

Is Hyperglycemia or Toxoplasmosis the Suspect of Spontaneous Abortion in Neonates in Baghdad Women?

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ABSTRACT

To identify the true contribution of toxoplasmosis or hyperglycemia to spontaneous abortion, we tested 45 women with 1st time abortion aged range(18-35) years old;(33.3%) of whom had a loss at 12th week of pregnancy, and 54 women had multiple abortion in the same age range; 33.3% of whom had abortion at 8th week of pregnancy. The study was included 45 normal pregnant women in the same age range, conducted in Baghdad, Capital of Iraq, from December 2008 till June 2009. All the women were examined for the presence of toxoplasma-specific IgG; 63.4% of all habitual abortion groups (n=99) showed no significant increase in positive IgG when compared with normal pregnant women (n=45). We also tested glucose level in each group of women; 60% of 1st abortion group with positive IgG had a non-significant hyperglycemia than normal pregnant women ($p>0.05$), 72% of 2nd abortion group with positive IgG had a significant hyperglycemia than normal pregnant women. The results indicate that the contribution of toxoplasmosis to spontaneous abortion in Baghdad is greatly overestimated, while hyperglycemia may be a real reason of repeated abortion.

Abbreviations : F.B.S fasting blood sugar, ELISA: enzyme link immune sorbent assay , IFA : Indirect fluorescent Antibody, IgM , IgG, and IgA : immunoglobulin M , G and A

KEY WORDS: hyperglycemia, Iraqi pregnant women, spontaneous abortion, toxoplasmosis.

INTRODUCTION

Spontaneous abortion is defined as the termination of pregnancy at less than 20 weeks' gestation in the absence of elective medical or surgical measures. After 20 weeks the pregnancy losses are called preterm deliveries. On the other hand, the term "miscarriage" is synonymous to spontaneous abortion and is often used with patients because the word "abortion" is associated with elective termination. "Spontaneous pregnancy loss" has been recommended to avoid the term "abortion" and acknowledge the emotional aspects of losing a pregnancy(I).

Toxoplasmosis is a common infection that occurs in humans and other warm-blooded animals usually caused by the parasite toxoplasma gondii (*T. gondii*). It represents one of the main causes of infectious reproductive failure in the world. It causes serious problems that lead to fetal abortion, stillbirth and neonatal mortalities, resulting in great economic losses (2, 3). Furthermore, there is a study indicated that a number of early abortions may be due to decontrolling of diabetic patients (4).

It was found that nearly one third of humanity has been exposed to *T. gondii*(5). This parasite is able to develop in a wide variety of vertebrate hosts but its definitive host is the house cat and certain other felidae(6). The transmission of *T.gondii* to humans occurs commonly via ingestion of food or water contaminated with oocysts shed by cats, by ingesting tissue cysts in undercooked, uncooked or raw meat, via contact with cats faeces (directly or indirectly through the soil) and via transplacental transfer, notably when the mother becomes infected for the first time while pregnant(7-11).

The diagnosis of toxoplasmosis infection in humans is, but seldom do by recovering of the parasite , usually done by serological tests, and in order to get a proper diagnosis it is necessary to follow-up the algorithm illustrated in Figure 1 (6).

In 1993, Dupouy-Camet and co-workers presented their study on the fact that acute infections with toxoplasmosis induce production of specific immunoglobulin A and M (IgA and IgM) antibodies(the peak of antibodies were produced from 1-18 weeks of pregnancy) while chronic infection cause elevation of specific immunoglobulin G (IgG)in serum (from 4-25 weeks of pregnancy)(12).

Most gynecologists working in the general hospital in Baghdad, Iraq, consider toxoplasmosis as a primary cause of abortion. They rely on the results of latex agglutination tests. Patients who tested positive will be submitted to long-time therapy with anti-toxoplasma drugs may be extended for the whole pregnancy unless there is abortion. In addition, this long-term therapy is highly expensive and affects the economic burden on families who may already be financially weak. Also, we can add another reason related to the side effect of the continuous therapy of

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toxoplasmosis on both pregnant women and her baby. For these reasons we aimed to identify the true contribution of hyperglycemia or toxoplasmosis infection in women had unexplained recurrent abortion, against control pregnant women supposed to be without infection, by the enzyme-linked immunosorbent assay (ELISA) for toxoplasma-specific IgG antibodies depending on recent recommendation by (13) for detection of specific anti toxoplasma IgG antibodies and diagnosis of active toxoplasmosis because latex agglutination test can be useful as screening test, but when the result is positive, it should be followed by a test for specific anti toxoplasma IgM or IgG antibodies. In our opinion, differentiation between active toxoplasmosis and past infection is important so that treatment can be limited to those having acute infection. This will definitely reduce the incidence of unnecessary therapy.

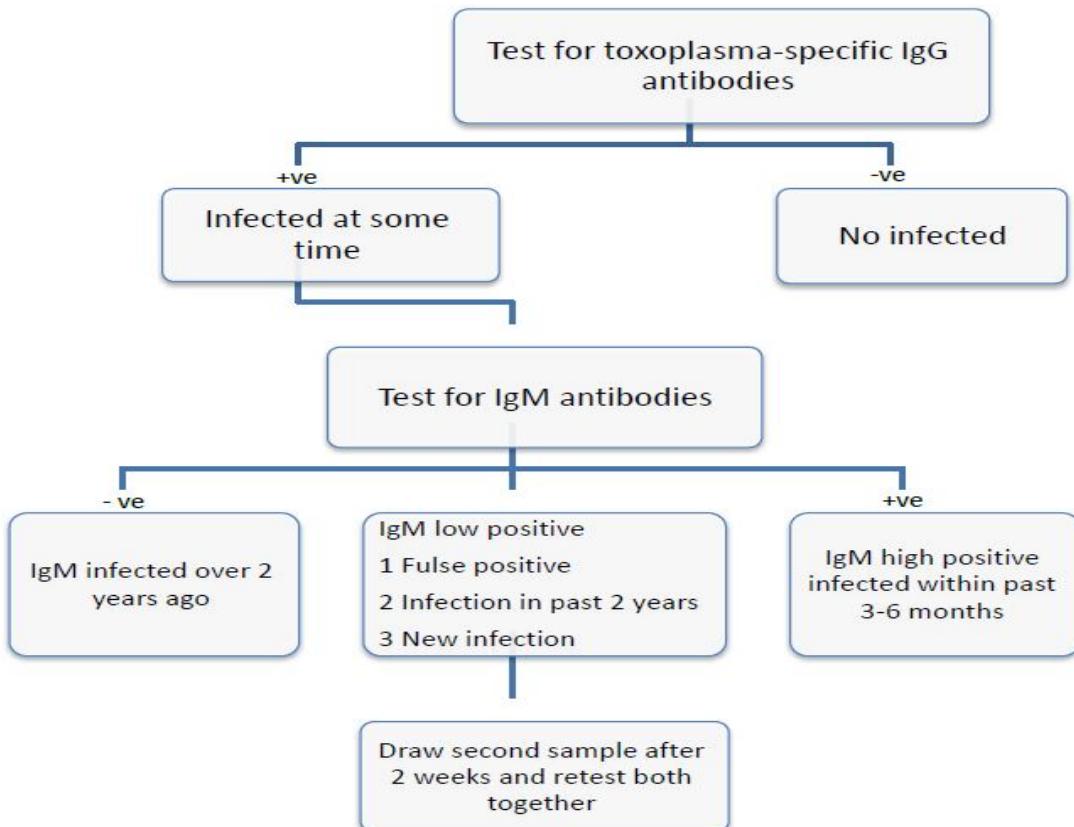


Figure 1: Algorithm represents the best way for testing toxoplasma infection, reproduced from Razzak *et al.*, 2005 (13).

MATERIALS AND METHODS

Study location, selection of subjects and inclusion criteria:

Baghdad is the capital city of Iraq, located in the middle of the country, having a population of about 17 million in 2007 and consisting of administrative, commercial, industrial and residential areas, with patches of agricultural land that are mostly grass fields.

This study was carried out for 141 women who had been referred to a gynecologist of Public Al-Karakh Hospital, most after testing positive by the latex agglutination test. For every patient a full history was taken by a gynecologist of our team covering age, and the fate of every pregnancy, as well as the health of her live-born children. The study comprised three groups: 1) 45 women with 1st spontaneous abortion, aged 18-35 years old. 2) 54 women were suffering from 2nd spontaneous abortion, aged 17-30 years old. 3) The study was also included another 45 healthy pregnant women, aged 18-30 years old. Clinical examination and laboratory investigations were carried out for those with habitual abortion in order to exclude other causes of fetal wastage such as malformation of the genital tract, diabetes mellitus, renal disease or Rhesus incompatibility. All the women examined were interviewed to ascertain sociodemographic, medical and obstetric information. This study was conducted during the period Dec. 2008-Jun 2009.

Serum samples: Sera were separated by blood centrifugation at 3000 rpm for 5 min. samples were stored at -20°C until needed. Blood glucose level testing was done using Biomerieux-laboratories kit.

Serological tests: For all the participants, the Toxoplasma specific IgG antibodies were studied by (ELISA) technique with a set of reagent Biochek.

Statistical analysis. Results are expressed as the mean \pm SD. The significance of differences was assessed by Students *t*-test for groups of non-paired observations. Association of serum toxoplasmosis with several variables-including patient characteristics, management of complications of hyperglycemia with toxoplasmosis was tested using Pearson's Correlation Coefficients. Differences were considered significant if $P < 0.05$. In addition frequency and ratio occurrences of each group were calculated. All the statistical analyses were performed by SPSS version 16 software.

RESULTS

The major characteristics of the habitual abortion group and the normal pregnancy group are compared in Table 1 which refers that the prevalence rate of 1st abortion (**73.4%**) and 2nd abortion (**55.5%**) in our groups occur around **19-21** old ages. Also we found that **33.3%** of the 1st abortion group lost their babies after **12** weeks of pregnancy, while the abortion time of 2nd group occur after week **8th**. In addition, **66.6%** of 1st group and **55.5%** of 2nd group have no baby, but **33.3%** and **38.8%** respectively, have one healthy baby.

Out of **99** patients with habitual abortion, **63 (63.4%)** had a positive IgG higher than normal values, Table 2. Among the normal pregnant group, **18** out of **45** women (**40%**) had a positive IgG. Analysis of toxoplasma antibody prevalence in relation to abortion history at presentation revealed that the prevalence rate increased with a greater number of previous abortions 1st abortion group had a mean of IgG = **20.33 ± 4.9**, the 2nd abortion group IgG = **24.00 ± 6.5** when compared with normal group IgG= **19.83 ± 4.0**, however, all the difference is statistically non-significant ($p > 0.05$), Table 3. The levels of F.B.S of different toxoplasmosis groups are illustrated in Table 4, in which we notice that the F.B.S of 2nd abortion patients is significantly higher than normal for positive anti-IgG (**108.10 ± 18.0, 60% vs. 96.66 + 1.66, 40%, p < 0.03**), but the difference with negative IgG corresponding to the normal pregnant women was non-significant (**99.16 ± 16.25 vs. 99.66 ± 13, p > 0.05**), Table 4.

Table 1 : Characteristic of women included in the study

| Characteristic | 1 st abortion group | | | 2 nd abortion group | | | Normal pregnancy group | | |
|-------------------------------|--------------------------------|-------|------|--------------------------------|-----|------|------------------------|-----|------|
| | Mean \pm s | No. | % | Mean \pm s | No. | % | Mean \pm s | No. | % |
| Age (years) | | | | | | | | | |
| 18-23 | 20.71 \pm 1.7 | 33 | 73.4 | 19.63 \pm 1.5 | 30 | 55.5 | 20.22 \pm 1.8 | 33 | 73 |
| 24-29 | 26.55 \pm 3.5 | 6 | 13.3 | 26.20 \pm 1.8 | 15 | 27.7 | 25.00 \pm 1.0 | 9 | 20 |
| 30-35 | 35.00 \pm 0.01 | 6 | 13.3 | 30.60 \pm 0.6 | 9 | 16.6 | 30.00 \pm 0.02 | 3 | 6.6 |
| Total | | 45 | 100 | | 54 | 100 | | 45 | 100 |
| Abortion time (weeks) | 14 th | | | 11.6 th | | | | | |
| 8th | | 9 | 20 | | 18 | 33.3 | | 9 | 20 |
| 12th | | 15 | 33.3 | | 12 | 22.2 | | 6 | 13.3 |
| 16th | | 14 | 26 | | 15 | 27.7 | | 15 | 33.3 |
| 20th | | 9 | 20.3 | | 9 | 16.6 | | 15 | 33.3 |
| Total | 20 th weeks | 45 | | | 54 | 100 | | 45 | 100 |
| Healthy born baby(No.) | | | | | | | | | |
| None | | 30 | 66.6 | | 30 | 55.5 | | 36 | 80 |
| 1 | | 15 | 33.3 | | 21 | 38.8 | | 6 | 13.3 |
| 2 | | ----- | | | 3 | 5.5 | | 3 | 6 |

Table 2 : Results of toxoplasmosis tests in different groups of women

| Toxo –test | Habitual abortion | | | Normal pregnancy group | | |
|---------------------|-------------------|-----|------|------------------------|-----|-----|
| | Mean \pm s | No. | % | Mean \pm s | No. | % |
| Positive IgG | 22.16 \pm 5.7 | 63 | 63.4 | 19.83 \pm 4.0 | 18 | 40 |
| Negative IgG | 6.57 \pm 2.4 | 36 | 36.4 | 7.37 \pm 1.9 | 27 | 60 |
| Total | | 99 | 100 | | 45 | 100 |

Table 3 : Frequency of toxoplasmosis antibodies in relation to a history of previous abortion

| Toxo -test | 1 st abortion group | | | 2 nd abortion group | | | Normal pregnancy group | | |
|--------------|--------------------------------|-----|------|--------------------------------|-----|------|------------------------|-----|----|
| | Mean± s | No. | % | Mean± s | No. | % | Mean± s | No. | % |
| Positive IgG | 20.33±4.9 | 24 | 53.3 | 24.00±6.5 | 39 | 72.2 | 19.83±4.0 | 18 | 40 |
| Negative IgG | 7.40±1.9 | 21 | 46.6 | 5.75±2.8 | 15 | 27.7 | 7.37 ± 1.9 | 27 | 60 |
| Total | | 45 | | | 54 | | | 45 | |

Table 4 : Results of F.B.S in different toxoplasmosis groups of women against normal group

| F.B.S mg/dl | 1 st abortion group | | | | | 2 nd abortion group | | | | | Normal pregnancy group | | | |
|----------------|--------------------------------|----|-----|-------|--------------|--------------------------------|----|------|--------|--------------|------------------------|-----|-----|--------------|
| | Mean± s | No | % | p | r | Mean± s | No | % | p | r | Mean± s | No. | % | r |
| Positive IgG | 108.77±32.6 | 27 | 60 | >0.05 | 0.015 N.S | 108.10±18.0 | 39 | 72.3 | <0.03* | 0.103 N.S | 96.66±1.6 | 18 | 40 | 0.576 N.S |
| Negative IgG | 99.16±16.2 | 18 | 40 | >0.05 | | 107.00±19.2 | 15 | 27.7 | >0.05 | | 96.66±13.0 | 27 | 60 | |
| Total | | 45 | 100 | | | | 54 | 100 | | | | 45 | 100 | |

* = indicate a significant value , N.S = non-significant

DISCUSSION

The results of this study, based on serological tests, confirmed our expectation that spontaneous abortion is indeed endemic in Baghdadi women, and that a substantial proportion of the referred patients **73.4 %** of 1st time, and **55.5 %** of 2nd time aborted women occur in age (**18-23**) , while **13.3%** of 1st time , and **27.7%** of 2nd time abortion group occur around (**24-29**) years age respectively, and the abortion is stabilized at **13.3-16.6%** in all the older age classes ,(Table 1). These results give a clear indication that there is no association between age and related abortion factors. Our results are therefore in a good agreement with the results of other studies which showed no apparent association between *Toxoplasma* infection and age of the pregnant women (14, 15). Previous findings in Ghana reported prevalence of 67.9% and 82.1% of toxoplasmosis in pregnant women aged 15-25 and 31-40 years, respectively (16).

Toxoplasmosis commonly occurs in adults, usually in a mild or asymptomatic form. It can cause fetal infection if it is acquired during pregnancy, with unpredictable manifestation in the fetus and neonate (17). Therefore, the detection of specific antibodies in the patient's serum is considered the proper method to the diagnosis of toxoplasmosis (18).

In our study, all **141** women tested by latex agglutination test were positive for *T.gondii* antibodies (which detect anti-*T. gondii* IgG only but not differentiate between recent and past infection) and were then referred to the laboratory in which the specific anti-*toxoplasma* IgG antibody test was done using ELISA. Recently diagnosis had been done traditionally by detecting IgM or IgG antibodies or both (19, 20), IgM antibodies are considered to reflect active current\ recent infection (21). There is another study revealed that IgG is five times more common than IgM antibodies where IgG antibodies reflecting earlier infection with *T.gondii* in Qatar(22), therefore this study is depending on the value of positive and negative IgG antibodies mainly.

It should be noted here that we prefer to use ELISA technique rather than other techniques because it is a laboratory method for the diagnosis of acute acquired *Toxoplasma* infection. In 1980, Noat and coworkers used three tests for the diagnosis of *Toxoplasma* serum antibodies (Dye test, IFA and ELISA). They found that chronic phase gave negative results with Dye and IFA tests, while it gave positive results with ELISA test, accordingly they concluded that ELISA is more sensitive than IFA test (23). In addition, Paul and coworkers have been confirmed in their study the usefulness of ELISA as a toxoplasmosis diagnostic test and they suggest that ELISA is a valuable method for the diagnosis of congenital toxoplasmosis (24).

It has been found from different studies that the prevalence and risk factors for the transmission of *T.gondii* vary substantially between countries and geographic regions. However, in the middle east, the toxoplasmosis infections have been reported in cats and / or people Egypt (25), Jerusalem (26), Lebanon (27), Saudi Arabia (28), Qatar (22) and in Iran (29).

The serological data of the present work of 1st time aborted women, indicate the incidence of toxoplasmosis by **53.3%**, while in the 2nd time abortion the incidence was **72.2%**, detecting by ELISA (Table 3) , however , *T.gondii* infection did not significantly (**p>0.05**) affect the frequency of spontaneous abortions. Previous works by a number of researchers showed similar observations which conclude that the contribution of toxoplasmosis to abortion is greatly overestimated and the true prevalence of active toxoplasmosis among aborted women is much lower than previously thought (13, 30-33). It was found that these findings are not consistent with other studies which found a clear association of toxoplasmosis to abortion (34-37).

All samples were handled in a similar manner, and there was no significant correlation between positive IgG and the corresponding blood glucose level in all groups of this study (**r = 0.015-0.103, p > 0.1**). Blood glucose level in those that secondly aborted group were significantly higher than normal group of positive IgG (**108.10± 18.08, 39% vs 96.66 ± 1.66, 40%, p<0.03**), while F.B.S of toxoplasmosis in the 1st abortion group was only insignificantly

higher than normal (**108.77± 32.6, 60% vs 96.66 ± 1.66, 40%**), Table 4 . Similar results have been reported by another previous studies (4, 38), and could be explained by the indication of the world health organization study (39) which explained that transplacental haemorrhage may occur in association with abortions of all types of Diabetes.

Another fact has been noted that all the aborted groups of negative IgG have a nonsignificant increasing level of F.B.S than normal group (**99.16 ± 16.25, 40% and 107.00 ± 19.23, 27.7% vs 96.66 ± 13, 60% respectively, p>0.05**).

Finally, our results have highlighted that diabetic control is poor and suggest that a number of spontaneous abortions may be due to this factor rather than affection by toxoplasmosis for the same group of aborted women. Such efforts could have good benefit to reducing spontaneous abortion in this group of patients.

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