption during the previous 6 months. The iron-deficient and control groups were comparable with respect to age and gender. The characteristics of the participants in the experimental and control groups are shown in Table 1.Routine blood tests, including complete blood cell count (CBC),serum iron, total iron binding capacity, ferritin and liver enzymes, were conducted.

All participants provided informed written consent for the study, which was approved by the Ethics Committee of Namık Kemal University Medical Faculty.

Measurement of Serum Levels of MIF and MCP-1

After overnight fasting, blood samples were obtained to determine MIF and MCP-1. Serum samples, obtained by centrifugation, were then immediately frozen at -80 °C until further analysis of MIF and MCP-1 was conducted.

***Table 1**. The characteristics of the participants in the experimental and control groups*

Groups	Age	n	M/F	MIF (ng/mL)	MCP-1 (pg/mL)
Control	30.1 ± 8.830		8/22	32.7 ± 5.8*	299 ± 40**
Anemia	33.6 ± 7.830		9/21	27.9 ± 5.3	271 ± 38

*MCP-1:Monocyte chemoattractant protein-1, MIF: Macrophage migration inhibitory factor , M.Male, F:Female, *p<0.005 **p<0.01*

The serum levels of MIF (QuantikineHuman MIF ELISA; R&D Systems) and MCP-1 (Quantikine Human CCL2/MCP-1ELISA; R&D Systems) were specified with commercial enzyme-linked immunosorbent assay kits. MIF and MCP-1 were measured in a sandwich-assay format, using two specific and high affinity antibodies, streptavidin peroxidase conjugate and a chromogenic substrate. The minimum detectable levels of MIF and MCP-1 were 0.016ng/ml and 1.7 pg/ml, respectively.

Biochemical tests and blood cell count

Serum iron and TIBC levels were measured using a Cobas C501 Roche biochemistry autoanalyser, and serum ferritin level was analysed using a Cobas e 6000 autoanalyser (Roche Diagnostics). CBC was determined usinga Roche Sysmex XT-2000i autoanalyser and commercial kits, also from Roche.

Statistical analysis

Statistical tests were performed with an SPSS 12.0 software package (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL).Data are expressed as mean ± standard deviation of the mean. The differences between groups were evaluated using the student t test. Values of $p < 0.05$ were considered significant.

RESULTS

There was no statistically significant difference in age and gender distribution between the experimental and control participants ($p>0.05$), as shown in Table 1. Serum MIF and MCP-1 concentrations were lower in the IDA group than in the controls ($p < 0.005$ and $p < 0.01$, respectively) (Table 1). The mean HGB, MCV, serum iron and ferritin levels were significantly lower, while the TIBC was higher, in the IDA group vs. the control group. Statistical analysis by t test showed significant differences between these two groups for these parameters ($p<0.001$), as shown in Table 2.

DISCUSSION

Iron is essential for almost all living organisms and plays a role in a number of important biological processes, as well as in both pathogen virulence and host antimicrobial responses (13). As a consequence, disturbances of iron homeostasis can alter the body's susceptibility to infectious disease. While there is evidence of an altered immune profile in iron deficiency, the exact immunoregulatory role of iron is poorly understood (14). Iron deficiency relating to disturbances in humoral- and cell-mediated immunity have been studied extensively in both humans and animals. Although little evidence exists to suggest major humoral deficiencies (15), impairment of cell-mediated immunity has been well documented in iron deficiency; reduced neutrophil and macrophage function, with decreased myeloperoxidase activity, impaired bactericidal activity, a decrease in circulating peripheral T-lymphocytes with thymic atrophy, defective T lymphocyte-induced proliferative response, and impaired natural killer cell activity have all been demonstrated (16-18).

It is well known that cytokines are involved in immune function, and iron deficiency is related to various alterations in serum cytokine levels. Decreased production of

***Table 2**. The mean HGB, MCV, serum iron and ferritin levels*

	Unit	Control (Mean ± SD)	IDA (Mean ± SD)
HGB	g/dL	13.2± 1.2	10.1 ± 1.4*
MCV	fL	92.9± 5.3	77.8 ± 5.1*
Serum iron	µg/dL	80.3± 15.6	18.6 ± 10.8*
TIBC	µg/dL	287± 51	426 ± 58*
Ferritin	ng/mL	38.6 ± 10.8	8.3 ± 6.8*

interleukin (IL)-2, IL-4 and IL-8 has been reported in iron deficiency, while there are conflicting data regarding production of IL-1, IL-6, tumour necrosis factor (TNF)-α and interferon(IFN)-γ (19-22). Jason et al. showed that iron-deficient children were capable of producing a higher percentage of IFN-γ with in vitro stimulation, although a lower percentage of lymphocytes produced IFN-γ in vivo (i.e. spontaneously) (22).

This study was designed to investigate the serum MIF and MCP-1 levels in individuals with IDA. A scan of the literature revealed that few in vivo and in vitro studies have evaluated MIF levels in iron deficiency, and no previous in vivo studies have been conducted to assess the serum MCP-1 levels of individuals with IDA. The present study revealed that serum levels of MIF were significantly lower in participants with IDA than in the control group. Polati et al. showed that incubation of mouse bone marrow macrophages with ferric ammonium resulted in an abundance of MIF (23). Conversely, Kasvosve et al. showed that ferroportin Q248H and low iron stores are both associated with lower circulating TNF-α, while only ferroportin Q248H is associated with lower circulating macrophage MIF in African children (24). Genetic differences and the differences in the characteristics of the study groups may be responsible for this discrepancy. Secretion of MIF is mediated by several pathways, in one of which TNF-α induces MIF gene expression, resulting in elevated levels of circulating plasma MIF (25). This mechanism led us to the hypothesis that decreased plasma MIF levels may be a consequence of decreased TNF-α levels, which is related to iron deficiency (26).

With regard to MCP-1, we showed that there was a decrease in serum levels in participants with IDA as compared to the controls. Decreased MCP-1 levels were negatively correlated with serum iron, total iron binding capacity and ferritin levels. Although no previous in vivo studies regarding MCP-1 in iron deficiency have been conducted, a cell culture study has revealed that decreased intracellular iron was related to decreased MCP-1 secretion (27). In a similar manner to MIF, decreased IL-1or TNF-α, due to the iron deficiency, may share responsibility for decreased levels of MCP-1, since MCP-1was shown to be secreted as a consequence of IL-1 or TNF-α stimuli (28).

In conclusion, iron deficiency depresses certain aspects of cell-mediated immunity and innate immunity, but the exact mechanism of iron deficiency-induced immune dysfunction and susceptibility to infection has not been well elucidated. Considering the role of MIF and MCP-1 in maintaining the normal immune response of the organism, a decrease in production of these cytokines in individuals with IDA may contribute to their immune dysfunction and susceptibility to infections.

Conflict of interest:

All authors have no conflict of interest to declare

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