Detection of *B1* gene of *Toxoplasma gondii* in blood of pregnant and abortive women infected with this parasite.

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Abstract

Background: Primary maternal infection with toxoplasmosis during gestation and its transmission to the fetus continue to be the cause of tragic yet preventable disease in offspring.

Objective: This study was aimed to investigate the utility of nested PCR (nPCR) technique for detection recent infection with *Toxoplasma gondii* in blood of pregnant and abortive women.

Methods: One hundred twenty women were included in this study with a history of single or repeated abortion and thirty women with normal pregnancy were used as a control. Blood samples were tested for specific anti-*Toxoplasma* IgM and IgG antibodies by an enzyme-linked immunosorbent assay (ELISA) and detection of *B1* gene of *T. gondii* by nPCR.

Results: The results indicated that 43.33% of abortive women were exposed positive for anti-*Toxoplasma* antibodies, 4.16% of them had IgM, 25.83% had IgG, and 13.33% had both IgM and IgG, and 56.55% had no antibodies. Subsequently, nested PCR analysis was used to detecting *T. gondii* DNA in blood of abortive women. It was found that 15.83%of abortive women exposed positive result for *B1* gene of *T. gondii*, those abortive women involved 10.52% of them with IgM, 31.57%with IgG, and 26.31% with both IgM and IgG, and 31.57% of them had none anti-*Toxoplasma* antibodies.

Conclusion: It can be concluded that nPCR assay in blood has advantage in detection of recent and active toxoplasmosis.

Key Words: *Toxoplasma gondii*, nested PCR, toxoplasmosis.

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Introduction

Toxoplasma gondii is an obligate intracellular protozoan responsible for common parasitic infections throughout the world; it can invade and multiply in the nucleated cells of virtually all worm blooded animals including humans, mammals and birds, with members of the cat family being the only known hosts for the sexual stage of *T. gondii* infection [(1)].

In general, *T. gondii* infections are asymptomatic and self-limiting especially among healthy immunocompetent hosts; however the infection may cause severe complications in pregnant women and immunocompromised patients [(2, 3)]. Fetal toxoplasmosis, particularly in early pregnancy can cause miscarriage, stillbirth, and birth defect. Infected babies may not develop any disease, or they may experience serious damage to the brain and eyes, to degree depending on the gestational age [(4)].

Direct methods to demonstrate the presence of the parasite in tissues or body fluids, are more useful. Whereas, radiological findings, histology, tissue culture, or inoculation into mice are difficult, time consuming, or impractical assays.

Diagnosis of *T. gondii* is routinely based on serological tests. Polymerase chain reaction (PCR) is used for direct detection of parasites in the tissues or other fluids.Lamoril *et al*. (1996) [(5)] used this technique to diagnose toxoplasmosis

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in immunocompromised and pregnant women.

Recently, researchers [6, 7] illustrated that the serological diagnosis of recent infection in early pregnancy could be confirmed by a positive *Toxoplasma* specific PCR result from blood samples in the first half of pregnancy. However, the reliability of the PCR is a crucial technique to evaluate the prevalence of *Toxoplasma* reactivation when the detection of circulating DNA is the only clue to its reactivation [8, 9].

B1 gene is highly conserved in all *T. gondii* strains [10, 11]. Approximately 35 copies of this gene are presented in the *T. gondii* genome, but absent in the other mammalian cells [10].

Thus, the aim of the present study was to determination the relationship between serological and molecular methods for detection of *T. gondii* in blood of abortive women.

Methods

- ***Study groups:***

The cases were collected during the period from 1st April to the end of November 2008 in the central Health Laboratory in Baghdad. They were included 120 women, who had spontaneous abortion; whom referred with a physician report for TORCH tests to determine the final diagnosis of pregnancy loss. In addition, 30 healthy women with a history of a normal pregnancy were attended to the outpatient clinics for routine gynecologic checking was considered as control. Relevant information about the cases and the possible risk factors were recorded for each woman. Any woman undergoing therapy against the disease was excluded from the study.

- ***Serological test***

The sera of all cases were tested for the presence of specific IgM and IgG anti-*Toxoplasma* antibodies via ELISA kits (Biokit Diagnostics Company, Spain) according to the manufacture's instructions.

- ***Isolation of genomic DNA from whole blood:***

DNA was extracted from the whole blood samples of the study groups using a commercial purification system (Wizard Genomic DNA purification kit, Promega, Madison, WI) following the manufacture's instruction for DNA purification from blood. Purified DNA molecules were resuspended in 100 µl of DNA rehydration solution (TE buffer) (10 mM Tris-HCL, 1mM EDTA, pH=8) and stored at -80°C, after estimation of DNA concentration and purity, thereafter they were separated by agarose gel electrophoresis.

- ***Amplification and detection of T-gondii DNA by Nested PCR (nPCR) assay.***

According to Burge *et al*. (1989) (10) method, nested PCR was performed for all DNA samples to amplify a fragment of *B1* gene by two steps with different primer pairs as follows:

First round

The primers used in the first round correspond to nucleotides 694-714 and 887-868 with the sequences: 5'-GGAACTGCATCCGTTCATGAG-3' and 5'-TCTTTAAAGCGTTCGTGGTC-3' (Promega, USA) respectively. These primers used to amplify 193bp of *B1* gene. 3µl of DNA template were added to a final reaction volume equal to 25 µl consisting of 2.5 µl PCR buffer (10 mM Tris-HCL, 50 mM KCL), 1 µl $MgCl_2$, 1 µl dNTPs mix, 2 µl for each primer, 0.25 µl Taq polymerase (5000 U/ml) and 13.25 µl of H_2O (nuclease free).A negative control reaction in each experiment was set up containing all components of the reaction except

template DNA.The cycling condition for the first round were 94°C for 2 minutes, followed by 40 cycles at 94°C for 10 seconds, 57°C for 10 seconds and 72°C for 30 seconds and a final extension at 72°C for 5 minutes.

Second round

Nested reaction was performed to amplify 96 bp of *B1* gene by using the primers which correspond to nucleotides 757-776 and 853-831 with the sequences 5'-TGCATAGGTTGCAGTCACTG-3', 5'-GGCGACCAATCTGCGAATACACC-3'respectively.

Three microliters of the first round products were used as a template for the second round PCR with the same components of the master mix in a total volume of 25 µl. Negative controls of sterile water were included in the nested reaction. The cycling condition for nPCR were 94°C for 2 minutes, followed by 40 cycles of 93°C for 10 seconds, 62.5°C for 10 seconds and 72°C for 30 seconds, and a final extension at 72°C for 5 minutes.PCR product was analysed on 2% agarose gel at 5 v/cm for 2 hours and stained with ethidium bromide.

Statistical analysis

Data were analysed with chi-square and P value ≤ 0.05 was considered statistically significant.

Results

Figure (1) indicate that 43.3% of abortive women were significantly (P ≤ 0.05) exposed positive for anti-*Toxoplasma* antibodies (IgM and IgG) in comparison with normal pregnant women 20%.

The level of specific anti- *Toxoplasma* antibodies was determinate. Figure (2) shows that 4.16% of abortive women revealed a positive result for IgM in comparison with non IgM in sera of normal pregnancy. IgG antibodies were seen in 25.83% of abortive women in comparison with 20% IgG in normal groups. While, IgM and IgG levels were formed 13.33% and 0% in abortive women and normal pregnancy respectively. Whereas, no antibodies were detected in 56.55% of abortive women and 80% of normal group.

Besides the serological diagnosis of *T. gondii* nested PCR (nPCR) technique was used to confirm the infection with *T. gondii* by detection of *T. gondii* DNA in the blood of abortive women. *Toxoplasma gondii* DNA was successfully extracted and analyzed by nPCR. It was noticed that 15.83% of abortive women had *T. gondii* DNA in their blood if compared with normal group who showed no toxoplasmosis (0%).Whereas, the negative nPCR analysis was significantly (P≤ 0.05) formed 84.16% in abortive women when compared with control group (100%). Moreover, the DNA products from the first and second round of nPCR analysis indicated that the abortive women had B1 gene of *T. gondii* with 193 bp and 96 bp in the first and second round of nPCR respectively (Figure 4).

On the other hand, 15.83% of abortive women who gave positive in molecular diagnosis were serologically detected. The results were significantly (P ≤ 0.05) showed that 10.52% of positive nPCR had IgM, 31.57% had IgG, 26.31% had IgM and IgG, and 31.57% had no antibodies in comparison with negative nPcR analysis (Figure 5).

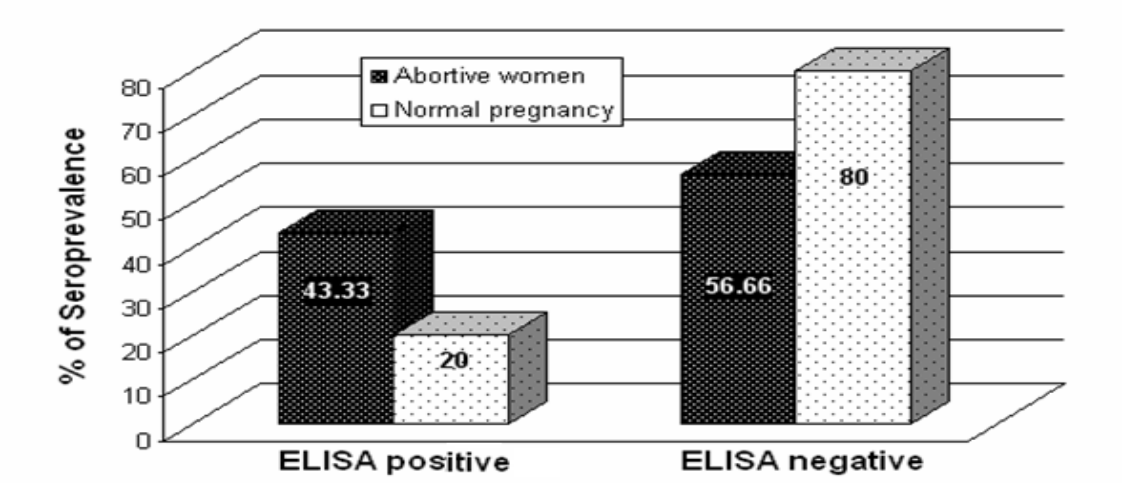

Figure 1: Seroprevalence of anti-*Toxoplasma* antibodies by ELISA in the abortive women and normal pregnancy.

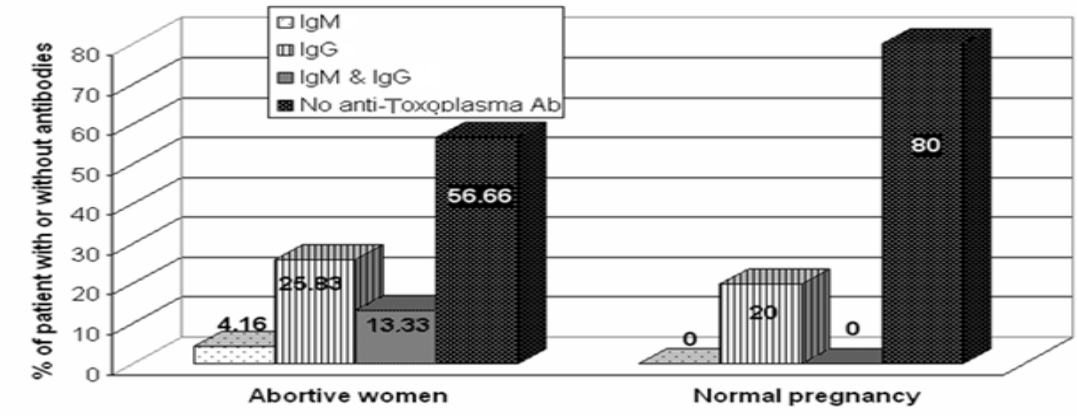

Figure 2: level of IgM and IgG antibodies detected by ELISA in abortive women and normal pregnancy.

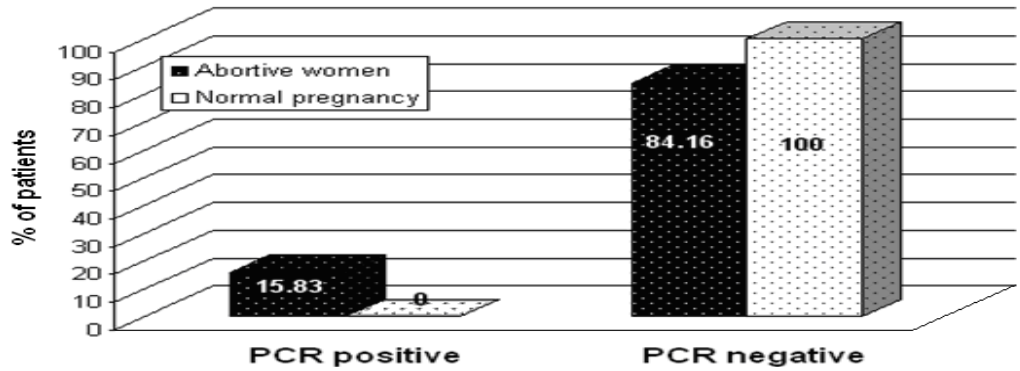

Figure 3: nested PCR analysis in the abortive and normal pregnancy groups.

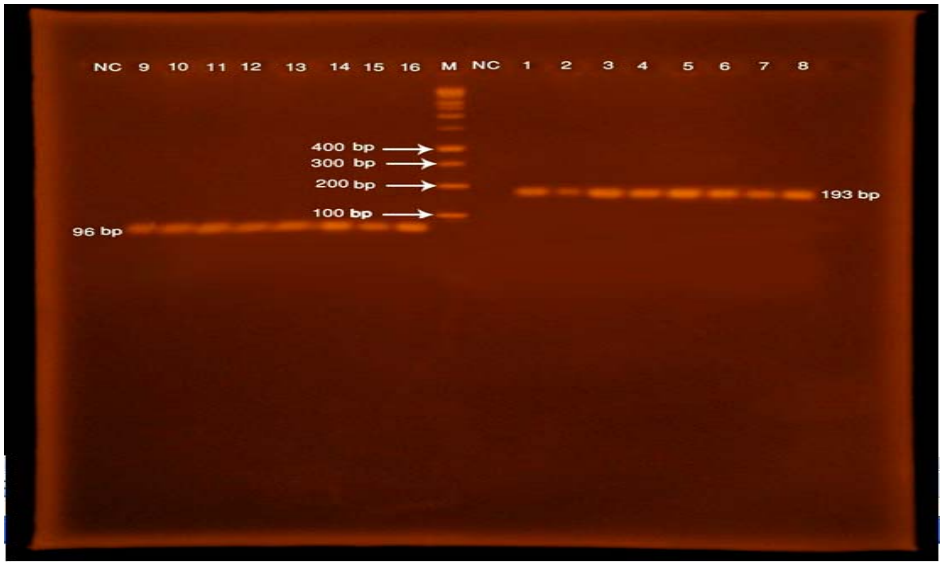

Figure 4: Amplification of *B1* gene of *Toxoplasma gondii* DNA from the blood of the infected abortive women. Lane-M, molecular weight marker (100 bp ladder), Lanes-NC negative control, Lanes 1-8 positive samples at 193bp in the first round of n-PCR , Lanes 9-16 positive samples at 96 bp in the second round of n-PCR. Running conditions: Agarose gel (2%), 5 v/cm for 2 hrs, stained with ethidium bromide.

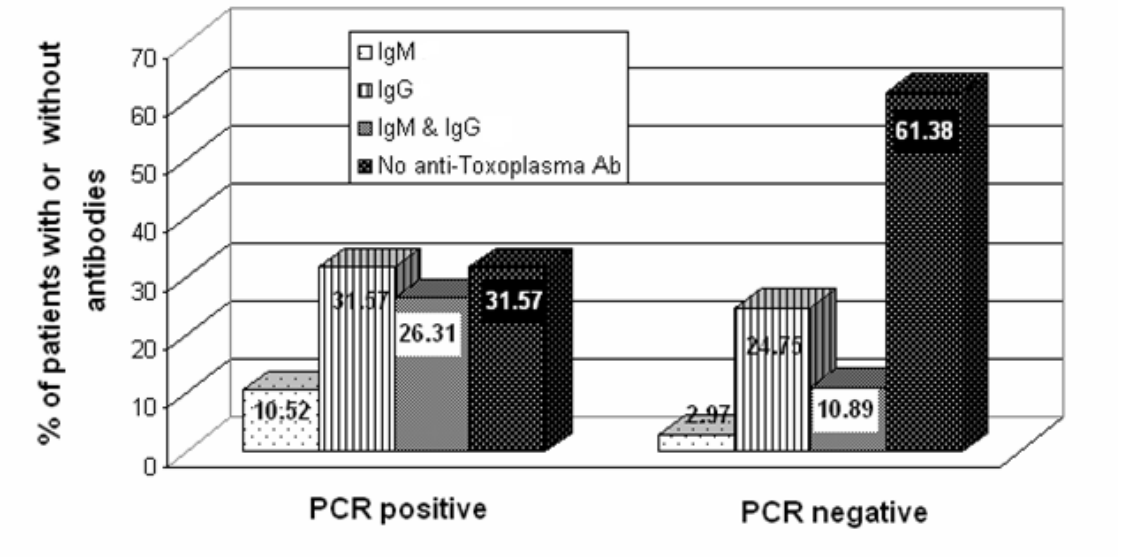

Figure 5: Nested PCR analysis distributed according to anti-*Toxoplasma* antibodies in abortive women.

Discussion

The most likely sources of human toxoplasmosis are ingestion of lightly cooked meat containing live *T. gondii* tissues cysts, ingestion of vegetable or fruits contaminated with oocysts derived from cat faeces that may be encountered in gardens. Toxoplasmosis is also recognized to be a water-borne zoonosis [12, 13].

High prevalence of *Toxoplasma* infection in women may be attributed to the socio-economic status, the unhygienic environment and low level of education in which they resides [14, 15].

It's rare that a woman who got toxoplasmosis before getting pregnant will pass the infection to her fetus, because she will have built up immunity to the infection. It can occur through, if a pregnant woman who's had a previous infection becomes immunocompromised and her infection is reactivated [4]. In the current study the presence of signification rates of anti-*Toxoplasma* antibodies are considered as indirect evidence of the organism being the cause of bad obstetric history in women of reproductive age group. These results are in agreement with [16, 17].

Although serological testing has been one of the major diagnostic for toxoplasmosis, it has many limitations; it may fail to detect specific anti-*Toxoplasma* IgM or IgG during the active phase of infection, because these antibodies may not be produced until after several weeks of parasitemia. Furthermore, the test may fail to detect *T. gondii* infection in certain immunocompromised patients due to the fact that the titers of specific anti-*Toxoplasma* antibodies may fail to rise in this type of patient.

Indeed detection of *T. gondii* DNA using nPCR minimizes the problems faced when using serodiagnostic assays and facilitates diagnosis in difficult cases.

Therefore, the negative results obtained by both PCR and ELISA rule out an infection in abortive women.

The fact that could explain the proportion of 2.97%of abortive women that revealed negative PCR results and positive IgM is the probability to find patients with residual IgM detected during prolonged periods of time. Also false positive IgM tests results have been reported previously [18].

Toxoplasma DNA was found in 31.57% abortive women with positive IgG indicates chronic infection since it is knows that patients with latent toxoplasmosis present intermittent parasitemia with low parasite burden.

Toxoplasma DNA was detected in 31.57% seronegative abortive women; it could correspond to a very recent infection at the time of serological leading to an insufficient production of immunoglobulin not detected by serology, or other explanation that those patients are not able to produce specific antibodies, representing a state of immunodeficiency.

Molecular tests that could detect the presence of circulating parasites would be of extreme application in this scenario. A positive serological result is only indicative of infection, whereas direct detection of *T. gondii* in blood or other clinical samples categorically confirms the parasite presence leading to the diagnosis of primary, reactivated or chronic toxoplasmosis [19].

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References

1. Dubey JP. *Toxoplasma, Hammonida,* Besnotia, Sarcocystis, and other tissue cyst forming coccidia of man and animal. In: Kreier, J.P. editor. Parasitic protozoa III New York: Academic Press; 1977. p. 101-237.

2. Holland GN, O'Connors GR, Belfort Jr. and Remington JS. Toxoplasmosis. In: Peprose, J.S., Holland, G.N. and Wilhelmus, K.R. editors. Ocular infection and immunity. Mosby Yearbook, St. Louis; 1996. p. 1183- 1223.

3. Espinoza L A. Toxoplasmosis. Caribbean AIDS education and training center. HIV primary care guide.2005; Chapter11, section 6:1-4.

4. Montoya JG and Remington JS. Management of Toxoplasma gondii infection during pregnancy. Clinical practice.Cyto. Incl. Dis. 2008; 47: 554-566.

5. Lamoril J, Molina JM, De Gouvello A, Garin YJ, Deybach JC, Modai J and Derouin F. Detection by PCR of Toxoplasma gondii in blood in the diagnosis of cerebral toxoplasmosis in patients with AIDS. J. Clin. Pathol. 1996; 49: 89-92.

6. Nimri L, Pelloux H and Elkhatib L. Detection of Toxoplasma gondii DNA and specific antibodies in high- risk pregnant women. Am. J. Trop. Med. Hyg. 2004; 71(6): 831-835.

7. Iqbal J and Khalid N. Detection of acute Toxoplasma gondii infection in early pregnancy by IgG avidity and PCR analysis. J. Med. Microbiol. 2007; 56: 1495- 1499.

8. Costa JM, Munoz C, Kruger D, Martino R, Held TK, Drade ML, Cordonnier C and Bretagne S. Quality control for the diagnosis of Toxoplasma gondii reactivation in SCT patients using PCR assays. Bone Marrow Transplantation. 2001; 28(5): 527-528.

9. Colombo FA, Vidal JE, De Oliveira A C P, Hernandez AV, Bonasser-Filho F Nogueira RS et al. Diagnosis of cerebral Toxoplasmosis in AIDS patients in Brazil: Importance of molecular and immunological methods using peripheral blood samples. J. Clin. Microbiol. 2005; 43(10): 5044-5047.

10. Burg JL, Grover CM, Pouletty P and Boothroyd J. Direct and sensitive detection of a pathogenic protozoan, Toxoplasma gondii, by polymerase chain reaction. J. Clin. Microbio. 1989; 27(8): 1787-1792.

11. Jones CD, Okhravi N, Adamson P, Tasker S and Linghtman S. Comparison of PCR detection methods for B1, P30, and 18S rDNA genes of T. gondii in aqueous humor. Invest. Opthalmol. Vis. Sci. 2000; 41: 634-644.

12. Qublan H S, Jumaian N, Abu-Salem A, Hamadelil F Y, Mashagbeh M and Abdel- Ghani F. Toxoplasmosis and habitual abortion. J. obst. Gyn. 2002; 22(3): 296-298.

13. Dubey, J.P. Toxoplasmosis- a waterborne zoonosis. Vet. Parasitol. 2004; 126: 57-72.

14. Yasodhara P, Ramalakshmi B A, Lakshmi V and Krishna T P. Socioeconomic status ans prevalence of toxoplasmosis during pregnancy. Indian J. Med. Microbiol. 2004; 22: 241-243.

15. Gollub EL, Leroy V, Gillbert R, Chene G and Wallon M. Effectiveness of health education on Toxoplasma related knowledge, behaviour and risk of seroconversion in pregnancy. Eur. J. Obstet. Gynecol. Reprod. Biol. 2008; 136: 137-145.

16. Mookherjee N, Gogate A and Shah PK. Microbiological evaluation of women with bad obstetric history. Indian J. Med. Res. 1995; 101: 103-107.

17. Kumar A, Arora V and Mathur M. Toxoplasma antibody levels in females with habitual or sporadic abortions and normal pregnancies. Indian J. Med. Microbiol. 2004; 22(4): 276- 277.

18. Emna S, Karim A, Mohammed K and Aida B. Difficulty in dating primary infections by Toxoplasma gondii in pregnant women in Tunisia. Tunis Med. 2006; 24: 85-87.

19. Bastien P. Molecular diagnosis of toxoplasmosis. Trans. R. Soc. Trop. Med. Hyg. 2002; 96: 205-215.