Serum Immunoglobulins in Patients with Iron Deficiency Anemia

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Abstract Iron deficiency is a common cause of anemia worldwide. Documentation shows that these patients have impaired immunity and are prone to infections. The aim of this study was to confirm whether serum immunoglobulins change in adult nonpregnant females with iron deficiency anemia. Based on patients' clinical history, CBC results, and serum ferritin, iron and total iron binding capacity, 45 healthy patients and 45 iron deficiency anemia (hypochrom microcytic) patients were entered into this case–control study. Serum IgG, IgA and IgM were measured in patient and control groups using the nephelometry method. Both of the groups were compared using suitable statistical test. Mean serum values of IgG, IgA and IgM in the patient group were 12.47 ± 7.67 , 1.93 ± 0.92 and 1.35 ± 0.90 g/l respectively. Mean serum values for the control group were 12.51 ± 6.85 , 2.14 ± 0.88 and 1.49 ± 0.73 g/l respectively. Although serum immunoglobulins were slightly lower in the patient group compared to the control group these differences were not significant with the *t* test. Our study showed serum immunoglobulin levels did not change in iron deficiency anemia.

Keywords Iron deficiency anemia · Humoral immunity · Immunoglobulin

Introduction

Iron is vital for all living organisms because it is essential for multiple metabolic processes, including DNA synthesis and oxygen and electron transport. Iron deficiency is a main nutritional deficiency disorder affecting large fractions of the world population and is a common cause of anemia. Iron deficiency anemia (IDA) is characterized by a defect in hemoglobin synthesis, resulting in red blood cells that are abnormally small (microcytic) and contain a decreased amount of hemoglobin (hypochromic). The prevalence of IDA varies between the sexes, age groups, and geography [1].

About half of anemia cases in developing countries are associated with iron deficiency, and for each case of IDA there are 1–2.5 cases of iron deficiency without anemia [2]. The prevalence of iron deficiency was reported in 9–11% of adolescent girls and women of childbearing age in the US, 19.3% in France, 20% in Poland, 35.6% in Lebanon, 27.5% in India, and 20.8% in Turkey [3–9]. There is not sufficient documentation on frequency of IDA in Iran but it seems common in Iran based on existing reports. For example, Jafari et al. [10] reported that in the south of Iran the prevalence rates of iron deficiency (low serum ferritin level) and IDA in 1,458 females between 15- to 45-year-old were 53.2 and 19.5% respectively.

Iron and other micronutrients have immunomodulating functions that influence the susceptibility of a host to infectious diseases [11]. Some studies suggest a link between iron deficiency and impaired immune system function [12]. It has been suggested that iron supplementation can improve iron levels and reduce morbidity from upper respiratory tract infections in children with or without infection [13]. Decreased cellular immune function characterized by decreased T-lymphocyte, reduced proliferative

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capacity of T cells and altered T cell subsets in iron deficiency were reported [14, 15]. Mullick et al. [16] and Tang et al. [17] showed a relationship between the severity of haematological and immunological compromise. Changes in serum complement levels were also observed in anemic patients [18].

The effects of iron deficiency on humoral immunity remain controversial. Some reports indicate iron depletion may be responsible for decreased humoral immunity while others did not report any change in serum immunoglobulins in IDA [17–20]. Most of the data were obtained from anemic children and few reports of nonpregnant adults with IDA exist. It is important to understand the affects of IDA on the immune system due to its high prevalence in the world. Serum levels of IgG, IgA and IgM in 45 premenopausal adult females with IDA were studied and the results were compared with the control group. The aim of this study was to show whether serum immunoglobulins change in adult nonpregnant female with IDA.

Materials and Methods

This case–control study was done in the Hematology Department of the Mashhad University of Medical Sciences, Mashhad, Iran. A total of 90 premenopausal adult females, who were referred to the Hematology Laboratory of Ghaem Hospital from February 2008 to May 2009 with informed consent, were entered into this study and humoral immunity (as shown by serum immunoglobulins concentrations) was evaluated in 45 patients with IDA (hypochrom microcytic anemia) and in 45 healthy controls.

Complete blood cell count (CBC) and red cell indices were measured with tripotassium ethylene diamine tetraacetic acid (K3-EDTA) anticoagulated blood using the Sysmex K-21 calibrated electronic counter (Toa Medical Electronics Co, Tokyo, Japan). Serum irons and total iron binding capacity (TIBC) were measured by the spectrophotometry method and serum ferritin level was measured by the ELISA method. Criteria for diagnosis of IDA were considered as hemoglobin <125 g/l, mean cell volume (MCV) <80 fl, mean cell hemoglobin (MCH) <27 pg, serum ferritin <20 µg/L, serum iron <50 µg/dl and TIBC >400 µg/dl. Clinical history was obtained for all patients. Other causes that may alter serum antibodies such as malignancy, congenital or acquired immunodeficiency disease, autoimmune disease, pregnancy, thalassemia, and acute or chronic infections recorded in patients' medical history and laboratory results were excluded.

Five milliliters of venous blood collected in clean tubes without any anticoagulant was collected from all patients. After centrifugation, the samples were individually stored in sterile tubes and frozen at -20°C until immunoglobulin

levels were measured. Serum IgG, IgA and IgM were measured with the nephelometry method and results of the patient group and control group were compared. Total human immunoglobulins G, A and M were measured for each case and control using the MININEPHTM Human IgG, IgA and IgM kit respectively that was designed for in vitro measurement of human IgG, IgA and IgM in serum (Binding Site Limited, Birmingham, UK).

Serum immunoglobulin levels in the two groups, IDA and control, were analyzed with SPSS (Statistical software for social analysis—Version 11.5) by a statistician and the *t* test and correlation test were used. A *P* value under 0.05 ($P \leq 0.05$) was considered significant.

Results

The age range in the control group was 20–51 years with 34.33 ± 10.76 years for mean $\pm$ standard deviation (SD). In the IDA group (case group) it was from 15 to 46 years with 34.27 ± 9.94 years for mean $\pm$ standard deviation (SD) and the *t* test showed no significant difference between the case and control group ($P = 0.97$). The mean value for hemoglobin, hematocrit, red cell indices, RBC (red blood cell) count and WBC (white blood cell) count was significantly lower in the iron deficient group; in contrast the PLT (Platelet) count and the relative count of Monocytes (MON) in the IDA group were significantly more than the control group (Table 1). The *t* test showed significant difference between these two groups and the *P* value was lower than 0.001 for all of these indices. No difference between mean relative count of Neutrophils ($P = 0.46$) and Lymphocytes ($P = 0.44$) existed. Serum ferritin level agreed with Hemoglobin ($P < 0.001$), Hematocrit ($P < 0.001$), MCV ($P < 0.001$), MCH ($P < 0.001$), MCHC

Table 1 CBC results in IDA patients and control group

Hematology indexes	Control Mean $\pm$ SD	IDA Mean $\pm$ SD
Hemoglobin (g/dl)	14.00 $\pm$ 0.80	10.93 $\pm$ 1.17
Hematocrit (l/l)	41.48 $\pm$ 2.24	35.57 $\pm$ 4.35
MCV (fl)	85.94 $\pm$ 4.19	73.57 $\pm$ 4.70
MCH (pg)	28.60 $\pm$ 1.98	23.10 $\pm$ 2.77
MCHC (g/dl)	33.38 $\pm$ 1.48	30.85 $\pm$ 1.77
RBC ($\times 10^{12}/\text{l}$)	4.85 $\pm$ 0.37	4.40 $\pm$ 0.22
PLT ($\times 10^9/\text{l}$)	245.62 $\pm$ 46.47	339.82 $\pm$ 105.56
WBC ($\times 10^9/\text{l}$)	7.02 $\pm$ 1.33	5.93 $\pm$ 1.21
Neutrophils (%)	58.93 $\pm$ 10.73	60.60 $\pm$ 10.89
Lymphocytes (%)	35.64 $\pm$ 8.68	34.05 $\pm$ 10.80
Monocytes (%)	2.71 $\pm$ 1.27	3.84 $\pm$ 1.22

Table 2 Comparison of results of patients and control group

Type of immunoglobulins	Control group			Patient group			<i>P</i> value
	Minimum	Maximum	Mean	Minimum	Maximum	Mean	
IgG (g/l)	1.23	28.95	12.51	1.22	27.25	12.47	0.98
IgA (g/l)	0.88	4.23	2.14	0.3	4.98	1.93	0.29
IgM (g/l)	0.37	3.17	1.49	0.2	4.31	1.35	0.44

($P < 0.001$), RBC ($P < 0.001$), WBC ($P < 0.001$), PLT ($P = 0.001$), and percent of monocytes ($P = 0.006$).

Mean serum IgG, IgA and IgM levels were 12.47 ± 7.67 , 1.93 ± 0.92 and 1.35 ± 0.90 g/l in the IDA patient group respectively. The results were 12.51 ± 6.85 , 2.14 ± 0.88 and 1.49 ± 0.73 g/l for the control group respectively. Although serum immunoglobulins were slightly lower in the patient group compared to the healthy subject, the *t* test showed there were not any significant difference between the mean of IgG (0.98), IgA (0.29) and IgM (0.44) in case and control groups (Table 2).

There was no agreement between serum IgG and IgM levels ($P = 0.87$), serum IgG and IgA levels ($P = 0.21$) and serum IgM and IgA levels ($P = 0.27$).

In next step we compared mean serum immunoglobulins level of ten patients with moderate to severe anemia (who had HGB < 10 g/dl) and control subjects. T test showed significant difference ($P = 0.02$) for IgM between case and control group (Table 3).

Discussion

The physiologic function of the immune system is to protect individuals from infectious pathogens and tumor cells. The mechanisms that are responsible for this protection fall into two categories: innate and adaptive immunity. The adaptive immune system consists of lymphocytes and their products, including antibodies. There are two main branches of adaptive humoral immunity and cell-mediated immunity responsible for defense against intracellular microbes, and humoral immunity that protects against extracellular microbes and their toxins [21]. Impaired immune function is reported in patients who are iron deficient, and there is documentation that these patients are

prone to infection, although few reports indicate otherwise [17, 22, 23].

This study showed IDA did not have major effects on humoral immunity and there were not any statistical differences (Table 2) between the IDA and control groups for serumic levels of IgG, IgA and IgM. This finding is in agreement with studies by Ekiz et al. [19] and Leush et al. [20] done on children and pregnant females respectively. Tang et al. [17] and Feng et al. [18] reported a lower level of IgG in pregnant women and children with IDA respectively. Also a decrease in serum IgA and IgG was found in children with iron deficit by Guzikowska et al. [24]. The population studied in our research was restricted to adult nonpregnant females. Similar studies on adult nonpregnant females were not found in the literature.

We found lower level of IgM in ten patients moderate to severe anemia in comparison with control subjects but this finding must be confirmed with complementary study and selection severe form of anemia. This significance was not showed by Ekiz et al. [19] and Leush et al. [20].

A relationship between iron deficiency and cell-mediated immunity was studied by Mullick et al. and Berge et al. They reported decreased mature T lymphocytes and helper-inducer T lymphocytes in iron-deficient versus iron-sufficient children. They did not find any differences in B lymphocyte subsets between iron-deficient and iron-sufficient children [16, 25]. Mullick et al. [16] and Sejas et al. [26] observed iron supplementation improved the number of T lymphocytes counts.

Iron and other micronutrients such as zinc and selenium can influence several components of innate immunity [27]. Kurtoglu et al. [28] reported a defect in bactericidal function of neutrophils (innate immunity) by reduced activity of neutrophilic NADPH oxidase (respiratory burst activity) in iron-deficiency anemia. Ahluwalia et al. [29] reported a link between IDA and impairment in cell-mediated and innate immunity.

The exact effects or mechanisms of iron deficiency on the immune system are not yet known, but some authors have suggested that altered levels of some interleukins (IL) and Cytokines (e.g. IL2, IL1, IL6, TNF-alpha and INF-gamma, IL-4, IL-12p40, IFN-gamma, IL-10) might lead to immune system impairments in iron-deficient patients [30–32]. Also, it has been suggested that altered cell marker expression may contribute to reduced T-cell proliferation

Table 3 Comparison of results of patients with moderate to severe anemia and control group

Type of immunoglobulins	Control group Mean	Patient group Mean	<i>P</i> value
IgG (g/l)	12.51 ± 6.85	12.03 ± 9.39	0.88
IgA (g/l)	2.14 ± 0.88	1.84 ± 0.93	0.38
IgM (g/l)	1.49 ± 0.73	0.85 ± 0.70	0.02

during iron deficiency [33]. Iron is also essential for enzymes such as ribonucleotide reductase, and it is involved in DNA synthesis; therefore, the proliferative phase of lymphocyte activation is a Fe-requiring step and it can be diminished during IDA [34].

Conclusion

Although our study showed serum immunoglobulins were slightly lower in the patient group compared to the control group, IDA did not have major effects on humoral immunity and we did not observe any statistical difference in the serumic levels of IgG, IgA and IgM in the IDA and control groups.

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