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# The effect of iron deficiency anemia on the function of the immune system

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We aimed to study the effect of iron deficiency anemia (IDA) on immunity. In 32 children with IDA and 29 normal children, the percentage of T-lymphocyte subgroups, the level of serum interleukin-6 (IL-6); and the phagocytic activity, the oxidative burst activity of neutrophils and monocytes and the levels of immunoglobulins were compared. There was no difference in the distribution of T-lymphocyte subgroups. The mean IL-6 levels was $5.6 \pm 3.9$ pg/ml in children with IDA and $10.3 \pm 5.3$ pg/ml in the control group ($P < 0.001$). The percentage of neutrophils with oxidative burst activity when stimulated with pma was $53.4 \pm 32.7\%$ in children with IDA and $81.7 \pm 14.3\%$ in the control group ($P = 0.005$). The percentage of monocytes with oxidative burst activity was $13.8 \pm 11.7\%$ in children with IDA and $35 \pm 20.0\%$ in the control group ($P < 0.001$) when stimulated with pma. and $4.3 \pm 3.1$ versus $9.7 \pm 6.0\%$ ($P = 0.008$) when stimulated with fMLP. The ratio of neutrophils with phagocytic activity was $58.6 \pm 23.3\%$ in the anemic group; and $74.2 \pm 17.7\%$ in the control group ($P = 0.057$). The ratio of monocytes with phagocytic activity was $24.3 \pm 12.0\%$ in the anemic group; and $42.9 \pm 13.4\%$ in the control group ($P = 0.001$). IgG4 level was $16.7 \pm 16.6$ mg/dl in children with IDA and $51.8 \pm 40.7$ mg/dl in healthy children ($P < 0.05$). These results suggest that humoral, cell-mediated and nonspecific immunity and the activity of cytokines which have an important role in various steps of immunogenic mechanisms are influenced by iron deficiency anemia.
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## Introduction

Protein-energy malnutrition is known to be associated with a significant impairment of cell-mediated immunity, phagocyte function, complement system, secretory immunoglobulin A antibody concentrations, cytokine production and an altered immune response. Deficiency of single nutrients as zinc, selenium, iron, copper, vitamins A, C, E and $B_6$, and folic acid have important influences on immune responses. Altered immune responses have considerable practical and public health significance.[1] Iron deficiency anemia due to nutritional deficiency is not just a disease of developing countries, it can also be seen in developed countries. Worldwide, over 40% of the children have iron deficiency anemia, frequently associated with infections. The prevalence of anemia was found to be between 79% in 1977 and 44.3% in 1997 among Turkish children.[2,3]

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The relationship between iron deficiency and infection susceptibility has been investigated as well.[4] Experimental evidence in the last decades show that iron is a fundamental element for normal development of the immune system. Its deficiency affects the capacity to have an adequate immune response. The role of iron in immunity is necessary for immune cell proliferation and malnutrition, particularly lymphocytes, associated with the generation of a specific response to infection.[5] Despite proven reversible functional immunological defects, a clinically important relationship between states of iron deficiency and susceptibility to infections remain controversial. There are several possible mechanisms that could explain the effects of iron deficiency on immune system. In adult animals or humans with intact immune systems, nonspecific immunity is affected by iron deficiency in several ways. Macrophage phagocytosis is generally unaffected by iron deficiency, but bactericidal activity of these macrophages is shown to be attenuated in some studies.[6] Neutrophils have a reduced activity of the iron-containing enzyme, myeloperoxidase, which produces reactive oxygen intermediates responsible for intracellular killing of pathogens.[7]

There is a decrease in both T-lymphocyte number and T-lymphocyte blastogenesis and mitogenesis in iron deficiency in response to a number of different mitogens.[5] On the other hand, others have found a normal T-lymphocyte proliferative response to mitogens.[8] Cytokines are important mediators of cellular immune activity. Little is known concerning the effects of clinical iron deficiency on cytokines, although it has been reported that the *in vitro* production of interleukin (IL)-2 by lymphocytes of iron-deficient children may be impaired.[9] Humoral immunity appears to be less affected by iron deficiency than is cellular immunity. In iron-deficient humans, antibody production in response to immunization with most antigens is preserved.[5–7]

Iron is also required for monocyte/macrophage differentiation and macrophages require iron as a cofactor for the execution of important antimicrobial effector mechanisms, including the NADPH-dependent oxidative burst.[10,11]

We aimed to make a prospective study to evaluate the effect of iron deficiency anemia on humoral, cellular, and nonspecific immunity; and on the cytokines which are the key factors of many immunologic steps.

## Materials and methods

A total of 32 children with a mean age of $13.8 \pm 4.3$ months (6–24 months) were diagnosed to have iron deficiency anemia (Hb levels below 2 s.d.'s of the age-matched levels, ferritin $< 10$ ng/ml, transferrin saturation $< \%10$) which was related to nutritional deficiency. All patients had normal growth and normal serum albumin levels. Nutritional iron deficiency was the only etiology of iron deficiency anemia, and the cases had no other nutritional deficits. Our exclusion criteria were previous iron replacement therapy, any recent illness or chronic disease. The control group consisted of 29 healthy children at the same ages. The control group's hemoglobin, hematocrit and MCV levels were within normal values for children of the same age and sex. The complete blood count (automatic cell analyzer 600), serum iron, total iron binding capacity (spectrophotometry), ferritin (RIA), serum albumine (colorometry) were measured. A measure of 1 ml of blood sample with EDTA-containing tubes was taken by venipuncture from each patient for complete blood count including differential cell counts, hemoglobin, hematocrit, serum IgG, subgroup of IgG, IgM, IgA, albumin and iron, iron binding capacity and ferritin levels, whole blood samples were collected. Serum immunoglobulins and subgroup of IgG were measured by using commercially prepared antisera to IgG, IgA, IgM and TurboxTM protein analyzer and radial immune diffusion method. Lymphocyte subsets were analyzed on a flowcytometer (Becton Dickinson) using Anti-CD3/CD4, Anti-CD3/CD8, Anti-CD3/CD19 and Anti-CD3/CD16 + CD56 (FITC/PE) antibody kits (Becton Dickinson, San Jose, CA, USA).

We used serum samples for determining Interleukin-6 (IL-6) levels with commercially available kit although serum, heparin, EDTA-anticoagulated plasmas and culture fluids can be suitable for this assay. In patients and control group serum IL-6 level ('CLB Pelikine Compact™ human IL-6 ELISA kit Netherlands'), oxidative burst (Burst test, Orpegen Pharma, Germany) and phagocytic activity of neutrophils and monocytes (Fagotest$^R$, Orpegen Pharma, Germany) were also studied. Phagocytic activity of neutrophils and monocytes were measured number of phagocytic cells by flowcytometry using Fagotest$^R$, Orpegen Pharma, Germany) kit. For determining oxidative burst floressain dye (Rhodamin 123) was used and florescein staining was measured by flow cytometry with commercially available kit (Bursttest, Orpegen Pharma, Germany).

The statistical analysis were carried out using Student's *t*-test for independent samples.

## Results

There was a statistically significant difference between all hematologic parameters in the two groups ($P < 0.001$) (Table 1).

There was no statistically significant difference in IgG, A, M and IgG1, G2, G3 levels of both groups. Mean serum IgG4 levels of the study group were $16.7 \pm 16.6$ mg/dl; the control group had mean serum levels of $51.8 \pm 40.7$ mg/dl. A statistically significant difference was detected between two groups ($P < 0.05$) (Table 2). The study group had serum IL-6 levels with a mean of $5.6 \pm 3.1$ pg/ml, while the control group had a mean IL-6 level of $10.3 \pm 5.3$ pg/ml in 27 cases ($P < 0.001$).

**Table 1** Hematological parameters in the study (anemia) group and control group

| | *Study group* (n = *32*) *Mean* ± *s.d.* | *Control group* (n = *29*) *Mean* ± *s.d.* |
|---|---|---|
| Hemoglobin (g/dl) | 8.8 ± 1.2 (6.2–10.8) | 12.1 ± 0.3 (11.5–12.6) |
| Hematocrite (%) | 28.2 ± 2.1 (23.1–30.9) | 35.8 ± 1.2 (34.2–37.7) |
| MCV (fl) | 62.9 ± 5.9 (50–72.9) | 78.5 ± 2.6 (72.4–82) |
| RDW | 16.6 ± 1.4 (12–18.1) | 12.2 ± 1.2 (10.1–14.2) |
| Serum iron ($\mu$g/dl) | 27.3 ± 9.5 (13–43) | 88.5 ± 23.9 (54–132) |
| Total iron binding capacity ($\mu$g/dl) | 469.9 ± 38.7 (411–576) | 325.7 ± 50.3 (268–462) |
| Ferritin (ng/ml) | 4.3 ± 2.4 (1–9.6) | 78.1 ± 33.7 (13.5–175) |
| Transferrin Sat. (%) | 5.9 ± 2.3 (3.3–10) | 28.1 ± 9.9 (17.8–49.2) |
| WBC | 9696 ± 2686 (6100–16 000) | 8941 ± 2209 (6500–15 500) |

**Table 2** Results of humoral immunity (mg/dl) and T-lymphocyte subgroup (%)

| | *Study group* *Mean±s.d.* | *Control group* *Mean±s.d.* | *P* |
|---|---|---|---|
| *IgG* | 811.1±187.6 (441–1199) | 885.3±240.0 (504–1620) | NS |
| IgG1 | 552.3±158.6 (238–714) | 671.1±207.4 (475–982) | NS |
| IgG2 | 107.0±31.9 (67.6–165) | 160.9±75.8 (55.6–241) | NS |
| IgG3 | 45.3±23.5 (7.9–91.7) | 38.4±19.1 (17–71.5) | NS |
| IgG4* | 16.7±16.6 (3.3–51.3) | 51.8±40.7 (3.3–132) | <0.05 |
| IgA | 51.1±42.5 (20.3–266) | 49.7±23.3 (17.3–130) | NS |
| IgM | 127.1±46.9 (53–217) | 111.7±40.8 (44–200) | NS |
| $CD3^+$ | 62.81±6.38 (51–72) | 62.96±7.69 (45–72) | NS |
| $CD4^+$ | 34.65±7.14 (22–48) | 36.34±9.78 (17–58) | NS |
| $CD8^+$ | 28.84±6.89 (10–37) | 26.96±7.63 (20–50) | NS |
| $CD3^+/CD8^+$ | 1.36±0.81 (0.6–2.4) | 1.47±0.57 (0.3–2.7) | NS |

*Statistically significant.
NS = nonsignificant.

**Table 3** Oxidative burst and phagocytic activity of phagocytic cells

| | *Oxidative burst* | | | *Phagocytic activity* |
|---|---|---|---|---|
| | *Bacteria* | *pma* | *fMLP* | *Bacteria* |
| *Neutrophil* | | | | |
| S | 85.7±8.6 (29–91) | 53.4±32.7 (0–38) | 10±5.6 (3–20) | 58.6±23.3 (21–95) |
| C | 90.4±11.0 (28–89) | 81.7±14.3 (7–42) | 17.8±15.5 (4–46) | 74.2±17.7 (33–90) |
| *P* | 0.215 | 0.005 | 0.104 | 0.057 |
| *Monocyte* | | | | |
| S | 59.3±19.6 (29–91) | 13.8±11.7 (0–38) | 4.3±3.1 (0–11) | 24.3±12.0 (6–42) |
| C | 68.8±20.1 (28–89) | 35±20.0 (7–42) | 9.7±6.0 (4–18) | 42.9±13.4 (19–58) |
| *P* | 0.129 | ⩽0.001 | <0.008 | <0.001 |

S = study group; C = control group.

There was no statistically significant difference in T-lymphocyte subgroup distribution between two groups (Table 2).

We have found that phagocytic activity of monocytes was significantly decreased in patients who had iron deficiency anemia. The oxidative burst activity of neutrophils and monocytes that are stimulated with pma (phorbol-12 myristate-13 acetate) and of monocytes stimulated with fMLP (*N*-formil-met-leu-phe) were also decreased (Table 3).

## Discussion

An increased susceptibility to infections has been observed in some patients with iron deficiency, etiology of which is not well known. Deficiency of iron and zinc are well documented to impair immune function in experimental animals and to the extent studied, in humans as well.[12] Iron plays an essential role in immunosurveillance, because of its growth promoting and differentiation inducing properties for immune cells and its interference with cell-mediated immune effector pathways and cytokine activities.[13]

Reported immune defects in iron deficiency include decreased cell-mediated immunity, mitogen responsiveness and natural-killer cell activity. Neutrophil phagocytosis and B lymphocyte function are reported to be generally intact, but lymphocyte bactericidal activity is decreased.[14]

In our study, we found no change in the T-lymphocyte numbers and distribution of subgroups in cases with iron deficiency anemia. The reported T-cell dysfunction may be the result of functional defects of T cells rather than quantitative defects. The first report investigating the effect of iron deficiency on T-cell function came from Joynson *et al*. in 1972. They showed negative effects of iron deficiency on adult cellular immunity.[14] In their study, the investigators found a decrease in DNA synthesis in the activated lymphocytes with PPD and in the formation of 'macrophage migration inhibition factor' and in delayed type of immune reaction after stimulation with PPD and Candida antigens. Higgs and Wells[15] reported impaired cellular immune functions in iron deficiency and its relation to mucocutaneous candidiasis. Following studies showed a decrease in T-cell numbers but Van Heerden in 1981 reported normal values.[16–18] Santos *et al*.[19] in 1990 reported a decrease in total lymphocyte numbers and $CD3^+/CD4^+$ cell ratios. Luraschi *et al*.[20] in 1991, showed decrease in $CD3^+$ and $CD8^+$ levels and increase in $CD4^+/CD8^+$ cell ratio. Kuvibidila *et al*.[21] in

1999, reported decreased T-cell number, blastogenesis and mitogenesis in response to different mitogens in T lymphocyte in iron deficiency.[21] This alteration is largely correctable with iron repletion.[7] Another study of T lymphocytes in iron deficiency noted that protein kinase C activity and translocation of both splenic and purified T cells were altered by iron deficiency.[21] On the other hand, others found normal T-lymphocyte proliferative response to mitogens.[22] In our study, a moderate decrease in IgG4 levels was found although humoral immunity appears to be less affected by iron deficiency than is cellular immunity. In order to study humoral immunity we investigated serum levels of IgG, IgA, IgM and IgG subgroups IgG1, IgG2, IgG3 in the study and the control group. There was no significant difference except IgG4 levels which were lower in the iron deficiency anemia group ($P = 0.035$). Reports have indicated that iron deficiency does not impair humoral immunity.[22,23] Chandra *et al*.[24] showed bactericidal defects in neutrophils of iron deficient patients and reported a resolution after parenteral iron treatment in 1973. In 1975 he also showed a decrease in serum immunglobulin levels.[25]

In our study, serum IL-6 levels in iron deficiency anemia group were significantly lower than the control group. IL-6 is a cytokine with a 22–30 kD weight which stimulates B-cells. Several investigators showed decreased levels of several cytokines.[26] Feng-Xue *et al*.[22] reported decreased levels of IL-6 in patients with iron deficiency anemia. T-cell dysfunction may be the result of low cytokine activity. Cytokines derived from T cells and monocytes regulate cellular iron homeostasis by affecting the expression of proteins involved in the uptake and storage of the metal. Proinflammatory cytokines such as tumor necrosis factor-$\alpha$ (TNF-$\alpha$), interleukin-1 (IL-1) or IL-6 directly stimulate the transcription and translation of the major iron storage protein ferritin.[27] Decreased IL-6 levels may also be a causal factor for the decreased cell-mediated immunity mitogen responsiveness and natural-killer activity.

A demonstration that the observed abnormalities are correctable with iron repletion would be the best evidence of cause–effect relationship between the observed changes in serum IL-6 changes. On the other hand, Sipahi *et al*.[28] demonstrated no difference in serum levels of IL-6 in iron deficiency anemia before and after iron supplementation ($P > 0.05$). Further studies shall elucidate this point.

In our study, we demonstrated a decrease in oxidative burst activity and phagocytic activity in both neutrophils and especially monocytes due to the defects in iron dependent enzymes. There are many studies reporting decreased phagocytic and bactericidal activity in neutrophils, whereas little about the relationship with monocytes can be found in the literature.[29–33] While there are some studies that found no effect of iron deficiency on oxidative burst; most studies, similar to our study, have shown an impairing effect on bactericidal activity in neutrophils.[32,33] In our study, we also found a striking decrease in monocyte function.

More investigations are needed to clarify these results.

Our study was conducted on children of 6 months–2 years. While the immune function was depressed in our study group, our sex and age-matched control group was immunocompetent. Thus, we have excluded any influence of age on immune function. Moreover, similar studies conducted upon adults conclude similarly to our study. In a recent study focused on iron-deficient elderly adult women, T-cell proliferation upon stimulation with mitogens and respiratory burst were significantly less than iron-sufficient women.[34]

Lactoferrin is an iron-binding glycoprotein and is present in specific granules of polymorphonuclear leukocytes. Leukocyte lactoferrin has been considered an important protein for the normal phagocytic process, and is thought to contribute to the prevention and treatment of microbiological infectious diseases. In iron deficiency decreased lactoferrin may result in decreased phagocytic activity.[35] This is another issue to be investigated.

## Conclusion

These results suggest that humoral, cell-mediated and nonspecific immunity and the activity of cytokines which have crucial roles in various steps of immunogenic mechanisms are influenced by the iron deficiency anemia[36]. There are many related issues which await investigation.

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**References**

1 Chandra RK. Nutrition and the immune system from birth to old age. *Eur J Clin Nutr* 2002; **56:** 73–76.
2 Çetin E. Investigation of the prevalence of anemia in children and adolescents living in Istanbul. Thesis of subspeciality for Hematology. Istanbul University Cerrahpaşa School of Medicine, Department of Pediatrics, 1997, pp 41–43.
3 Gedikoglu G, Koc (Agaoglu) L. Screening of iron deficiency at Marmara Region II. *Med Bull Istanbul* 1977; **10:** 172–183.
4 Oppenheimer SJ. Iron and its relation to ımmunity and infectious disease. *J Nutr* 2001; **131:** 6168–6355.
5 Beard JL. Iron biology in immune function, muscle metabolism and neuronal functioning. *J Nutr* 2001; **131:** 568S–580S.

6 Hallquist NA, McNeil LK, Lockwood JF, Sherman AR. Maternal-iron-deficiency effects on peritoneal macrophage and peritoneal natural-killer-cell cytotoxicity in rat pups. *Am J Clin Nutr* 1992; **55:** 741–746.
7 Spear AT, Sherman AR. Iron deficiency alters DMBA-induced tumor burden and natural killer cell cytotoxicity rats. *J Nutr* 1992; **122:** 46–55.
8 Canonne-Hergaux F, Gruenheid S, Govoni G, Gros P. The NRAMP 1 protein and its role in resistance to infection and macrophage function. *Proc Assoc Am Phys* 1999; **111:** 283–289.
9 Jason J, Archibald LK, Nwanyanwu OC, Bell M, Jensen RJ, Gunter E *et al*. The effects of iron deficiency on lymphocyte cytokine production and activation: preservation of hepatic iron but not at all cost. *Clin Exp Immunol* 2001; **126:** 466–473.
10 Kramer JL, Baltathakis I, Alcantara OS, Boldt DH. Differentiation of functional dendritic cells and macrophages from human peripheral blood monocyte precursors is dependent on expression of p21 (WAF1/CIP1) and requires iron. *Br J Haematol* 2002; **117:** 727–734.
11 Collins HL. The role of iron in infections with intracellular bacteria. *Immunol Lett* 2003; **85:** 193–195.
12 Gerald TK. The history of nutrition: malnutrition, infection and immunity. *J Nutr* 2003; **133:** 336S–340S.
13 Weiss G. Iron, infection and anemia – a classical triad. *Wien Klin Wochenschr* 2002; **114:** 357–367.
14 Joynson DHM, Jacobs A, Walker M, Dolby AE. Defect of cell-mediated immunity in patients with iron deficiency anaemia. *Lancet* 1972; **2:** 1058–1059.
15 Higgs JM, Wells RS. Chronic mucocutaneous candidiazis. Associated abnormalities of iron metabolism. *Br J Dermatol* 1972; **86** (Suppl 8)**:** 88.
16 Dallman PR. Iron deficiency and the immune response. *Am J Clin Nutr* 1987; **46:** 329–334.
17 Krantman HJ, Young SR, Ank MJ. Immune function in pure iron deficiency. *Am J Dis Child* 1982; **136:** 840–844.
18 Van Heerden C, Oosthuizen R, Van Wyk H, Prinsloo P, Anderson R. Evaluation of neutrophil and lymphocyte function in subjects with iron deficiency. *S Afr Med J* 1981; **59:** 111–113.
19 Santos PC, Falcao RP. Decreased lymphocyte subsets and K-cell activity in iron deficiency anemia. *Acta Haematol* 1990; **84:** 118–121.
20 Luraschi A, Borgotti P, Gioria A, Fedeli P. Determination of lymphocyte subpopulations, defined with monoclonal antibodies, in patients with iron deficiency anemia. *Minerva Med* 1991; **82:** 557–563.
21 Kuvibidila SR, Kitchens D, Baliga BS. *In vivo* and *in vitro* iron deficiency reduces protein kinase C activity and translocation in murine splenic and purified T cells. *J Cell Biochem* 1999; **74:** 468–478.
22 Feng Xue-bin, Yang Xi-qiang, Shen Jin. Influence of iron deficiency on serum IgG subclass and pneumococcal polysaccharides specific IgG subclass antibodies. *Chin Med J* 1994; **107:** 813–816.
23 Bagchi K, Mohanram M, Reddy V. Humoral immune response in children with iron deficiency anaemia. *Br Med J* 1980; **280:** 1249–1251.
24 Chandra RK. Lymphocyte subpopulations in human malnutrition. Cytotoxic and supressor cells. *Pediatrics* 1977; **59:** 423–427.
25 Chandra RK, Saraya AK. Impaired immunocompetence associated with iron deficiency. *J Pediatr* 1975; **86:** 899–902.
26 Kochanowski BA, Sherman AR. Phagocytes and lysozyme activity in granulocytes from iron deficient rat pups. *Nutr Res* 1984; **4:** 511–520.
27 Ludwiczek S, Aigner E, Theurl I, Weiss G. Cytokine-mediated regulation of iron transport in human monocytic cells. *Blood* 2003; **101:** 4148–4154.
28 Sipahi T, Akar N, Eğin Y, Cin S. Serum interleukin-2 and interleukin-6 levels in iron deficiency anemia. *Pediatr Hematol Oncol* 1998; **15:** 169–173.
29 Chandra RK. Reduced bactericidal capacity of polymorphs in iron deficiency anemia. *Arch Dis Child* 1973; **48:** 864–866.
30 Kulapongs P, Suskind R, Vithayasi V, Olson RE. Cell-mediated immunity and phagocytosis and killing function in children with severe iron deficiency anaemia. *Lancet* 1974; **II:** 680–691.
31 Moore LL, Humbert JR. Neutrophil bactericidal dysfunction towards oxidant-radical sensitive microorganisms during experimental iron deficiency. *Pediatr Res* 1984; **18:** 684–689.
32 Walter T, Arredondo S, Arevalo M, Stekel A. Effect of iron therapy on phagocytosis and bactericidal activity in neutrophils of iron deficient infants. *Am J Clin Nutr* 1986; **44:** 877–882.
33 Yetgin S, Altay C, Ciliv G, Laleli Y. Myeloperoxidase activity and bactericidal function of PMN in iron deficiency. *Acta Haematol* 1979; **61:** 10–14.
34 Ahluwalia N, Sun J, Krause D, Mastro A, Handte G. Immune function is impaired in iron-deficient, home-bound, older women. *Am J Clin Nutr* 2004; **79:** 516–521.
35 Bethell DR, Huang J. Recombinant human lactoferrin treatment for global health issues: iron deficiency and acute diarrhea. *Biometals* 2004; **17:** 337–342.
36 Canonne-Hergaux F, Gruenheid S, Govoni G, Gros P. The NRAMP 1 protein and its role in resistance to infection and macrophage function. *Proc Assoc Am Physicians* 1999; **111:** 283–289.