Original Article

Molecular detection of Toxoplasma in women with abortions

Reyam Abdul Khuder Mohammed[1], Ali Alaa Alhashmi[2], Mustafa Salah Hasan[3]
[1]B.V.S, M.Sc. Oral Microbiology, Al-Mustaqbal University College, Hilla, Iraq.
[2]Community Health Nursing Branch, College of Nursing, University of Al-Ameed, Iraq
[3]University of Fallujah, College of Vet. Med.
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Abstract

The current study aims to molecular detection of Toxoplasma in women with abortions. Seventy-five aborted women visited the private clinics between 2021 and 2022. Each female filled out a questionnaire that asked about her age, address, blood type, how many abortions she had, how many cats she had at home, and if she was a carrier. Venous blood samples (5ml) were carefully drawn into EDTA tubes and centrifuged at 300 rpm for 5 minutes to extract the clear serum. The sera were then analyzed to determine the amount of antibodies against T. gondii infection. The Latex Agglutination Test, Cassette IgG\IgM combo rapid test were done for detection of toxoplasmosis. PCR was done for detection of B1 gene that is representative for toxoplasma diagnosis. The current results showed that the positive results of latex agglutination test were significantly higher in results than Cassette IgG\IgM combo rapid. The findings revealed 49 (63.3%) for IgG and 11 (14.7%) for IgM. Out of the 75 pregnant and aborted women who were 34 (45.3%) instances in the study were PCR positive.

In conclusion, PCR was the gold test for diagnosis of toxoplasmosis.

Keywords: Toxoplasma, Molecular, PCR..

INTRODUCTION

According to Dardé and Ajzenberg, the protozoan parasite Toxoplasma gondii causes the disease toxoplasmosis. Toxoplasma is a parasite that thrives in hot, humid climates and may spread to a variety of vertebrate hosts. (Liu et al., 2012)

Numerous vertebrate species, including animals and humans, serve as intermediate hosts, with cats and other members of the Felidae family serving as the ultimate hosts (Knoll and others, 2019). (Roberts and Janovy ,2005). The majority of congenital Toxoplasma abnormalities (2% to 3%) are caused by parental infections (Ocak and other, 2007).

The parasite is transmitted to humans either by undercooked, raw meat consumption (Dubey and Beattie, 1988), blood transfusions and organ transplants, or oocyst intake from contaminated food or water (Dubey and Jones,2008). The chance of having feces on your hands is quite high (Torrey, 2007).

The primary maternal T. gondii infection during pregnancy is often linked to the fetus being exposed to it (Pappas, 2009).

According to Monteya and Remington (2008), the rate of transmission of maternal infection to the fetus is estimated to be around 45%, of which 60% are subclinical infections, 9% result in fetus death, and 30% have serious consequences like intracerebral calcification, hydrocephalus, chorioretinitis, and mental retardation. IgG and IgM antibodies are often found during the laboratory diagnosis of acute and latent toxoplasmosis.

The identification of antibodies against Toxoplasma gondii in pregnant women has been done using serological techniques, including the LATEX agglutination test, indirect fluorescent antibody test (IFA), ELISA (Hajsoleimani, 2012), and hemagglutination test (Kadir, 2012).

High Toxoplasma gondii levels are among the illnesses that may cause people with acquired immunodeficiency syndrome (AIDS) die syndrome (Luft and Remington ,1992).

The current study aims to molecular detection of Toxoplasma in women with abortions.

Materials and Methods:

Seventy-five aborted women visited the private clinics between 2021 and 2022. Each female filled out a questionnaire that asked about her age, address, blood type, how many abortions she had, how many cats she had at home, and if she was a carrier.

Venous blood samples (5ml) were carefully drawn into EDTA tubes and centrifuged at 300 rpm for 5 minutes to extract the clear serum. The sera were then analyzed to determine the amount of antibodies against T. gondii infection.

The Latex Agglutination Test should be used in accordance to manufacturing instructions. Toxoplasma antibodies in serum may be determined qualitatively and quantitatively using the one-step quick LATEX particle agglutination test on a slide. The basic way of testing for positive results with latex is when agglutination droplets are seen on the slide.

Cassette IgG\IgM combo rapid test: IgG and IgM anti-Toxoplasma gondii antibodies may be quickly and easily distinguished from one another in human serum, plasma, or whole blood with this quick test.

In this work, we use the PCR to several female samples in the manners listed below:

PCR is a method used to quickly multiply copies of a particular DNA sequence.

DNA extraction from entire blood using a commercial small kit from Geneaid.

Half gm of Agarose powder, 50 ml of X1 TBE, and 3 ml of red safe are required to create Agarose gel with a concentration of 1%. Use a heating source while stirring to bring the mixture to a boil, then let it to cool between 25 and 38 degrees.

After attaching the specific comb to create wells in the margins of the gel, the gel solution is poured into the tray during electrophoresis.

All of the research samples' DNA concentrations were adjusted to (50) Nano g/L by diluting with TE buffer solution for the PCR process.

Prepare the Master reaction mixture for each sample for each PCR reaction using the relevant gene primer and master mix in a side Eppendorf of 0.2 ml.

Utilizing the particular protocol for each reaction, make the mixture in the microfuge for 5–3 seconds before adding the reaction tubes to the thermocycler to create the polymerase reaction.

carried sample in previously prepared 1% concentration Agarose gel wells. Add the ladder DNA to one well after that.

Amplification of the Toxoplasma gondii B1 gene was carried out using two primers with the following sequences in blood samples from pregnant and aborted women.

F: TTTTGACTCGGGCCCAGC

R: GTCCAAGCCTCCGACTCT

Place the tubes in the thermocycler device to start the reaction as directed by its unique program:

Stage	Temperature (c)	Time	Cycle
Initial denaturation	90	7 min.	
denaturation	90	50 Sec.	

Annealing	58	1 min.	35
Extension	73	1 min.	
Hold	4	5 min.	

Statistical analysis was done by using SPSS software.

Results and Discussions:

The current results showed that the positive results of latex agglutination test were significantly higher in results than Cassette IgG\IgM combo rapid (Table 1).

Table 1. Serological tests for detection Toxoplasmosis

Test	**Number of positive**	**Percentage**
Latex	65	86.7 A
IgM	11	14.7 C
IgG	49	65.3 B

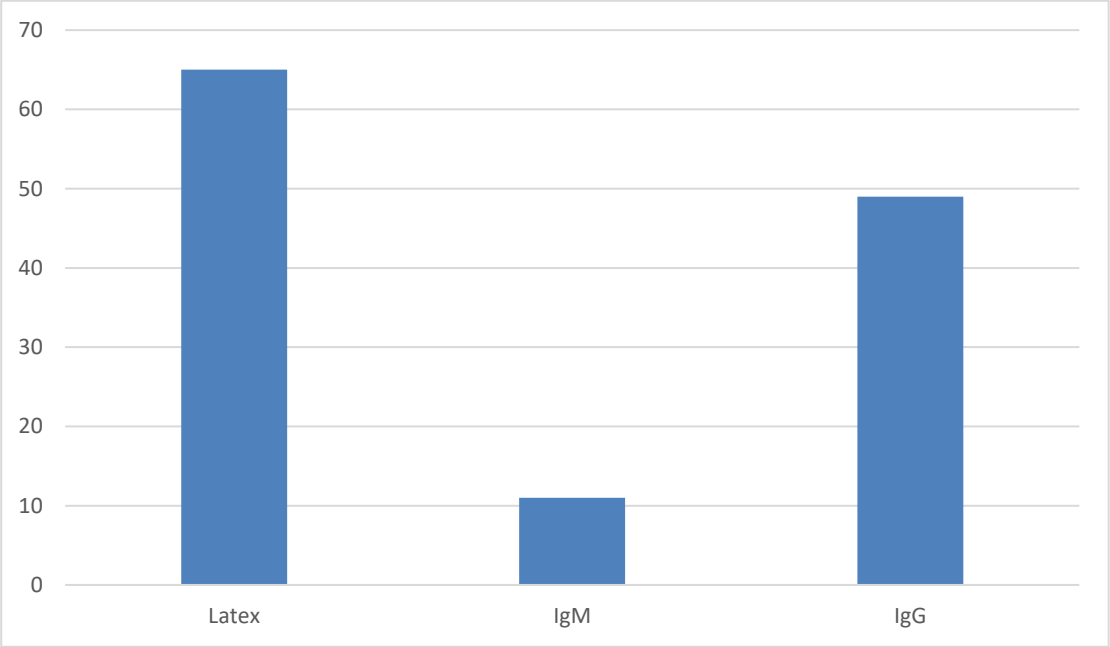

Figure1. Serological tests for detection Toxoplasmosis

The body initially developed detectable IgM antibodies in the blood one to two weeks after infection; a few months later, the IgM became undetectable and was replaced by IgG antibodies that would be present for a longer duration. The findings revealed 49 (63.3%) for IgG and 11 (14.7%) for IgM.

If the infection is persistent or renewed, the IgM antibodies could resurface. Because there is a considerable chance (30–40%) of passing the illness to developing babies, which may result in a variety of complications or even death, toxoplasmosis detection is especially crucial during pregnancy. The findings of this research agreed with those of a survey conducted in New Zealand in which 500 women who had had abortions underwent ELISA testing. 500 women were examined, and it was discovered that 2.5% and 33% of them had anti-toxoplasma antibodies, IgM and IgG, respectively (Morris and Croxson, 2004). This study's findings on IgG antibodies were consistent with those from Cameroon, where 100 pregnant women were tested using an ELISA and 70% of them had positive IgG results, indicating high rates of prior infection. This demonstrated that a high prevalence of toxoplasma infection may also be related to geography, poor hygiene, low education levels, an increase in stray cats, and low socioeconomic position (Mahmood et al., 2010; Al-Taie, 2011).

The findings made it abundantly evident that the molecular approach, which deals with particular Toxoplasma genomic DNA, is characterized by great sensitivity and specificity with no possibility for false positive or false negative results. As a result, the PCR test is regarded as the gold standard for diagnosis.

Out of the 75 pregnant and aborted women who were 34 (45.3%) instances in the study were PCR positive (Figure 2).

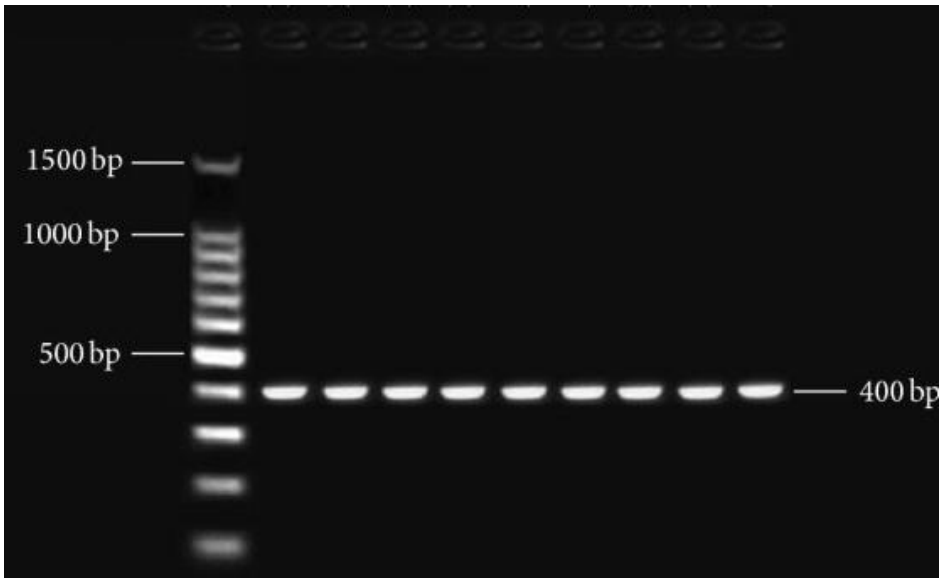

Figure 2. T. gondii diagnosis using gel electrophoresis analysis of PCR products with 400 bp for B1 gene identification and M: 100 bp DNA ladder size marker.

The findings of the present research were agreed with those of a study conducted in Saudi Arabia in which 137 pregnant and aborted women were evaluated by PCR for BOH (poor obstetric history). They demonstrated that 42% of the patients were positive for PCR (Dajem and Al-Mushait, 2012), while 45.3% of the participants in our study had PCR-positive results. Also our results were in agreement with results of AL-SAEED et al., 2016 who revealed that 41% of the cases were PCR positive.

Regarding the PCR test, this study's findings differ from those of a study conducted in Shiraz, Iran (Asgari et al.,, 2013), which found that 14.4% of the 542 pregnant and aborted women tested positive for the test, whereas the results of the current study showed that 45.3% of pregnant and aborted women tested positive.

Conclusion:

The findings made it abundantly evident that the molecular approach, which is distinguished by great sensitivity and specificity with no possibility for false positives or negatives, is the consequence of dealing with particular genomic DNA of Toxoplasma.

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