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Iron Deficiency Anemia in Children and Alteration of the Immune System

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Abstract

Background and objectives: While there is evidence of an altered immune profile in iron deficiency, the precise immunoregulatory role of iron is not known. Information particulary in children who are vulnerable to iron deficiency and infection, is lacking. We aimed to study the effect of iron deficiency anemia (IDA) on immunity.

Methods: In 101 children with IDA (iron deficiency anemia), (study group), and 99 normal children (control group), the percentage of lymphocytes, monocyte, neutrophile, level of immunoglobulin and serum iron, ferritin, Hb, MCV, HT, MCHC, MCH were compared.

Results: There was a significant difference between the two groups (control and study) in the distribution of various hematological and biochemical parameters (serum iron, ferritin, Hb, MCV, Ht, MCHC, MCH), ($P<0.05$).

The percentage of monocyte levels was $5,529 \pm 3,720$ in children with IDA and $4,574 \pm 2,910$ in the control group ($p=0.044$). The percentage of neutrophils was $45,045 \pm 15,982\%$ in children with IDA and $57,562 \pm 16,267\%$ in the control group (($p<0.0001$). The percentage of lymphocytes with was $43,681 \pm 17,936\%$ in children with IDA and $38,199 \pm 16,699\%$ in the control group ($P<0.026$). There was no difference in the distribution of éosinophile and basophile ($p>0.05$). The percentage of EOSINOPHILE was $0.881 \pm 1,385\%$ in the anemic group; and $0.877 \pm 0.938\%$ in the control group ($P=0.979$). The level of BASOPHILE was $0.307 \pm 0.522\%$ in the anemic group; and $0.387 \pm 0.603\%$ in the control group ($P=0.318$). IgG level was $548,772 \pm 91,885$ mg/dl in children with IDA and $852,714 \pm 181,424$ mg/dl in normal children ($P<0.0001$). IgA level was $74,123 \pm 35,109$ mg/dl in study group, and in $94,936 \pm 64,452$ mg/dl control group ($P<0.0049$). IgD level was $4,729 \pm 6,53$ mg/dl in anemic group and in control group was $7,191 \pm 6,439$ mg/dl ($P<0.0081$).

Conclusion: These results suggest that humoral, cell-mediated which have an important role in various steps of immunogenic mechanisms are influenced by iron deficiency anemia.

Introduction

Protein-calorie malnutrition (PCM) is associated with a significant impairment of cell mediated immunity, phagocyte function; complement system, secretary immunoglobulin a concentrations, cytokine production and an altered immune response [1]. Deficiency of various essential vitamins and minerals such as iron, zinc, selenium, copper, vitamins A, C, E and pyridoxine, and folic acid individually have been shown to have important influences on immune responses and risk of infection. Iron deficiency is associated with impairment of cell mediated immunity and the bactericidal activity of neutrophils, thereby increasing the susceptibility to infections [2,3]. Iron deficiency might play an important role in defense mechanism and thus, the term "nutritional immunity" was coined to highlight the importance of iron deficiency to prevent bacterial growth.

Iron is also required for monocyte/macrophage differentiation and macrophages require iron as a cofactor for the execution of important antimicrobial effectors' mechanisms, including the NADPH-dependent oxidative burst. [4,5].

We aimed to make a prospective study to study the effect of iron deficiency anemia on humoral and cellular immunity.

Patients and Methods

The study was conducted in the departments of pathology and paediatrics of University hospital center and the service of Immunology; Oran (west of Algeria) during the period February - May 2014 and the sample consisted of 200 children.

All children received in the Department during the study period and our exclusion criteria were previous iron replacement therapy, any recent illness or chronic disease. A total of 101 children (12 months and 5 years) were diagnosed to have iron deficiency anemia (hemoglobin levels below 11 g/dl, regardless of sex; mean corpuscular volume (MCV) less than 80 femtoliters (FL); mean corpuscular hemoglobin (MCH) less than 27 picograms (pg); iron serum less than 58 μg 100 ml, serum ferritin less than 07 μg/l).

The complete blood count (automatic cell analyzer 600), serum iron (spectrophotometry) and ferritin (RIA) 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, IgM, IgA, and ferritin levels, whole blood samples were collected. Serum immunoglobulins were measured by using commercially prepared antisera to IgG, IgA, IgM. and radial immune diffusion method.

For statistical analysis, data were analyzed by the Statview software

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(1997). Frequencies and the percentages were calculated and Student's t test was performed to investigate the significance in the association of the iron deficiency anemia and immune system. Correlations were considered significant if the observed significance level *p* was <0.05. Chisquare test was used as test of significance at 5% level. Pearson's correlation coefficient was used to study the relation between immunoglobulin and iron status.

Results

A total of 101 children were diagnosed to have iron deficiency anemia (study group) which was related to nutritional deficiency. The control group consisted of 99 healthy children at the same ages (control group).

The mean age of anemic patients is (23.3 ± 16.6 months) and the control group is (24.09 ± 16.6 months), (p=0.75). In relation to anthropometric characteristics (age and sex), no significant difference was found between two group (study and control), (p>0.05).

The severity of the anemia has been classified in three following phases: • severe anemia defined by a rate of Hb < 7 g/dl • moderate anemia sets a rate of 7 < Hb < 10 g/dl • mild anemia corresponds has a rate 10 < Hb < 11 g/dl. According to the severity, it was noted as 21.78% of patients have severe anemia, 63.36% of patients with moderate anemia and 14.85% of patients with mild anemia (Figure 1).

Biochemical and hematologic parameters measured in our study population are reported in Table 1. There was a statistically significant difference between all hematologic parameters in the two groups (study and control), (p<0.0001). Our study noted that, the numbers of white blood cells were significantly lower in the iron deficient group (study group), (9.66 ± 4.75×10^{12}/l). In the control group the numbers were (11.19 ± 4.33 ×10^{12}/l), (p<0.04).

Result of cellular immunity was summarized in Table 2. Mean serum neutrophil levels of the study group were 45.04 ± 15.98%; the control group had mean serum levels of 57.56 ± 16.26%. A statistically significant difference was detected between two groups (p<0.0001). There was statistically significant difference in monocytes and lymphocyte distribution between two groups (p<0.044, p<0.026 respectively).

There was no statistically significant difference in basophils and eosinophils levels between two groups (study and control), (p>0.05).

Table 3 shows results of humoral immunity. There was statistically significant difference in IgG, IgD, IgA levels of both groups (study and control), (p<0.0001, p<0.008, p<0.0049 respectively).

The study group had serum IgE levels with a mean of 0.223 ± 0.270 mg/100 ml, while the control group had a mean IgE level of 0.294 ± 0.314 mg/100 ml (p > 0.05).

Mean serum IgM levels of the study group were 73,343 ± 31,013 mg/100 ml; the control group had mean serum levels of 76,911 ± 32,700 mg/100 ml. No statistically significant difference was detected between two groups (p>0.05), (Table 3).

In our study, a correlation has been noted between iron status parameters (serum iron, ferritin, Hb, MCV, MCH, MCHC, Ht) and IgG (r=0.63, r=0.48, r=0.68, r=0.43, r=0.44, r=0.30, r=0.62 respectively), (Figures 2-5).

A linear correlation was found between the level of Hb and Ht (r=0.88); between Hb and serum iron (r=0.85), between Hb and MCV (r=0.62), between Hb and MCH (r=0.62), between Hb and MCHC

(r=0.57), between Hb and ferritin (r=0.74). Also a correlation was detected between IgA and IgG (r=0.26).

A negative correlation was noted between lymphocytes and neutrophils (r=-0.35). But no correlation was found between lymphocytes and hemoglobin (r=-0.18).

Discussion

Iron deficiency is the most common micronutrient deficiency in the world. Children, particularly infants living in developing countries are highly vulnerable to infectious diseases. Therefore, understanding the relationship between iron deficiency and alteration of immune system
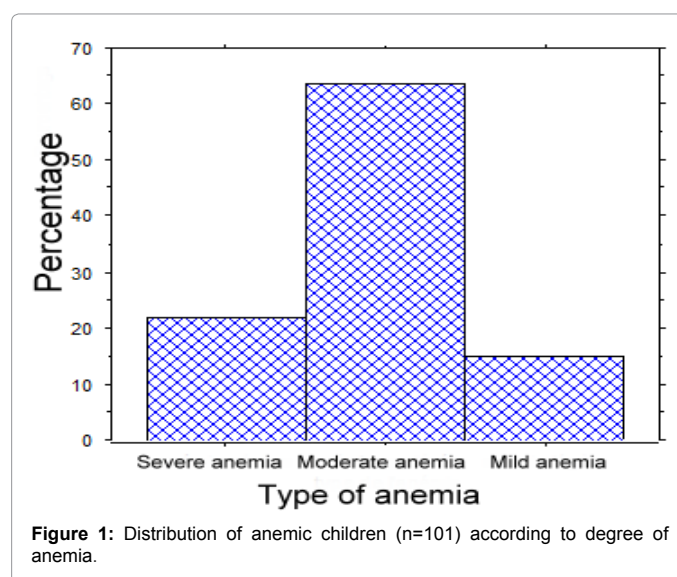

Figure 1: Distribution of anemic children (n=101) according to degree of anemia.

	Study Group *(n=101)* Mean ± Standard Deviation	Control Group *(n=99)* Mean ± Standard Deviation	*P*
Hemoglobin (g / dl)	08.40 ± 1.54	13.27 ± 1.17	<0.0001 S
Hematocrit (%)	27.61 ± 5.80	41.07 ± 4.21	<0.0001 S
RBC (x 10^{12}/l)	3.78 ± 0.89	4.98 ± 0.66	<0.0001 S
MCV (fl)	72.97 ± 12.45	87.52 ± 8.78	<0.0001 S
MCH (pg)	24.72 ± 4.82	30.05 ± 3.59	<0.0001 S
MCHC (%)	30.58 ± 2.87	33.37 ± 2.29	<0.0001 S
FERRITIN (mg / l)	28.78 ± 25.07	106.60 ± 47.52	<0.0001 S
SERUM IRON (μg/100 ml)	45.67 ± 25.49	129.82 ± 31.20	<0.0001 S

Abbreviations: MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; MCV: Mean Corpuscular Volume; RBC: Red Blood Cell.

Table 1: Iron status parameters in the study group (anemic) and control group (no anemic).

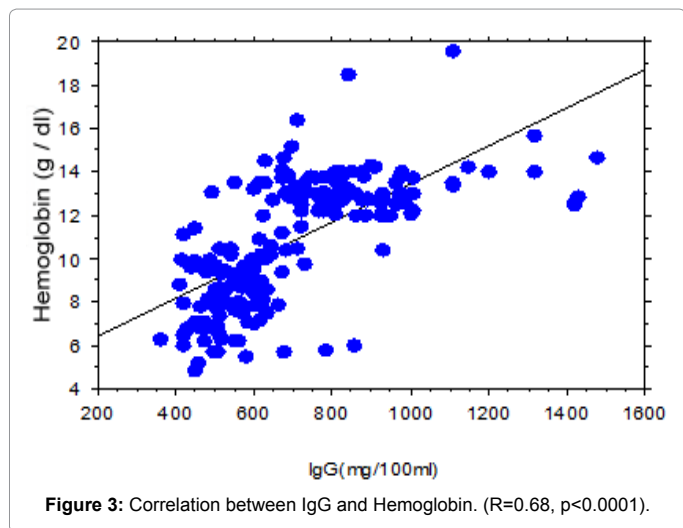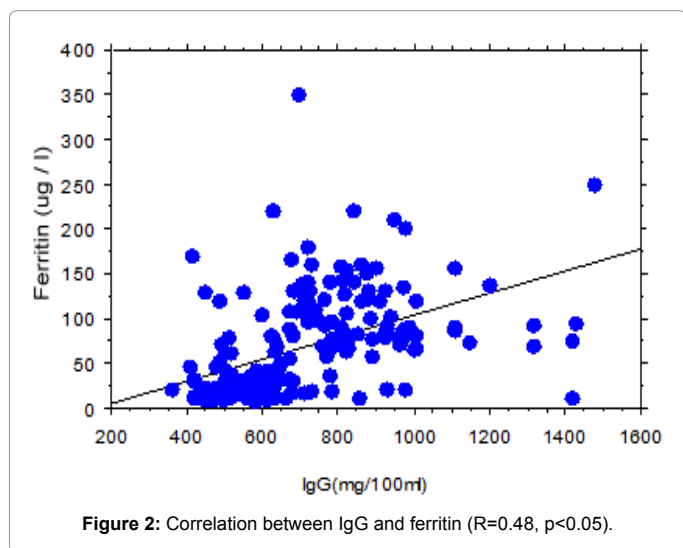|	Study Group *(n=101)* Mean ± Standard Deviation	Control Group *(n=99)* Mean ± Standard Deviation	*P*
NEUTROPHII (%)	45.04 ± 15.98	57.56 ± 16.26	<0.0001 S
LYMPHOCYTE (%)	43.68 ± 17.93	38.19 ± 16.69	0.026 S
MONOCYTE (%)	5.52 ± 3.72	4.57 ± 2.91	0.044 S
EOSINOPHIL (%)	0.88 ± 1.38	0.87 ± 0.93	0.979 NS
BASOPHIL (%)	0.30 ± 0.52	0.38 ± 0.60	0.318 NS

S: significant; **NS**: not significant

Table 2: Result of cellular immunity (%).

	Study Group (n=101) Mean ± Standard Deviation	Control Group (n=99) Mean ± Standard Deviation	P
IgG (mg/100 ml)	548.772 ± 91.885	852.714 ± 181.424	<0.0001 S
IgE (mg/100 ml)	0.223 ± 0.270	0.294 ± 0.314	0.0874 NS
IgA (mg/100 ml)	74.123 ± 35.109	94.936 ± 64.452	<0.0049 S
IgD (mg/100 ml)	4.729 ± 6.53	7.191 ± 6.439	<0.0081 S
IgM (mg/100 ml)	73.343 ± 31.013	76.911 ± 32.700	0.429 NS

Abbreviations: IgG: Immunoglobulin G; IgE: Immunoglobulin E; IgA: Immunoglobulin A; IgD: Immunoglobulin D; IgM: Immunoglobulin M; S: Significant; NS: Not Significant.

Table 3: Results of humoral immunity (mg/100 ml).

Figure 2: Correlation between IgG and ferritin (R=0.48, p<0.05).

Figure 3: Correlation between IgG and Hemoglobin. (R=0.68, p<0.0001).
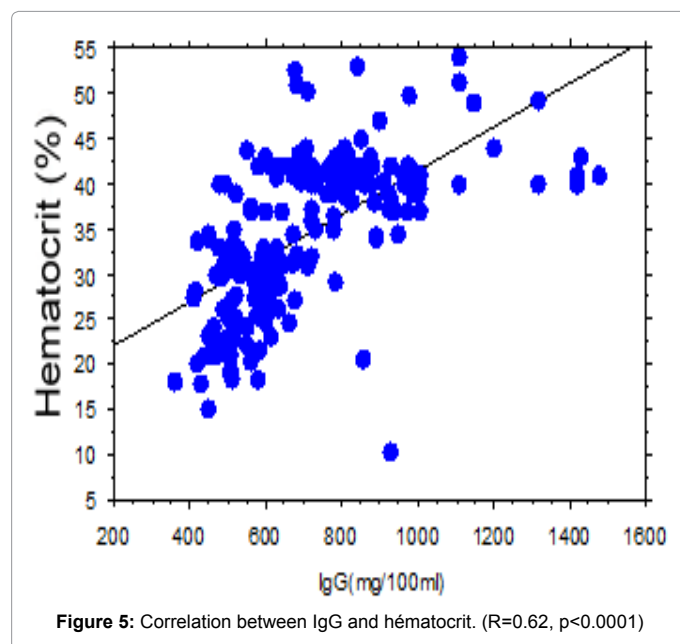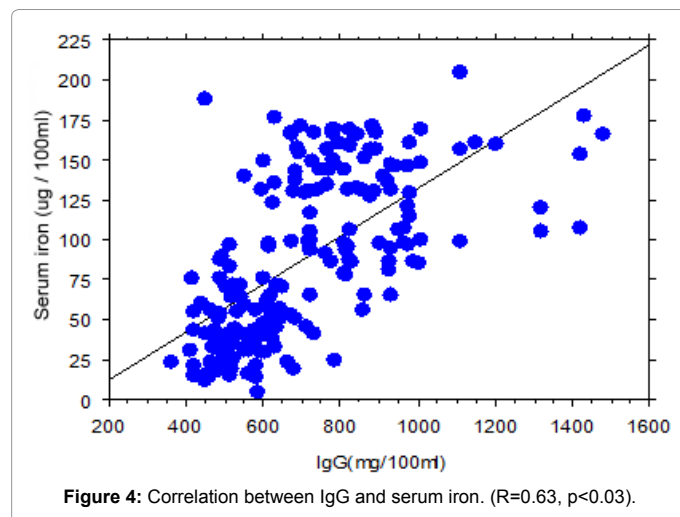
is of great importance. Iron deficiency is associated with impairment of innate (natural) immunity and cell mediated immunity, thereby contributing to increased risk of infections [6,7].

Deficiency of various essential vitamins and minerals such as iron, zinc, selenium, copper, vitamins A, C, E and pyridoxine, and folic acid individually have been shown to have important influences on immune responses and risk of infection. Iron deficiency is associated with impairment of cell mediated immunity and the bactericidal activity of neutrophils, there by increasing the susceptibility to infections [8,9].

Our study showed that 50.5% of children are anemic and 49.5% are not anemic. In developing countries are the most affected due to low bioavailability of iron in the diet, and the high incidence of parasitic diseases in some countries (malaria and intestinal parasites).

In our study, serum neutrophils level in anemic group was significantly lower than the control group. 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 [10].

In order to study humoral immunity we investigated serum levels of IgG, IgA, IgM, IgD in the study and the control group. There was statistically significant difference in IgG, IgD, IgA levels of both groups (study and control). Chandra et al. showed bactericidal defects in neutrophils of iron deficient patients and reported a resolution after parenteral iron treatment [2]. In 1975 he also showed a decrease in serum immunglobulin levels [7,11,12]. Ekij et al. showed significantly reduced interleukin-6 levels, immunoglobulin G levels, oxidative

Figure 4: Correlation between IgG and serum iron. (R=0.63, p<0.03).

Figure 5: Correlation between IgG and hématocrit. (R=0.62, p<0.0001)

burst activity of neutrophils and monocytes, and phagocytic activity of monocytes in 6-24 months children [13]. The major limitation of both these studies is small number of subjects. However, another study indicated that iron deficiency does not impair humoral immunity [14,15].

Humoral immunity appears to be less affected by iron than cellular immunity deficiency. In humans iron deficiency, the production of antibodies in response to vaccination with most of the antigens is preserved [7,16-18].

Neutrophil and macrophage dysfunction has been associated with low iron levels, as evidenced by deficient nitroblue tetrazoleum reduction and hydrogen peroxide formation in these respective cell lines [19]. Ribonucleotide reductase activity has been discovered to be iron dependent. Iron levels have also been shown to alter the proliferation of T helper (Th)-1 and Th-2 subsets, likely related to the difference in dependence of cells on transferrin-related iron uptake [20,21].

In the present study, we noted that the rate of lymphocytes and *Monocyte* are significantly different between two groups (study and control).

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 [8,22]. 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 reported impaired cellular immune functions in iron deficiency and its relation to mucocutaneous candidiasis [12,23]. Following studies showed a decrease in T-cell numbers but *Van* Heerden in 1981 reported normal values [14,24,25]. In our study, we demonstrated that neutrophils levels were reduced in study group. 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 [26,27].

There are several mechanisms that may explain the effects of iron on the immune system deficiency. DNA synthesis, ribonucleotide reductase enzyme containing iron-initiated is a factor limiting the speed in cell replication and can be limited by an iron deficiency. Control of cell differentiation is influenced by iron and iron transport in cells via the Transferrin receptor [28-30]. GALAN and Al reported a reduction in the production of interleukin-2 by lymphocytes activated in iron-deficient patients. The release of interleukin-2 is fundamental to communication between lymphocyte subpopulations and natural killer cells, but it doesn't seem to be the only cytokine which is modified by the iron status [29-31].

Humoral and cell mediated immunity both have been studied extensively, mainly *in vitro*, in relation to iron deficiency in both humans and animals. Impairment of cell mediated immunity have been well described in iron-deficient humans, however, little evidence exists for major humoral deficiencies. 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 [27,30].

In summary, iron deficiency depresses certain aspects of cell-mediated immunity and innate immunity. Because there are some conflicting effects of iron deficiency on defense systems, it becomes more important to review the relationship between iron deficiency and infection risk.

The proposed interventions rely primarily on enhancing iron intake either through supplementation or fortification of food. To conclude, we can say that iron deficiency can affect some humoral immunity and cell-mediated settings. More investigations are needed to clarify these results.

Conclusion

These results suggest that humeral and cell-mediated which have a crucial role in the different stages of the Immunogenic are affected by iron deficiency anemia. Iron deficiency is quite frequent in our study population. A comprehensive research in our country is needed on how to improve existing iron supplementation programs and nutritional status of children.

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