

Molecular Testing for Toxoplasma Diagnosis in Aborted Fetuses- Taleghani Maternity Hospital- Arak- Iran

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Abstract

Background: The diagnosis of toxoplasmosis is most critical in pregnant women who acquire infection during gestation and also in fetuses and newborns who are congenitally infected. This study described the performance of molecular and confirmatory serologic testing for toxoplasma infection in the tissues of human spontaneous aborted fetuses and their mothers' blood.

Material and Methods: 87 random samples from the tissues of body of spontaneous aborted fetuses (less than 14 weeks) in a separate container of preservative solution were collected from the delivery room of the university maternity hospital, Arak- Iran, during autumn 2012 to 2013. In the ward, 3 ml of blood sample of their mothers were collected and the sera were separated and analyzed by ELISA method for the detection of specific IgG. DNA extraction from the tissues of fetuses was performed and stored until use. The PCR reaction was performed by a pair of primers. PCR products were analyzed by electrophoresis and stained with safe stain. It is necessary to mention first that the written consent was obtained from their mothers and after recovery, a demographic questionnaire was completed.

Results: Most of the mothers were 20-29 years of age and the correlation between the location of residence, contact with cats and eating undercooked food with abortion, not significant. Serological tests on the sera of 87 mothers for anti-Toxoplasma IgG showed 39.08% positive results. The results of PCR amplification showed that none of the 87 samples from aborted fetuses were infected with *Toxoplasma gondii*.

Conclusions: In aborted fetuses, we did not observe any evidence of *Toxoplasmosis* and it appears that *Toxoplasma gondii* was not the cause of spontaneous abortion in this area of Iran but considering the importance of the infection during pregnancy, the control measurements during pregnancy is required.

Keywords: *Toxoplasma gondii*; Abortion; Fetus

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Introduction

If women become infected with *T. gondii* during pregnancy, the parasite can cause abortion or seriously damage the fetus (1). The risk of intrauterine infection of fetus depends on the time of maternal infection during pregnancy, the immunological competence of mother during parasitaemia, the number and virulence of parasites transmitted to the fetus, and the age of fetus at the time of transmission. If not treated, the risk of

intrauterine infection of the fetus increases during pregnancy (2, 3). The transmission risk of parasite to the fetus increases with increasing gestational age. But the disease severity and harms for infants are greater in early pregnancy (4). Toxoplasmosis causes abortion, but abortion due to toxoplasmosis is unclear in many areas where acquired toxoplasmosis is high. The risk of *Toxoplasma* infection in healthy pregnant women and reactivation of latent toxoplasmosis in

infected pregnant women is high. The prevalence of toxoplasmosis in pregnant women is about 38% (based on IgG anti-toxoplasma) in Arak City, so abortion due toxoplasmosis may be significant in this region (5). In the present study, the role of *Toxoplasma gondii* in abortion in human was evaluated by molecular method and confirmatory serologic testing in Arak city (central province).

DNA detection is faster than isolation and can be considered as a useful technique for diagnosing pathogens such as *Toxoplasma gondii* (6).

Materials and Methods

87 random samples from the tissues of body of spontaneous aborted fetuses (less than 14 weeks) were studied. Samples in a separate container of preservative solution were collected from the delivery room of the university maternity hospital, Arak- Iran during autumn 2012 to 2013.

At first, written consent was obtained from their mothers and after recovery, a demographic questionnaire was completed. In the ward, 3 ml of blood sample of their mothers were collected and sera were separated by centrifugation at room temperature. These sera were tested by ELISA for the detection of specific anti-Toxoplasma IgG. The ELISA kits were provided by Pishtaz Teb CO. LTD., Iran. The procedure was performed according to the manufacturer's instructions.

The DNA extraction was performed by DNA extraction kit. This kit was provided by STRATEC Molecular (former Invitek GmbH), Germany. The procedure was performed according to the manufacturer's instructions. The extract DNA was stored at 4 °C until use. The PCR reaction was performed by a pair of primers:

TOX4 (5'-CGCTGCAGGGAGGAAGACGAAAGT TG-3') TOX5 (5'-CGCTGCAGACACAGTGCATCT GTT-3')

The target of PCR was the 529 bp fragment of the highly conserved 35 fold repetitive B1 gene (AF179871). The final reaction volume was 25 µl. The amplification was performed for 5 min at 94 °C initial step, followed by 35 cycles: denaturation for 30 s at 95 °C, annealing for 30 s at 58 °C and extension for 30 s at 72°C. PCR products were analyzed by electrophoresis in 1.5% agarose gel and stained with safe stain.

Results & Discussion

Serological tests on the sera of 87 mothers showed that 39.08% of them had anti-Toxoplasma antibody. The demographic characteristics of mothers who had abortion are shown in Table 1.

The results of PCR amplification showed that none of the 87 samples from aborted fetuses were infected

with *T. gondii* (Figure 1).

In the current study, the main objective was to determine the toxoplasmic infection in aborted fetuses and their mothers. Molecular method was performed for detecting *T. gondii*. 39.08% of mothers had anti-Toxoplasma antibody (IgG).

Table 1. Demographic characteristics of mothers who had abortion.

Variable		N	%
Age (year)	20-29	41	47.12
	30-39	32	36.78
	>40	14	16.09
Habitat	Urban	52	59.77
	Rural	36	40.23
Keeping cat	Yes	4	4.6
	No	83	95.4
	Yes	34	39.08
Eating undercooked food	NO	53	60.02

The seroprevalence of toxoplasmosis based on IgG among pregnant women in Iran is relatively high (7). This rate in Zahedan, southeast of Iran, was reported to be 27% while in the northern part of the country was 48.3% (based on IgG) (8). In central province (Arak), the prevalence of toxoplasmosis in pregnant women was about 38% (7).

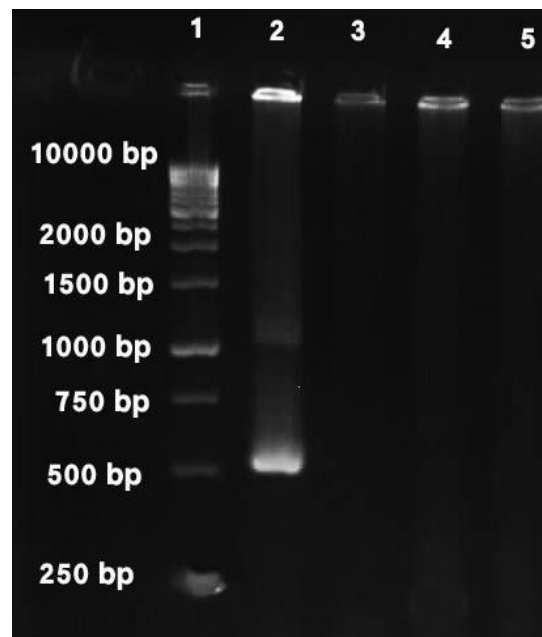


Figure 1. PCR products of aborted fetuses' samples on agarose 1%. Lane 1, molecular weight marker 1 kb; Lane 2, Positive control; Lane 3, negative control; Lanes 4, 5 aborted fetus samples

In Shiraz, southern Iran, the seroprevalence of toxoplasmosis among pregnant women was reported to be 77.2 % (9). This rate is lower in high school girls in Shiraz (11).

In a study on pregnant women in New York, 0.6% of mothers acquired *Toxoplasma* during pregnancy and 13% of their infants were born with congenital toxoplasmosis. So, the rate of congenital toxoplasmosis was reported to be 7 per 10,000 live births (7). A study in Colombia showed that 61 out of 15,333 umbilical cord blood samples have specific IgM for anti-*Toxoplasma*. This contributed a rate of 39 per 10,000 live births for congenital toxoplasmosis in this area (11). A study on the seroprevalence of toxoplasmosis in Kosovo in pregnant women demonstrated that 1.2% of women acquired toxoplasmosis during their pregnancy (12). In our study, the rate of *Toxoplasma* infection in aborted fetuses was 0%. But the result of research of Asgari et al. on aborted human placenta in Shiraz showed that the rate of *Toxoplasma* infection in aborted placenta was 14.4%. (4). In one study by Sarkari et al., no parasite cyst or tachyzoites were detected in PCR positive sample by immunohistochemistry in 50 aborted fetuses in Shiraz, Iran. This might have contributed to the low level of parasite in the aborted tissues (13). In livestock, the results indicate that *T. gondii* DNA was the most frequently detected in ovine fetuses (18.1%) (14). The detection of *Toxoplasma* DNA in placental tissue may not exclusively be connected to abortion because it may be the parasite that enters the placenta but not the fetus. Moreover, all items such as type of sample, method of sampling, and size of sample may affect the outcome of research, as was observed in this study. In addition, the sensitivity of PCR is significantly higher when maternal infection occurs between 17 and 22 weeks' gestation (15), whereas in our research, the fetuses were under 14 weeks. However, false-positive and false-negative tests do occur with PCR (16). A negative PCR at any gestation cannot completely rule out congenital infection, and obstetric providers should consider continued follow-up *via* serial ultrasounds, prophylaxis with spiramycin therapy, and neonatal testing (15). In this study, we did not find evidence of *Toxoplasma* trace in aborted fetuses, and it appears that *Toxoplasma* was not the cause of spontaneous abortion in this area of Iran in less than 14 weeks, but considering the importance of the infection during pregnancy, control measures during pregnancy are required.

Acknowledgments

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Conflict of Interest

The authors declare that there is no conflict of interest.

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