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Original Article

C3 and C4 Complement Levels in Iron Deficiency Anemia

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ABSTRACTS

Background and Objectives: **Complement proteins are some of the most important plasma proteins of the innate immune system. Impaired immune function is reported in subjects who are iron deficient, and there are documents that these patients are prone to infection. This study was conducted to show whether serum C3 and C4 complement change in adult nonpregnant female with iron deficient anemia or not.**

Methods: **Forty five normal subjects and 45 iron deficient anemia (hypochrom microcytic) cases were entered in this case and control study by using patients' clinical history and also results of CBC, Serum ferritin, iron and total iron binding capacity. Serum C3, and C4 were measured in case and control subjects with nephlometry method, finally comparison between result of patients group and control group was done with using suitable statistical test.**

Results: **Mean serum C3 and C4 in patient group was 1.28 ± 0.81 and 0.28 ± 0.23 g/L respectively and for control group was 1.39 ± 0.87 and 0.35 ± 0.25 g/L respectively. Although serum complements were slightly lower in patient groups in compared to control group but this differences was not meaningful with t test.**

Conclusion: **This study showed serum C3 and C4 complements levels were not changed in iron deficiency anemia.**

Keywords: Iron Deficiency Anemia, C3 Complement, C4 Complement

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Introduction

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. Complement proteins are some of the most important plasma proteins of the innate immune system (1). However, it plays an essential role in enhancing humoral immunity that is a part of adaptive immune system (2). Impaired immune function is reported in subjects who are iron deficient, and there are documents that these patients are prone to infection, although few reports indicate otherwise (3-5).

Iron is vital to the health of living cells because it is essential for multiple metabolic processes, including oxygen transport, electron transport, and DNA synthesis. Iron deficiency is considered one of the main nutritional deficiency disorders affecting large fractions of the world population and is probably the most 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, between age groups, and by geography (6). The prevalence of iron deficiency was reported in 2-5% 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 (7-13). Although there was not well document about overall frequency of IDA in Iran but it seems common there based on some reports. For example the prevalence rates of iron deficiency (low serum ferritin level) and IDA in 1458 females between 15 to 45 years old were reported 53.2% and 19.5% respectively in south of Iran (14).

Iron and some other micronutrients have immunomodulating functions and therefore influence the susceptibility of a host to infectious diseases (15,16). Therefore, it is suggested that iron supplementation can be improved iron status and reduced morbidity from upper respiratory tract infections in children with or without infection (17). Decreased cellular immune function that characterized by decreased in T-lymphocyte, reduced in the proliferative capacity of T cells and altered T cell subsets in iron deficiency was reported by some authors (18,19). Furthermore some researchers showed a relationship between the severity of hematological and immunological compromise (3,20).

The effects of iron deficiency on serum complement levels remain controversial. Although few reports indicate Iron depletion may be responsible for a decrease serum complement levels and activity but others did not any change in serum complement levels in IDA (21,22). The most of previous data were obtained from children with anemia and there are few reports in nonpregnant adults with IDA. It is important to know about affects of IDA on immune system due to high prevalence of IDA in world population.

In order to study innate immune system we investigated serum complement levels of C3 and C4 in the 45 young adult female with IDA and compared results with the control group. This study was done to show whether serum complement levels change in nonpregnant female with IDA or not.

Materials and Methods

This case control study was conducted in the laboratory of Ghaem Hospital, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran. A total of 90 young adult female referred to the Hematology Laboratory of Ghaem Hospital from June 2008 to May 2009 were entered in this study and one part of innate immunity (as shown by serum complement concentrations) was evaluated in 45 patients with IDA (hypochrom microcytic anemia) and in 45 normal controls. All case and control subjects gave informed voluntary consent to participate in our study according to the protocol approved by the local Ethics Committee of MUMS and according to the standards of the Helsinky Declaration.

Complete blood cell count (CBC) and red cell indices were measured with tripotassium ethylene diamine tetra-acetic acid (K3-EDTA) anticoagulated blood using a calibrated electronic

counter of Sysmex (K-21) company. Serum irons and total iron binding capacity (TIBC) were measured by spectrophotometry method and serum ferritin level was measured by Radioimmunoassay method (RIA). Clinical history was obtained for all patients. Clinical finding, laboratory result (such as hemoglobin electrophoresis) and familial history of patients were used for including and excluding patients.

Inclusion criteria: 1)Gender: Female, 2) Age: young aged (Premenopausal age), 3) Anemia: Hemoglobin (HGB) <125 g/L, 4) Hypochrom microcitic: mean cell volume (MCV) <80 Femtoliter and mean cell hemoglobin (MCH) < 27 Picogram, 5) Iron deficient: Serum ferritin< 20 µg/L , serum Iron < 50 µg/dl and TIBC > 400 µg/dl.

Exclusion criteria: We excluded other causes that might alter serum antibodies such as malignancy, congenital or acquired immunodeficiency disease, autoimmune disease, pregnancy, and acute or chronic infection by patients' past medical history and patients' laboratory results. The other causes of hypochrom microcytic anemia such as thalassemia, sideroblastic anemia and anemia in chronic disease (ACD) excluded by personal medical history and familial history (especially for rule out of thalassemia) and by results of CBC, serum iron, TIBC and hemoglobin electrophoresis.

Forty-five healthy young women considered as a control group. The laboratory inclusion criteria for control group were HGB range = 125-160 g/L, MCV =80-95 femtoliter and MCH= 27-33 picogram, serum ferrittin= 20-300 µg/L, serum Iron = 50-160 µg/dl, and TIBC =250- 400 µg/dl.

Five milliliter of venous blood collected in clean tube without any anticoagulant was collected for all patients. After centrifugation separate were preserved in sterile tubes and stored frozen at −20 °C until used for measurement of complements. Serum C3 and C4 were measured with nephlometry method and finally comparison between result of patients group and control group was done. Total human complements of C3 and C4 was measured for each case and control individuals using the MININEPH™ Human C3 and C4 Kit respectively that designed for in vitro measurement of human C3 and C4 in serum (Binding Site Limited, Birmingham, UK).

Serum complements level in two groups of IDA and control were analyzed with SPSS (Statistical software for social analysis – Version 11.5) by a specialist of statistics and using one-sample Kolmogorov-Smirnov test for evaluation normality of the data distribution and then using *t* test for comparing mean value in two group of case and control. A *P*-value under 0.05 ($P \leq 0.05$) was considered significant.

Results

The age range in control group was from 18 to 51 years with 34.11 ± 10.79 years for mean ± standard deviation (SD) and in IDA group (case group) was from 19 to 50 years with 32.73 ± 9.50 years and *t* test showed no significant differences between case and control group ($P=0.59$). The mean Serum ferritin, serum iron and TIBC in IDA patients were 10.19 µg/L , 36.50 µg/dl and 420.00 µg/dl respectively and these results for control group were 48.65 µg/L, 108.87 µg/dl and 319.40 µg/dl respectively. The mean hemoglobin, hematocrit, red cell indices, RBC (Red blood cell) count, and WBC (White blood cell) count were significantly lower in the iron deficient group; in opposite the PLT (Platelet) count in IDA group were more than control group (Table 1). The *t* test showed significance differences between these two group and *P* value was lower than 0.001 for all of these indexes. There were not any differences between mean of the relative count of neutrophils($P=0.77$), lymphocytes ($P=0.57$) and monocytes ($P=0.37$). Serum ferritin level had concordance with hemoglobin($P<0.001$), hematocrit($P<0.001$), MCV($P<0.001$),MCH($P<0.001$), MCHC($P<0.001$), RBC ($P<0.001$), WBC ($P<0.001$) and PLT ($P=0.017$), and had not concordance with concordance with neutrophils (0.79) , lymphocytes (0.07) and monocytes (0.28).

Table 1: The hematologic finding of control group and patients with iron deficiency anemia (IDA).

Hematology indexes	Control	IDA
Hemoglobin (g/dL)*	14.23 ± 0.83	10.98 ± 1.15
Hematocrit (L/L)*	42.00 ± 2.66	35.71 ± 4.32
MCV (fL)*	86.10 ± 3.89	73.76 ± 4.18
MCH (pg)*	29.16 ± 1.39	23.11 ± 2.80
MCHC (g/dL)*	33.80 ± 1.24	30.74 ± 1.91
RBC ($x10^{12}$ /L)*	4.94 ± 0.26	4.28 ± 0.18
PLT ($x10^9$/L)*	245.62 ± 46.47	339.82 ± 105.56
WBC ($x10^9$/L)*	7.02 ± 1.39	5.97 ± 1.27
Neutrophils (%)	58.61 ± 11.26	59.27 ± 10.60
Lymphocytes (%)	35.59 ± 9.71	35.78 ± 10.47
Monocytes (%)	3.02 ± 1.31	3.33 ± 1.78

*Statistically significant. (P<0.05)

Mean serum C3 and C4 level were 1.28 ± 0.81 and 0.28 ± 0.23 g/L in IDA patient group respectively and these results were 1.39 ± 0.87 and 0.35 ± 0.25 g/L respectively for control group. Although serum complements were slightly lower in patient groups in compared to normal subject but *t* test showed there was not any meaningful differences between the mean of C3 (P=0.54) and C4 (P=0.18) in case and control groups (Table 2). There were not any concordance between serum C3 and C4 level (P=0.25). In next step, we compared complement level in two groups of control and patients with moderate to severe anemia that was defined as HGB lower than 10 g/dL (Table 3).

Table 2: Compare result of patients and control group

Type of Complements	Control group Minimum	Maximum	Mean	Patients group Minimum	Maximum	Mean	*P* value
C3 (g/L)	0.28	4.03	1.39	0.27	3.23	1.28	0.54
C4 (g/L)	0.08	1.03	0.35	0.05	0.88	0.28	0.18

Table 3: Compare result of control group and six patients with HGB<10 g/dL

Type of Complements	Control group Mean	Patients group Mean	*P* value
C3 (g/L)	1.39	0.84	0.036
C4 (g/L)	0.35	0.11	<0.001

Discussion

The complement system is a complex cascade involving proteolytic cleavage of serum glycoproteins often activated by cell receptors. This cascade ultimately results in induction of the inflammatory response, phagocyte chemotaxis and opsonization, and cell lysis. Three pathways have been elucidated through which the complement cascade can be initiated; Classical, Alternate and Lectin Pathways. All three pathways merge through at common intersection, complement C3, therefore complement component C3 plays a central role in the activation of complement system. People with complement deficiency are susceptible to bacterial infection (23).

In this study, we compared six patients with HGB<10g/dL and control group (Table 3). The

finding of Table 3 showed lower complement level in IDA patients than control group but it must be confirmed with complementary study and larger case group. In the other hand, although immunity can be failed in IDA patients but overall finding of our study showed iron deficiency anemia have not major effect on serum level of complements as a part of innate immunity and there are not statistically differences between two groups of IDA and control subjects for serumic level of C3 and C4. Our finding is concordance with Tang *et al*. and Galan *et al*. studies that were done on pregnant females and children respectively(3,24). In another study, a lower level of c3 in children with iron deficiency was reported (25). Population studied in our research was restricted to adult non-pregnant female. We did not find in literature review any similar study that worked on adult non-pregnant female. Protein-energy malnutrition is known to be associated with a significant impairment of cell-mediated immunity, phagocyte function, complement system. Low serum level of C3 also was reported in the severely malnourished children (26). Methodology of these previous studies was immunodiffusion. We measured complements with using nephlometery method and we think one of superiority of our study was our methodology. It is reported that nephlometery had greater sensitivity than radial immunodiffusion method for IgG, IgA, IgM, C3 and C4 measurement (27).

Relationship between iron deficiency and cell-mediated immunity were studied by Mullick *et al*. and Berge *et al*. They reported the decreased of mature T lymphocytes and helper-inducer T lymphocytes in iron-deficient versus iron-sufficient children. They did not find any differences in B lymphocytes subsets between iron-deficient and iron-sufficient children (20,28). Iron supplementation can be improved the number of T lymphocytes counts (20,29).

"Iron and other micronutrients such as zinc, selenium can influence several components of innate immunity" (30). Kurtoglu *et al*. reported defect in bactericidal function of neutrophils (innate immunity) by reduced activity of neutrophilic NADPH oxidase (respiratory burst activity) in iron-deficiency anemia (31). Defect in oxidative burst of neutrophils in IDA patients was found by Paino *et al*. (32). Furthermore, association between IDA and impairments in cell-mediated and innate immunity is reported by Ahluwalia *et al*. (33).

The exact effects or mechanisms of iron deficiency on 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 (34-36). In addition, it has been suggested that altered cell marker expression may contribute to reduced T-cell proliferation during iron deficiency (37). Iron also is essential for enzymes such as ribonuleotide reductase, involved in DNA synthesis therefore proliferative phase of lymphocyte activation is a Fe-requiring step and it can be diminished during IDA (38).

Conclusion

Our study showed although serum complements were slightly lower in patient groups in compared to control group but iron deficiency anemia have not major effect on complements level and there are not any statistically differences between two groups of IDA and control subjects for serum level of of C3 and C4. The larger study on IDA patients with moderate to severe iron deficiency anemia (hemoglobin lower than 10 g/dl) can be helpful for definite decision about affect of iron deficiency anemia on serum complement level.

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