Study of Humoral Immune Response in Complete and Incomplete Aborted Women with History of Toxoplasmosis

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Abstract

Humoral immune response was evaluated in three groups of pregnant women in Baghdad between June and September 2005: group A (pregnant women had history of complete abortion and positive anti-toxoplasma IgM), group B (pregnant women had history of incomplete abortion and positive anti-toxoplasma IgM) and group C (normal pregnant women with negative anti-toxoplasma) by examining IgM, IgG, IgA, C3 and C4. Which were at the minimum average in group C (144.5 ± 56.2 mg/dl), (815 ± 141.5 mg/dl), (225 ± 39.7 mg/dl), (106.2 ± 7.7 mg/dl) and (25.9 + 6.3 mg/dl) respectively, while the average of IgM, IgG, IgA, C3 and C4 in group A were (486 ± 113.7 mg/dl) ,(1893.2 ± 385.09 mg/dl) , (452.4 + 46.78 mg/dl) ,(184.58 ± 23.39 mg/dl) and (64.4 ± 4.6 mg/dl) respectively, and in group B the average were(443.03 ± 60.6 mg/dl) , (1626.26 ± 242.7 mg/dl) ,(361.2 ± 130.3 mg/dl) ,(181.76 ± 43.4 mg/dl) and (51.8 + 12.01 mg/dl) respectively .

Introduction

Toxoplasmosis is a universal zoonotic disease, caused by Toxoplasma gondii which is an obligate intracellular coccidian parasite [1]; it can take several different forms: the oocyst; the tachyzoite and the cyst [2]. The infection is worldwide especially in warm and moist climates; Domestic cats are the definitive host of the parasite and are the main source of infection via oocyst passed in their faeces [3]. Human infection may be acquired by many routs, mainly via contact with infected cats, ingestion of tissue cyst (bradyzoites) in undercooked or raw livestock's meat [4]. Most infection in human are asymptomatic but parasite can produce devastating disease [5]. In pregnancy, infection can result in congenital infection, and it is a frequent cause of encephalitis in severely immunosuppressed patient [6]. Furthermore Toxoplasmosis has been implicated in abortion in human [7].abortion is an interruption of pregnancy before the fetus has attained a stage of viability, there are many types of abortion: threatened, Habitual, complete (elimination of the enteric contents of the uterus), incomplete (expulsion of the fetus while all or part of the placenta remain in uterus), and therapeutic [8].

The immune response to Toxoplasma gondii infection is individual, complex and compartmented [9] Cellular immunity is the key component of the host's immune reaction in the event of attack by Toxoplasma [10]. While antibodies play minor role but remain the essential means for diagnosis in human. These antibodies are the best activators of the complement system. such antibodies, with complement, kill extracellular T. gondii and enhance phagocytosis [11,12].

The concept of the study is the evaluation of non specific Ig Level, C3 and C4 in pregnant women with history of complete and incomplete abortion in Baghdad.

Subjects & Methods

We examined twenty three female age range (21-40) years, thirteen with history of Toxoplasmosis as detected by the presence of anti-toxoplasma specific IgM, and history of complete or incomplete abortion, and ten normal pregnant women who had no history of abortion. All the examined women were interviewed to ascertain medical information. This study was carried out between June and September 2005.

Serum samples

Sera were separated by blood centrifugation at 3000 rpm for 5 minutes. Samples were stored at -20 C until needed.

IgM anti-toxoplasma antibodies

Specific IgM anti-toxoplasma antibodies were determined using ELISA technique (ETI-TOXOK-M reverse, Sorin Biomedica, France) according to the manufacturer's instructions. Briefly, 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. The wells were then washed carefully with the washing 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 450 nm 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.

IgG, IgM, IgA, C3, C4 evaluation

IgG, IgM, IgA, C3, C4 were determined by using Single radial immunodiffusion (Sanofi

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Diagnostic Pasture) according to the manufacturer's instructions.

Apply 5 ml of serum sample or control, then close the lid firmly, incubate at 4 C for (48-72) hour, then measure the diameter accurately with suitable device.

Statistical analysis

Descriptive statistic was use to describe the quantitative variables. T-test and ANOVA were used as appropriate p values where 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 compared in table (1). Out of 23 pregnant women, 7(30.43%) had history of complete abortion and positive anti-toxoplasma IgM, 6(26.08%) had history of incomplete abortion and positive anti-toxoplasma IgM and 10(43.47%) pregnant women with no history of any type of abortion and negative anti-toxoplasma IgM.

Non Specific Ig Level

It was clear that the normal pregnant women with negative anti-toxoplasma IgM (group C) had the lowest average of each of IgM (144.5 + 56.2 mg/dl), IgG (815 = 141.5 mg/dl) and IgA (225 + 39.7 mg/dl),while in group A (women had history f complete abortion and positive anti-toxoplasma IgM) were (486.9 = 113.7 mg/dl), (1893.2 = 385.09 mg/dl) and (452.4 ± 46.7 mg/dl) for IgM, IgG and IgA respectively. And in group B (had history of incomplete abortion and positive anti-toxoplasma IgM) the average of each IgM,IgG and IgA were (443.03 ± 60.6 mg/dl) , (1626 ± 242.7 mg/dl) and (361.2 ± 130.3 mg/dl) respectively. Statistical analysis showed significant differences at p< 0.05 between group (A & C) and Group (B & C). Figure (1, 2, and 3).

C3 and C4 Level

The results also showed significant differences at p< 0.05 between group (A & C) and Group (B & C) in average of C3 and C4 level. Group C had the lowest average of each of C3 and C4 level, which were (106.2 ± 7.7 mg/dl) and (25.9 ± 6.3 mg/dl) respectively. While these level were more in group A and B (184.5 - 23.3) and (181.7 ±43.4 mg/dl) for C3 respectively and (64.4 ± 4.6 mg/dl), (54.8 + 6.3 mg/dl) for C4 respectively. Figure (4, 5).

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

Group	Subject		Age Range /year	Anti-toxoplasma IgM	Number of Abortion	Type of Abortion
	Number	percentage				
A	7	30.43 %	22-40	+	1	Complete
B	6	26.08 %	21-40	+	2	Incomplete
C	10	43.47 %	25-35	-	-	-

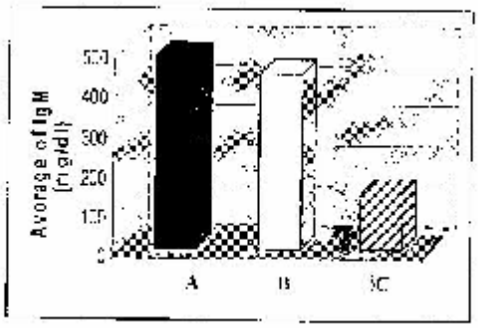

Figure (1): Average of IgM (mg/dl) in Groups A, B and C

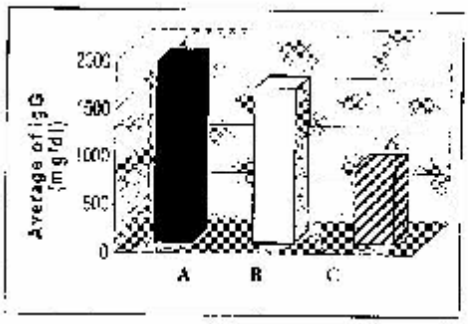

Figure (2): Average of IgG (mg/dl) in Groups A, B and C

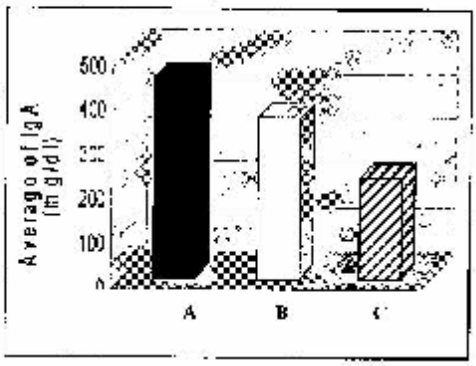

Figure (3): Average of IgA (mg/dl) in Groups A, B and C

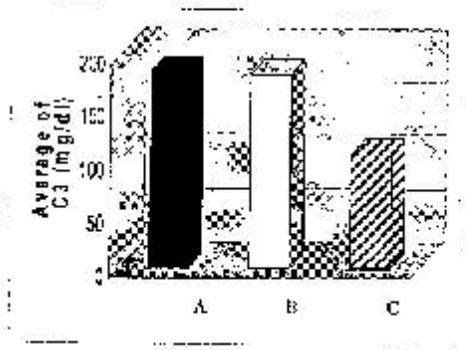

Figure (4): Average of C3 (mg/dl) in Groups A, B and C

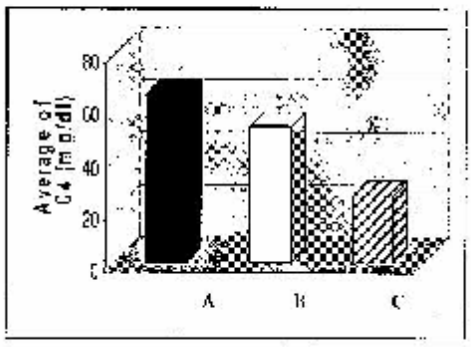

Figure (5): Average of C4 (mg/dl) in Groups A, B and C

Discussion

The study showed that the development of non specific Ig and complement levels varies greatly among pregnant women with different age and different history of abortion (complete, incomplete) and pregnant women had no history of abortion with or without Toxoplasmosis. It was clear that the normal pregnant women with negative anti-toxoplasma IgM (group C) had the lowest average of each of IgM, IgG, IgA, C3 and C4 in comparison with group A (women had history f complete abortion and positive anti-toxoplasma IgM) and group B (women had history of incomplete abortion and positive anti-toxoplasma IgM).

We must know that many different factors may influence the immune response to T.gondii infection, the virulence of parasite strain, the concentration of parasite in the inoculum, and the infective form of the parasite (oocyst or cyst) play a role [13].

During pregnancy, the fetoplacental unit orchestrates immune mechanism via T and B lymphocytes, natural killer cells, a variety of soluble immunoregulatory factors, antibodies and complement [14,15]. Levels of many sex hormones ,most notably estrogen and progesterone, are vastly increased during pregnancy , and consequently their effects on the immune system can be profound [16], there is a local T-helper (Th2) bias, with fetoplacental tissues spontaneously producing the Th2 associated cytokines interleukins (IL-4), (IL-5) and (IL-10) .[17]. These cells provide optimal help for humoral immune response including IgG1 and IgE isotype switching and mucosal immunity, stimulation of mast cell and eosinophil growth and differentiation IgA. [18].

In other hand there is increasing evidence that immunologic factors play an important role in the failure of natural pregnancies [19]. These factors include various humoral abnormalities such as antiphospholipids antibodies, antithyroid antibodies, antinuclear antibodies,antiovarian antibodies and increase in IgM levels [20]. These finding(increase in IgM level) is very similar with our results , in each of group A and B there is an immune response provoked by abortion and other immune response provoked by Toxoplasma infection, in group C (normal pregnancy ,no history of Toxoplasmosis) the immune response is lower than each of group A and B.

Two conclusions can be drawn from our results: (i) the amount of non specific IgM,IgG and IgA increase in aborted women with toxoplasmosis,(ii) C3 and C4 developed frequently with increasing of non specific IgM,IgG and IgA in complete and incomplete aborted women with toxoplasmosis .

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الخلاصة

تضمنت هذه الدراسة قياس تركيز كل من الفوسفوليبيدات المناعي IgM ،IgG ، IgA ، المتمم C3 و C4 في ثلاث مجموعات من النساء الحوامل في بغداد ، اجريت هذه الدراسة بين شهري حزيران و آب من عام 2005 وكانت المجموعات كما يأتي:

مجموعة A : موجبة للفحص المصلي لطفيلي Toxoplasma gondii ، نوع الأجهض Complete.

مجموعة B : موجبه للفحص المصلي لطفيلي Toxoplasma gondii ، نوع الأجهاض Incomplete

مجموعة C : سالبه للفحص المصلي لطفيلي Toxoplasma gondii ، تمثل تجميع اجهاضات سابقه.

تميزت المجموعة (C) بأنها كانت ذات معدلات قليلة لكل من IgM (141.5 ± 56.2 ملغم/ ديسيلتر):IgG (815 ± 141.5 ملغم/ ديسيلتر) ، IgA (225 ± 39.7 ملغم/ ديسيلتر)، C3 (106.2 ± 7.7 ملغم/ ديسيلتر)، C4 (25.9 ± 6.3 ملغم/ ديسيلتر) وبالمقارنة مع كل من المجموعة (A): IgM (486 ±113.7ملغم/ ديسيلتر)، IgG (1893.2 ±385.09 ملغم/ ديسيلتر) ، IgA (184.5 ±23.39 ملغم/ ديسيلتر)، C3 (452.4 ± 16.78 ملغم/ ديسيلتر)، C4 (64.1 ± 4.65 ملغم/ ديسيلتر) و المجموعة (B) : IgM (443.03 ± 60.6 ملغم/ ديسيلتر) ، IgG (1626.39 ± 242.1 ملغم/ ديسيلتر) ، IgA (361.2 ± 130.3 ملغم/ ديسيلتر) ، C3 (181.76 ± 43.4 ملغم/ ديسيلتر) C4 (51.8 ± 12.01 ملغم/ ديسيلتر).