Estimation of C3 and C4 Level in non-Pregnant Women with Iron Deficiency Anemia

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Abstract

Background: Iron deficiency is one of the most common nutritional disorders in menstruating women and adolescents. Iron and other micronutrients have immunomodulating functions that influence the susceptibility of a host to infectious diseases. Complement C3 and C4 are the components most frequently measured which are capable of lysis of bacteria or red cells or can opsonize bacteria or cells so that they are phagocytosed.

Objective: To determine the level of complements proteins (C3&C4) in adult non-pregnant women with iron deficiency anemia.

Patients and methods: A total of 57 young adult non pregnant females were enrolled in this case control study .Serum complements C3 and C4 were evaluated in 32 women with iron deficiency anemia and 25 age matched normal women as a control group. Measurement of complements (C3&C4) was done by using quantitative determination by radial immune diffusion plates.

Results: The mean ± standard deviation for C3 &C4 were 95.02± 27.35& 21.03± 9.72 respectively for the group with iron deficiency anemia while for control group was 112.79 ± 32.46 for C3 &26.32 ± 8.38 for C4, with statistically significant differences, p- value 0.0297 for C3 & 0.0034 for C4.

Conclusion: Adult non pregnant women with iron deficiency anemia have low level of C3 and C4 in compare to normal adult non pregnant women.

تقدير مستوى المتممات (سي٣ وسي٤) لدى النساء غير الحوامل المصابات بفقر دم عوز الحديد

الخلاصة

خلفية الدراسة: يعد النقص الحديدي أحد الاضطرابات المغذية الاكثر شيوعا في الدول الصناعية وخصوصا لدى النساء الحائضات والمراهقات. للحديد والمغذيات زهيدة المقدار وظيفة التحوير المناعي والتي تؤثر بسهولة على المضيف للإصابة بالأمراض المعدية. تعد المتممات نوع سي ٣ وسي ٤ من اكثر المتممات قياسا. ان المتمم نوع سي ٣ هو الاكثر وفرة في المصل الانساني ويستعمل كفحص تنظيري. المتمم نوع سي ٤ هو الثاني من حيث الوفرة في المصل.

الهدف: تحديد مستوى البروتينات المتممة نوع سي ٣ وسي ٤ لدى النساء البالغات غير الحبلى المصابات بفقر دم عوز الحديد.

المرضى و طرق العمل: سجلت ٥٧ شابة انثى غير حبلى في هذه الدراسة من نوع سيطرة الحالة٠ تم تقييم المتممات نوع سي ٣ وسي مصل ٣٢ امرأة مصابة بفقر دم عوز الحديد و ٢٥ امرأة متناظرة في العمر كمجموعة قياسية. تم قياس المتممات نوع سي ٣ وسي ٤ باستعمال التقدير الكمي بصحون الانتشار الشعاعية المنيعة.

النتائج: كان المتوسط ± الانحراف المعياري للمتممين سي ٣ و سي٤ هو (٩٥,٠٢± ٢٧,٣٥) و (٢١,٠٣ ± ٩,٧٢) على التوالي لمجموعة المريضات أما للمجموعة القياسية فقد كانت النتائج (١١٢,٧٩ ± ٣٢,٤٦) للسي ٣ و (٢٦,٣٢ ± ٨,٣٨) للسي ٤ مع اختلافات احصائية هامة حيث كانت القيمة التنبؤية للسي ٣ (٠,٠٢٩٧) وللسي ٤ (٠,٠٠٣٤).

الاستنتاجات: للنساء البالغات الحبلى المصابات بفقر دم عوز الحديد مستوى منخفض للمتممين سي ٣ وسي٤ مقارنة بالنساء الطبيعيات البالغات الحبلى قبل سن اليأس.

Introduction

Iron deficiency is one of the most common nutritional disorders in industrialized countries, especially in menstruating women and adolescents.[1] In iron deficiency anemia (IDA) the red cells becomes obviously microcytic and hypochromic and poikylocytosis become more marked, the MCV and MCH are reduced and target cells may be present, the reticulocytes count is low for the degree of anemia.[2] Iron and other micronutrients have immunom-odulating functions that influence the susceptibility of a host to infectious diseases [3]. Iron is essential for proper cell differentiation and cells growth; it is an important component of peroxidase-generation enzymes and nitrous oxide-generating enzymes that are critical for proper enzymatic functioning of immune cells. [4] Anemia and infection are coexistent among underprivileged populations and several epidemiologic, clinical, and experimental studies have linked their association with increased susceptibility to infections. Studies demonstrating the reversal of such susceptibility following correction of iron deficiency have established a causal role for iron in the predisposal to infections. Simultaneous reversal of immunologic defects following administration of iron supplements further supports the role of iron deficiency in promoting morbidity due to infections.[5] Complement consists of plasma proteins constituting an amplification enzyme system which is capable of lysis of bacteria or red cells or can opsonize bacteria or cells so that they are phagocytosed. The complement sequence consists of nine major complements C1, C2, etc. which is activated in turn and form a cascade resembling the coagulation sequence. [6] Complement C3 and C4 are the components most frequently measured; they are synthesized in the liver as single polypeptide chains. Complement C3 is the most abundant complement protein in human serum and used as a screening test. Complement C4 is the second most abundant complement protein in serum. Because of its pivotal position in the complement cascade; C3 is consumed by activation of either the classical or alternative pathway. However, C3 levels are not the most sensitive indicators of classical pathway activation and a decreased complement C4 level is frequently found to be a more sensitive measure of mild classical pathway activation.[7,8] The prevalence of IDA has been reported to be 57% of adult females in South Asia, 2%–5% of adolescent girls and women of childbearing age in the US, 19% in France, 20% in Poland, 36% in Lebanon, 28% in India, and 21% in Turkey.[9-14] Adult female considered to have iron deficiency anemia when hemoglobin concentration is < 12 g/dl and PCV<0.34L/L, hypochromic microcytic on blood film , MCV< 80 fl , MCH< 27pg,red cell distribution width (RDW) > 15% , serum iron concentration is reduced in iron deficiency and the total iron binding capacity(TIBC) is often increased, saturation of transferrin is always reduced to <16%. Ferritin levels are the single best serum measure of storage iron, in iron deficiency anemia, the value is <I5 ng/ml in the absence of a complicating disease. [10, 15]

Aim of the Study

To determine the level of complements proteins (C3&C4) in adult non-pregnant women with iron deficiency anemia.

Patients and Methods

A total of 57 young adult non pregnant females were enrolled in this case control study, from January 2012 to February 2013. Serum complements C3 and C4 were evaluated in 32 women with IDA and 25 age matched normal women as a control group. Consent was taken from all patients and control group to participate in this study. A 5 ml blood sample was aspirated from patients and controls groups, which was separated in two parts; 2.5 ml was put in a tube containing (K3-EDTA) anticoagulant for estimation of hematological indices using ABX Micros ES 60 automated counter (Horiba company/Germany) ,blood film and reticulocytes count were done using leishmans stain and new methylene blue respectively. The remaining blood sample centrifuged and separated as serum to be used for measurements of serum iron using photometric coloimetric assay by HUMAN kit with a normal reference values for females (37-145µg/dl), and for total iron binding capacity (TIBC) using Biomaghreb kit with normal reference values (249-412µg/dl). Ferritin was measured by one-step enzyme immunoassay using enzyme linked florescent immunoassay by sandwich technique, with normal reference values (12-150 ng/ml). The women have IDA when (Hb <12 g/dl, MCV <80fl, MCH< 27 pg. S. iron <37µg/dl, TIBC> 412µg/dl, S. ferritin< 15 ng/ml and transferrin saturation< 16% and blood film showing hypochromic microcytic RBC). Clinical history was obtained, regarding pregnancy, malignancy, autoimmune disease and acute or chronic infection, in presence of these conditions; patients were excluded from the study. Other causes of hypochromic anemia (anemia of chronic disease and thalassemia) were excluded by history and reticulocyte count that was done for all patients.

Twenty five adult healthy normal women were included in this study as a control group, whose hemoglobin > 12 g/dl, MCV >80fl, MCH >27pg, S. iron(37-145)µg/dl ,TIBC (249-412) µg/dl and S. ferritin (15-120) ng/ml. The remaining serum samples for patients and control group were stored at -20 C for measurement of complements (C3&C4) using quantitative determination by radial immune diffusion plates (IMMUCHEM kit) with normal reference values for C3 (91-156) mg/dl and for C4 (20-50)mg/dl.

Statistical analysis

The data were collected, organized, and tabulated using the Statistical Package for the Social Sciences version 19 software (SPSS Inc, Chicago, IL). The results are expressed in the form of ranges, and the mean ± standard deviation. A *P* value < 0.05 was considered to be statistically significant and *P* value< 0.01 to be highly significant.

Results

A total of 57 adult female were enrolled in this study , their age ranging between (16-41) years with mean ± standard deviation 27.22 ±7.31 years for patients and (23-40) years with mean ± standard deviation 28.84±3.88 years for control group, with no statistically significant differences between two groups (*P* value, 0.32).

Table-1 shows that Hb, HCT, RBC count, reticulocyte count and red blood cells indices (MCV, MCH, MCHC), were significantly lower in patients with IDA comparing with control group (*P* value< 0.01). Red distribution width (RDW) were statistically significantly higher in patients with IDA than control group (*P* value <0.01). There were statistically

significant differences (*P* value < 0.01) between patients with IDA and control group regarding serum iron, TIBC, serum ferritin and transferrin saturation percentage (% TS) in opposite to WBC count which has no statistically significant differences between two groups (*P* value 0.12).

The mean ± standard deviation for C3 &C4 were 95.02± 27.35& 21.03± 9.72 respectively for the group with IDA while for control group was 112.79 ± 32.46 for C3 &26.32 ± 8.38 for C4, comparing these results between two groups ,showing statistically significant differences (p value 0.0297 for C3 & 0.0034 for C4),Table- 2.

Table- 1 Parameters of Red Blood cells Indices and Iron Status between Patients with Iron Deficiency Anemia and Control Group.

Variables		Patients	Control	P-Value
Hemoglobin (g/dl)				
	Range	6.3-10.9	12-14.1	0.00001
	Mean ± SD	8.94 ± 1.25	12.86 ± 0.67	
HCT	**Range**	17.5-33	36.8-45	
	Mean ± SD	28.52 ± 3.19	39.7 ± 2.29	0.000001
RBC	**Range**	2.08-4.8	3.8-5.9	
	Mean ± SD	4.11 ± 0.56	4.62 ± 0.43	0.0004
WBC	**Range**	4.4-10	4.9-10.2	
	Mean ± SD	6.99 ± 1.28	7.54 ± 1.40	0.128
MCV	**Range**	53-80	81-98	
	Mean ± SD	67.74 ± 8.22	88.60 ± 4.37	0.000001
MCH	**Range**	14.9-27.0	26.9- 36.4	
	Mean ± SD	21.20 ± 4.18	29.01 ± 2.29	0.000001
MCHC	**Range**	23-33.2	30-36	
	Mean ± SD	29.28 ± 2.54	33.35 ± 1.42	0.000001
RDW	**Range**	15.1-21.10	10.6-15.2	
	Mean ± SD	17.0 ± 1.75	12.99 ± 1.30	0.000001
Retic.	**Range**	0.5-1.2	1.2-2.4	
	Mean ± SD	0.82+0.24	1.88 ± 0.27	0.000001
Serum Iron	**Range**	20-36.8	64-90	
	Mean ± SD	28.9+5.94	74.12 ± 6.16	0.000001
TIBC	**Range**	415-445	245-380	
	Mean ± SD	426.67+8.60	304.64 ± 30.80	0.000001
Serum Ferritin	**Range**	2.20-14.82	15.08-55.3	
	Mean ± SD	8.62+3.26	33.13 ± 11.86	0.000001
% TS	**Range**	4-11.5	19-31	
	Mean ± SD	6.91+2.01	24.20 ± 3.70	0.000001

HCT: hematocrit, **RBC**: red blood cell, **WBC**: white blood cell, **MCV**: mean cell volume, **MCH**: mean corpuscular hemoglobin, **MCHC**: mean corpuscular hemoglobin concentration, **RDW**: red cell distribution width, **Retic**.: reticulocyte count, **TIBC**: total iron binding capacity, **% TS**: transferrin saturation percentage

Table 2 Complement Level (C3 and C4) in Patients with Iron Deficiency and Control Group.

Variables		Patients	Control	P-Value
C3	**Range**	41.70-149.50	51.9-178.3	
	Mean ± SD	95.02+27.35	112.79 ± 32.46	0.0297
C4	**Range**	3.50-44	11.6-48.6	
	Mean ± SD	21.03+9.72	26.32 ± 8.38	0.0034

Discussion

Iron deficiency is one of the most common preventable nutritional deficiencies in developed and developing countries. IDA can cause irritability, headache, and fatigue that change social behavior and impair the ability of adults to do physical work, an increased susceptibility to infections has been reported in some IDA patients, the etiology of which is not well-known.[16] The complement system is a part of the immune system that is concerned with the innate immunity. It consists of numerous proteins that are activated by several triggering factors and start a chain of events in the human body. Because the pathway finally culminates in the creation of a cytolytic "membrane attack complex", it is a vital mechanism to fight infection.[17] In the current study we compared the levels of complements proteins C3 &C4 in two groups of adult pre-menopausal non pregnant women, one with IDA and the second normal women as a control group ,the findings were statistically significant lower levels in anemic patients than control group, this result concordance with study done by Mohammad Hadi Sadeghian et al[18]. in 6 patients out of 45 patients enrolled in his study with Hb < 10 g/dl and control group but they mentioned no statistically differences in serum level of C3 &C4 between two groups regarding the remaining patients with Hb > 10 g/dl. Mohammad Hadi Sadeghian et al. had used nephlometry technique for complements measurements while radial immune diffusion method is used in our study. Our study is concordance with Seyad et al. regarding C4 level [19], with no significant differences regarding C3 level between two groups. Seyad et al. study was done on pregnant women while the current study restricted on non-pregnant adult females. Our results goes with study done by V. Jagadeesan and Vinodini Reddy[20],which was done on children aged less than 10 years . 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, IL12p40, IFN-gamma, IL-10) might lead to immune system impairments in iron-deficient patients.(21) Iron also is essential for enzymes such as ribonuleotide reductase, involved in DNA synthesis therefore proliferative phase of lymphocyte activation it a ironf-requiring step and it can be diminished during IDA.[22]

Conclusion

Adult pre-menopausal non pregnant women with iron deficiency anemia have low level of C3 and C4 in compare to normal adult pre-menopausal women.

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