EVALUATION OF NON- SPECIFIC IGG AND IGM ANTIBODIES IN PREGNANT WOMEN INFECTED WITH *TOXOPLASMA GONDII*[+]

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Abstract

IgG and IgM Antibodies were evaluated in (30) pregnant women divided into: {Group A (pregnant women with history of abortion and positive anti toxoplasma IgM), Group B (normal pregnancy women with no history of abortion or Toxoplasmosis.), Group C (pregnant women with history of two abortion and positive anti toxoplasma IgM) ,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)}.the highest level of IgG(1922.983 ± 169.303 mg/dl) and IgM (486.433 ± 41.578 mg/dl) were in group D while the less IgG (1608.300 ± 91.098 mg/dl) and IgM level (324.700 ± 35.919 mg/dl) were in group E.

المستخلص

تضمنت هذه الدراسه قياس تركيز كل من الغلوبيولين المناعي IgG و IgM في مجموعة من النساء الحوامل ، حيث تم تقسيمهن الى مجموعات :

مجموعة A : موجبة للفحص المصلي لطفيلي ***Toxoplasma gondii*** ، وعدد مرات الأجهاض (مرة او مرتين).

مجموعة B : سالبه للفحص المصلي لطفيلي ***Toxoplasma gondii*** ، ليس هناك اي اجهاضات سابقة.

مجموعة C : موجبة للفحص المصلي لطفيلي ***Toxoplasma gondii*** ، وعدد مرات الأجهاض (مرتين فقط).

مجموعة D : سالبة للفحص المصلي لطفيلي ***Toxoplasma gondii*** ، وعدد مرات الأجهاض (مره واحده فقط).

مجموعة E : سالبه للفحص المصلي لطفيلي ***Toxoplasma gondii*** ، وعدد مرات الأجهاض (مره واحده فقط).

تميزت المجموعة D بتفوقها على المجموعات الأخرى في تركيز كل من الغلوبيولين المناعي IgG و IgM حيث كان (١٩٢٢,٩٨٣ ± ١٦٩,٣٣ ملغم/ ديسيلتر) ، (486.433 ± 41.578 ملغم/ ديسيلتر) على التوالي ، بينما تميزت المجموعة E بأنها الأقل في تركيز كل من الغلوبيولين المناعي IgG (١٦٠٨,٣٠٠ ± 91.098 ملغم/ ديسيلتر) و IgM (324.700 ± ٣٥,٩١٩ ملغم/ ديسيلتر).

Introduction:

Toxoplasma gondii is an obligate intracellular protozoan that belongs to the phylum Apicomplexa, subclass coccidian. It can take several different forms: the oocyst; the tachyzoite and the cyst [1]. The infection with this parasite called Toxoplasmosis, the infection is worldwide, particularly in warm

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and moist climates [2]. T. gondii infection is found in 30%-50% of the human population worldwide, most of the 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, prematurity, still birth and early postnatal mortality [5]The frequency of toxoplasmosis is acquisition during pregnancy ranges from 1-4 per 1000 pregnancy, and congenital infection has a prevalence of 0.2-2 per 1000 births [6]

Abortion is generally defined as interruption of pregnancy before the fetus has attained a stage of viability [7].spontaneous abortion has been associated with maternal transmission of T. gondii to the fetus [8]. Controversial reports have appeared regarding its role in habitual and sporadic abortion [9]. Serological studies indicate that there is a clear association between Toxoplasma infection and habitual abortion, few studies have been conducted on the prevalence of toxoplasmosis in Iraq [10], and therefore, the aim of this study was to determine the prevalence of Toxoplasma antibodies and evaluation of non specific IgM and IgG in a group of pregnant women with abortion in Baghdad.

Subjects & Methods:

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

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

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 capture ELISA technique (ETI-TIOXOK-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 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 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.

IgM and IgG antibodies:

IgM and IgG were determined by using Single radial immunodiffusion (Sanofi 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.

Methods of statistical analysis:

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

Descriptive statistic was used 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 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

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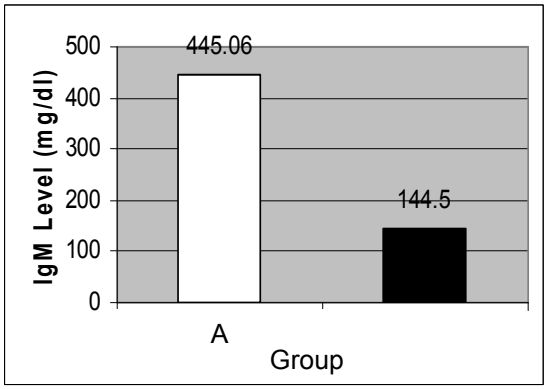
The results were presented by measuring the differences of non specific IgM and IgG among group A (pregnant women with history of abortion and positive anti toxoplasma IgM) and group B (normal pregnancy women with no history of abortion or Toxoplasmosis.) it was clear that the group A had the highest mean of IgM level (445.060± 31.444 mg/dl) and IgG level (1849.650 ± 110.414 mg/dl) while it was less in group B IgM level (144.500 ± 17.786 mg/dl) and IgG level (815.000 ± 44.752 mg/dl). Significant differences were noticed statistically between these two groups at $P < 0.05$. (Figure 1).

Statistical analysis showed significant IgM and IgG differences between group C [pregnant women with history of recurrent abortion (2 abortion) and positive anti toxoplasma IgM] and group D [pregnant women with history of only one abortion and positive anti- toxoplasma IgM] at $p< 0.05$. Group D showed the highest mean of IgM level (486.433 ± 41.578 mg/dl) and mean of IgG level (1922.983 ± 169.303 mg/dl).While group C showed less mean of IgM (415.483 ± 35.069 mg/dl) and IgG level (1738.933 ± 78.271 mg/dl). (Figure 2).

The results also showed significant differences in IgM and IgG between group D and group E (pregnant women with history of only one abortion and negative anti- toxoplasma IgM) at $p < 0.05$. The latest group showed less mean of IgM (324.700 ± 35.919 mg/dl) and IgG level (1608.300 ± 91.098 mg/dl) than group D. (Figure 3).

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



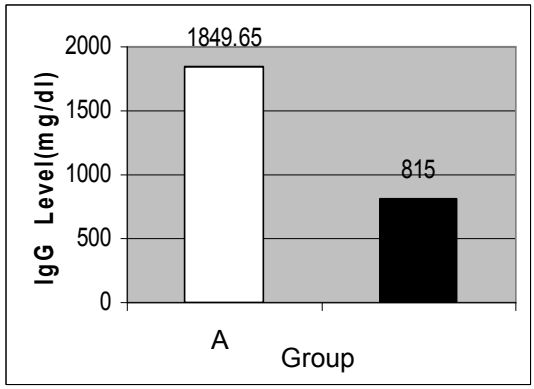
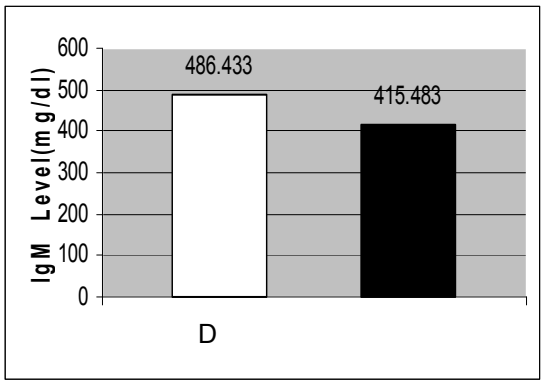
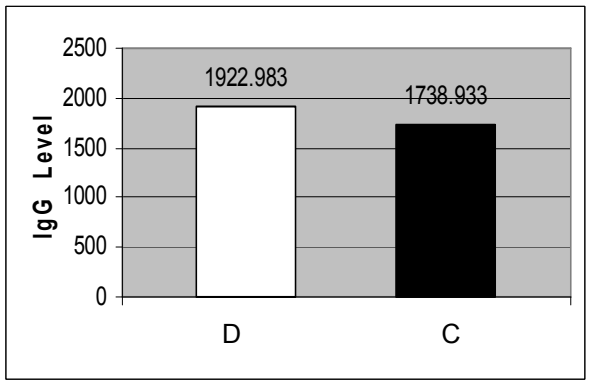
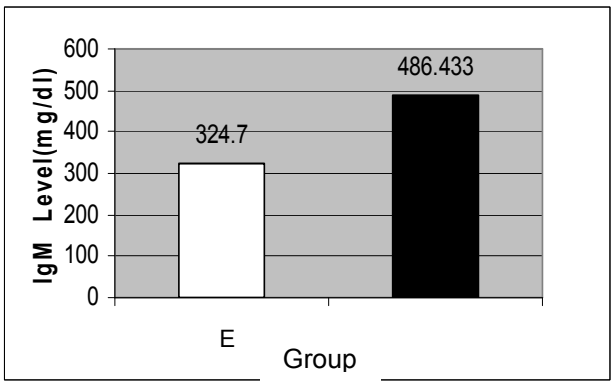


Figure (1): IgM and IgG Level in Group A and B







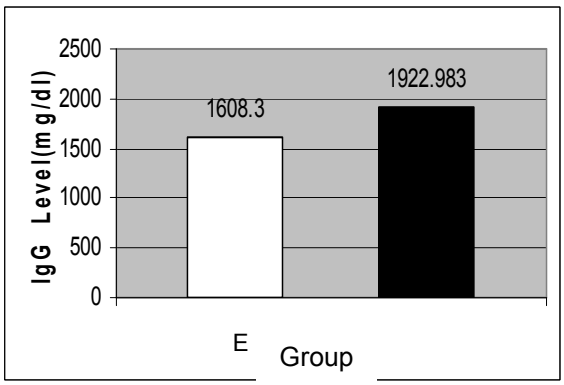


Figure (3): IgM and IgG Level in Group E and D

Discussion:

Toxoplasma infection is complex and depends on the genetic background of the host, his immune status and also parasite factors including virulence, Toxoplasma have the

capacity to spread in all the tissues and each tissue compartment has its own specific immune response [11].

In the present study, an Immunological comparison was made in different groups of pregnant women. and it was clearly found that pregnant women with history of abortion and positive anti toxoplasma IgM had higher antibody titer (IgM and IgG) than had in group of normal pregnancy. This finding is supported by Al-Hamadani & Mahdi [10].

The result also showed that there were a significant IgM and IgG differences between group C [pregnant women with history of recurrent abortion (2 abortion) and positive anti toxoplasma IgM] and group D [pregnant women with history of only one abortion and positive anti- toxoplasma IgM] at $p< 0.05$. Group D showed the highest mean of IgM and mean of IgG level when compared with group C. There is considerable confusion and uncertainty concerning T. gondii as a cause of multiple abortions, sterility and other reproductive failures because most data are based on serology [12]. On other hand there are articles indicating that there is a clear association between Toxoplasma infection and habitual abortion [13, 14].also, if a women foetal loss is proven due to T.gondii infection, her subsequent pregnancies are safe as far as this parasitic is concerned, until she becomes immunocopromised during subsequent pregnancies [15].

The results also showed significant differences in IgM and IgG between group D and group E (pregnant women with history of only one abortion and negative anti- toxoplasma IgM) at $p < 0.05$. The latest group showed less mean of IgM (324.700 ± 35.919 mg/dl) and IgG level (1608.300 ± 91.098 mg/dl) than group D., and this due to the non- specific immune response provoked by Toxoplasma infection, this non specific immune response being immediately following the first contact between the parasite and the host it peaks at the first week, and then slowly reduce [11]. But it may persist for month or even years [16]. Consequently, it has been demonstrated that pregnant mice are more susceptible to infection with T. gondii and show higher mortality than similarly infected non pregnant female mice [17]. The effects of sex- and pregnancy-associated hormones on the immune response are not limited to reproductive tissues but are clearly evident in the systemic immune system [18].

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