Evaluation of complements serum level (C3 and C4) in pregnant women with history of toxoplasmosis

تقييم مستوى المتمم C3 و C4 في مصول مجموعة من النساء الحوامل المصابات بداء المقوسات

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Abstract

C3 and C4 serum level were evaluated in 30 pregnant women which divided into groups: Group A16 (53.3%) (Pregnant women with history of abortion and positive anti toxoplasma IgM antibodies), Group B10 (33%) (Normal pregnancy women with no history of abortion or Toxoplasmosis.), Group C6 (20%) (Pregnant women with history of two abortion and positive anti toxoplasma IgM), group D 10 (33.3%) (Pregnant women with history of only one abortion and positive anti- toxoplasma IgM), group E 4 (13.3%) (Pregnant women with history of only one abortion and negative anti-toxoplasma IgM). The results showed that highest level of both C3 and C4 in women with positive anti toxplasma IgM and history of one or two abortion/abortions while the lowest level of these two complements were in women with negative anti toxplasma IgM even they had one abortion or no abortion. There is significant differences in concentration of C3 (189.7 ± 20.3 mg/dl) and C4 (59.3 ± 7.5 mg/dl) in group A and C3 (189.6 ± 17.7 mg/dl) and C4 (63.08 ± 4.7 mg/dl) when compared with group B and E, and the result showed statistical differences in C4 concentration between group C and D at P< 0.05. We conclude that complement was play role in immune response of pregnant women especially against toxoplasmosis that cause abortion to these women.

المستخلص

تم تقييم مستوى كل من المتمم C3 و المتمم C4 في مصل الدم عند 30 مجموعة من النساء الحوامل ، والتي قُسِّمت إلى مجاميع: مجموعة(A) نساء حوامل ذات تاريخ إجهاض وموجبة للفحص المصلي لطفيلي *Toxoplasma gondii* ، وكان عددهم 16 (53,3 %) ، مجموعة (B) نساء ذات حمل طبيعي غير مجهضات و غير مصابات بداء القطط و سالبه للفحص المصلي لطفيلي *Toxoplasma gondii* ، وكان عددهم 10 (33%) ، مجموعة (C) نساء حوامل مجهضات مرتين وموجبة للفحص المصلي لطفيلي *Toxoplasma gondii* ، وكان عددهم 6 (20%) ، مجموعة (D) نساء حوامل مجهضات لمرة واحدة و موجبة للفحص المصلي لطفيلي *Toxoplasma gondii* ، و كان عددهم 10 (33,3%) ، مجموعة (E) نساء حوامل مجهضات لمرة واحدة وسالبه للفحص المصلي لطفيلي *Toxoplasma gondii* ، وكان عددهم 4 (13,3%) . أظهرت النتائج إرتفاع مستوى كل من العامل المتمم الثالث والرابع في النساء ذوات الفحص المصلي الموجب للطفيلي و لديهن اجهاضات سابقه (مرة او مرتين) بينما ظهر إنخفاض في مستوى كلا العاملين في النساء ذوات الفحص المصلي السالب للطفيلي وليس لديهن اجهاضات سابقه او لديهن اجهاض سابق واحد ، هناك فروق معنوية في تركيز الجزء الثالث (C3) من المتمم والجزء الرابع (C4) في المجموعة A و D من المريضات عند مقارنتها بالمجموعة B و E من المريضات ، لكن هنالك فرق إحصائي في الجزء الرابع من المتمم في كل من المجموعتين C و D بأحتمالية P<0.05 . نستنتج من هذه الدراسة أن المتمم يلعب دور في الأستجابة المناعية في النساء الحوامل خصوصاً ضد داء القطط الذي يسبب الأجهاض لهن .

Introduction

Toxoplasma gondii is an obligate intracellular protozoan that belongs to the phylum Apicomplexa; subclass coccidian can take several different forms: the oocyst; the tachyzoite and the cyst [1]. The infection with this parasite called Toxoplasmosis, the importance of *T. gondii* as a human pathogen has stimulated a huge number of researches [2], most of the *T. gondii* infected people being clinically asymptomatic and spontaneous recovery [3, 4]. Infection with this protozoan usually occurs by ingestion of food or water contaminated with its oocyst. Infection with *T. gondii* is major cause of fetal death since *T. gondii* can be transmitted to the fetus through the placenta from an infected mother [1]. Furthermore toxoplasmosis has been implicated in abortion, and early postnatal mortality [5].

Cellular immunity is the key component of the host's immune reaction in the event of attack by *Toxoplasma* [6]. While antibodies play minor role but remain the essential means for diagnosis in human [7], these antibodies are the best activators of the complement system [8]. Such antibodies, with complement, kill extracellular *T. gondii* and enhance phagocytosis [9]. The role of complement at the placenta level as in any other tissue in the body is to protect both the fetus and the mother against infectious and other toxic agent the placenta is the subject of complement mediated immune attack at the feto-maternal interface with the potential risk of fetal loss. Uncontrolled complement activation is prevented in successful pregnancy by three regulator protein DAF, MCP and CD59 positioned on the surface of the trophoplasts. Extensive complement activation in the placenta places the fetus at risk for growth restriction or death [10].

We choose the pregnant women in this study because toxoplasmosis was showed risk factor for fetus and lead to abortion. The aim of this study is to evaluate C3 and C4 in a group of pregnant women with history of Toxoplasmosis in Baghdad.

Matereal & Methods

This study was carried out on 30 pregnant women attending outpatient gynecology of Baghdad teaching hospital between June - September 2005.

The study group consists of 20 pregnant women with history of abortion and 10 normal pregnancy women who had no history of abortion. Their ages ranged from 21-40 years. All the women examined were interviewed to ascertain medical information.

Serum samples

Two ml of blood were taken from each woman and put in plane tube. Then sera were separated by centrifugation at 3000 rpm for 5 minutes. Samples were stored at -20 Cº until use.

IgM anti-toxoplasma antibodies

Specific anti-toxoplasma IgM antibodies were determined using ELISA technique (ETI-TIOXOK-M reverse, Sorin Biomedica, France). According to the manufacturer's instructions. Patient samples, blank, negative and positive controls were diluted with sample diluents and dispensed into their corresponding wells, followed by incubation for (1) hour at 37°C. Wells were washed carefully with the wash buffer. The antigen/ tracer was dispensed into all wells except the blank well. Wells were incubated for 1 hour at 37°C, followed by proper washing. Then 100 ml of chromogen substrate were

added to each well and incubated at room temperature for 30 minutes. The reaction was stopped by the addition of the blocking reagent. The optical density (absorbance) was determined at 450nm 2 hours after the addition of the blocking buffer. The presence or absence of the anti-toxoplasma IgM antibodies was determined by relating the absorbance value of the unknown samples to that of the cut-off control values.

C3 and C4 evaluation

C3 and C4 concentration were determined by using single radial immune diffusion (Sanofi Diagnostic Pasture) according to the manufacturer's instruction, five µl of serum sample or control were applied. The lid was closed firmly, incubated at room temperature 25Cº and the diameter was measured accurately within 0.1 mm with a suitable device.

Statistical analysis

Descriptive statistic was used to describe the quantitative variables. T-test and ANOVA were used as appropriate p values were set to be < 0.05 throughout the study. SPSS version 10 was used for data analysis.

Results

The major characteristics of pregnant women in this study are mentioned in Table (1). Out of 30 pregnant, 6 (20) % had history of recurrent abortion 2 abortion and positive anti toxoplasma IgM, 10 (33.3)% had a history of only one abortion and positive anti-toxoplasma IgM, 4 (13.3)% had history of one abortion and negative anti- toxoplasma IgM, and 10 (33.3)% normal pregnancy women with no history of abortion or Toxoplasmosis.

Significant differences C3 and C4 were noticed between Group A (pregnant women with history of abortion and positive anti toxoplasma IgM) and Group B (normal pregnancy women with no history of abortion or Toxoplasmosis.) Group A showed high C3 (189.7 ± 20.3 mg/dl) and C4 level (59.3 ± 7.5 mg/dl) while it was less in Group B, C3 (116.3 ± 19.8 mg/dl) and C4 level (28.2 ± 5 mg/dl) Table (2).

The results also showed significant differences in C3 and C4 level between Group D (pregnant women with history of only one abortion and positive anti- toxoplasma IgM) and Group E (pregnant women with history of only one abortion and negative anti- toxoplasma IgM) $p< 0.05$. The latest group showed less mean of C3 (97.85 ± 6.8 mg/dl) and C4 level (37.9 ± 15.9 mg/dl) while group D showed higher mean of both of C3 (189.6 ± 17.7 mg/dl) and C4 level (63.08 ± 4.7 mg/dl) Table (2).

However, the results showed no significant differences in C3 level between Group C (pregnant women with history of two abortion and positive anti toxoplasma IgM) and group D, but there was statistical differences in C4 level between these two groups $p< 0.05$ Table (2).

Table (1): Major characteristics of pregnant women in this study

Group	Subject		Anti-toxoplasma IgM	History of Abortion
	Number	percentage		
C	6	20%	+	2 time
D	10	33.3%	+	1 time
A (C+D)	16	53.3%	+	1or 2 time
B	10	33.35	-	-
E	4	13.3%	-	1 time

Table (2): C3 and C4 Levels in Different group in this study

	Groups	Average of Complement Level (mg/dl)	
		C3	C4
A	Pregnant women with history of abortion and positive anti toxoplasma IgM	189.7 ± 20.3	59.3 ± 7.5
B	Normal pregnancy with no history of abortion or toxoplasmosis	116.3 ± 19.8	28.2 ± 5
C	Pregnant women with history of two abortion and positive anti toxoplasma IgM	187.06 ± 27.8	53.2 ± 8.3
D	Pregnant women with history of only one abortion with positive anti toxoplasma IgM	189.6 ± 17.7	63.08 ± 4.7
E	Pregnant women with history of only one abortion and negative toxoplasma IgM	97.85 ± 6.8	37.9 ± 15.9

Discussion

The study showed that the complements level (C3, C4) varies among pregnant women with different history of abortion (one or two abortion) and presence of anti-toxpoplasma IgM (negative or positive). It was clear that Group B (normal pregnancy women with no history of abortion or Toxoplasmosis and Group E (pregnant women with history of only one abortion and negative anti- toxoplasma IgM) had the lowest of each of C3 and C4 in comparison with the other groups: Group A (pregnant women with history of abortion and positive anti toxoplasma IgM), Group C (pregnant women with history of two abortion and positive anti toxoplasma IgM), and Group D (pregnant women with history of only one abortion and positive anti- toxoplasma IgM).

It is know that many factors influence the immune response to *T.gondii* infection. Virulence of parasite strain and concentration of parasite in the inoculums as well as, age, sex and level of many sex hormones all may affect on immune response of the host [11]. Pregnant females are more susceptible to infection with *T. gondii* and this is associated with a reduction in IFN-ɣ production. There is increasing evidence that immunological factors include various humoral abnormalities may play central role in failure of natural pregnancies [12, 13].

Our finding agreed with AL-Warid (2006) who focused on the type of abortion in relation with immunological status of *Toxoplasma* infected women, The results indicated that the amount of non specific antibodies increased in aborted women with

history of toxoplasmosis , and both complements (C3,C4) increased of these non specific antibodies [14] because antibody can enhance phagocytosis by macrophage , and phagocytosis is increased even more by addition of complement, these effects are mediated by Fc and C3 on the macrophage , which may increase in number as a result of macrophage activation [15]. The requirement for (C4,C2), indicated that the classical complement pathway was involved in the killing of *Toxoplasma* , and the activator system for Ab to *Toxoplasma* in human serum is identical with the classical complement pathway and functions completely independently of properdin and the alternative complement pathway [16].

We conclude that complement may play a good role in immune response against toxoplasmosis especially in aborted women.

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