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Comparison of ELISA Diagnostic Methods (IgG, IgM) and Real Time PCR in Identifying *Toxoplasma* in Women with Abortion

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ABSTRACT

Background: Toxoplasmosis as a frequent parasitic infection is caused by *Toxoplasma gondii*, transmitted through contaminated food or water. Maternal infection during pregnancy can lead to serious complications including miscarriage and congenital defects, requiring accurate diagnosis for proper management.

Methods: This study analyzed 120 women with spontaneous abortion (ages 18-45, gestational age 6-20 weeks) who were referred to Baqiyatallah Hospital in Tehran from February 2021 to February 2022. Blood samples were collected and assessed using ELISA for IgG/IgM antibodies and real-time PCR for *T. gondii* B1 gene detection, with statistical analysis performed using Stata software to evaluate diagnostic accuracy and associations.

Results: This study investigated *Toxoplasma gondii* infection in 120 females with abortion, revealing a 1.67% prevalence rate. Diagnostic performance analysis showed that both IgG and IgM antibodies demonstrated excellent sensitivity (100%) when compared to PCR as the gold standard, with IgG showing 83.90% specificity and IgM achieving 100% specificity. Clinical symptoms were significantly higher in infected women, and age-stratified analysis revealed 0% prevalence in women under 30 years versus 2.99% in older women.

Conclusion: This study revealed a low prevalence of *Toxoplasma gondii* infection (1.67%) among females with abortion, with higher rates in women over 30 years (2.99%). Clinical symptoms were significantly more common in infected women, and both IgG and IgM serology demonstrated excellent sensitivity (100%) compared to PCR, highlighting their reliability for initial screening despite low positive predictive values.

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Introduction

Toxoplasmosis as a parasitic disease is caused by *Toxoplasma gondii*. This obligate intracellular opportunistic organism infects about one-third of the global population and is the commonest parasitic infection in humans (1). Infection with *T. gondii* can occur mainly through consumption of undercooked, cyst-contaminated meat or ingestion of oocysts via contaminated food, soil, and water. The incidence of this infection varies between countries depending on socio-economic and health status (2, 3). Seroprevalence ranges between different countries from 10% to 80%. Lower seroprevalence rates exist in Southeast Asia, North America, and Northern Europe, while the highest levels are observed in tropical countries of the African continent and Latin America (4, 5). In a 2021 study in Iran aimed at determining the incidence of toxoplasmosis in pregnant women, the frequency of this infection was reported as 14.5%(6). Maternal infection in the 1st or 2nd trimester of pregnancy is linked to stillbirth rates of respectively 5% and 2% (7, 8). The incidence of congenital toxoplasmosis is between 0.1% and 0.3% per 1000 live births, with the risk of mother-to-fetus transmission increasing from 15% to 70% between 13 and 36 weeks of gestation, respectively (9, 10). Most acquired toxoplasmosis cases are either asymptomatic or exhibit mild symptoms; but, in cases with weakened immune systems, like those with HIV or transplant recipients, the disease can be life-threatening, leading to ocular and cerebral damage. Additionally, reactivation of latent infections can result in serious complications with a poor prognosis. Infection during pregnancy can also result in congenital toxoplasmosis in the fetus, which can lead to stillbirth, spontaneous abortion, microcephalus, hydrocephalus, and neurological symptoms that may be identified either in utero or at birth (11, 14). Its early diagnosis, whether during pregnancy or following birth, is critical for inhibiting and mitigating serious complications in the newborn or fetus and for make an improvement in the infection prognosis (15). Overall, maternal serological screening for toxoplasmosis, particularly among mothers who are seroconverting throughout pregnancy, is vital to preventing fatal outcomes using prophylaxis or appropriate medical treatment (16).

The diagnosis of toxoplasmosis typically relies on clinical and serological assessments. While *Toxoplasma*-specific antibodies (immunoglobulins) can be found in the pregnant women's serum during 1–2 weeks following the infection exposure, the serological findings for these antibodies mostly do not differentiate between chronic and acute infections (15, 16). The presence of IgM antibodies or both IgG and IgM display an acute infection, while negative findings could suggest either no infection altogether or a very recent infection (17). Nonetheless, positive IgM findings are always be reliable because of variations in commercial test kits or because IgM remains identifiable in the serum even following the acute infection has resolved (16, 17). To improve the serological evaluations' sensitivity and specificity, more confirmatory tests, like serological conversion testing and IgG affinity test, are needed (18, 17). Furthermore, when unusual ultrasound results like calcifications, microcephaly, and hydrocephalus, are noted, a polymerase chain reaction (PCR) test positive for *T. gondii* in amniotic fluid is expected (18). In recent years, DNA-oriented molecular techniques, offering greater specificity and sensitivity than serological techniques, have been utilized for more accurate detection of infections during pregnancy, focusing on various targets within the *T. gondii* genome (19). Consequently, this study was conducted to compare the diagnostic effectiveness of ELISA (IgG, IgM) and real-time PCR in detecting *Toxoplasma* in women who have experienced a miscarriage.

Materials and Methods

This research was conducted on 120 women referring to Baqiyatallah Hospital in Tehran from February 2021 to February 2022. The study population included women aged 18-45 years with gestational age of 6-20 weeks who presented with spontaneous abortion. Inclusion criteria consisted of confirmed pregnancy loss and written informed consent, while exclusion criteria included known immunodeficiency disorders, recent antibiotic therapy, and multiple gestations. Peripheral blood samples (5cc) were collected from all participants by an experienced nurse using sterile syringes and transferred to the parasitology laboratory under aseptic conditions. Serum specimens were isolated by centrifugation (3000 rpm / 10 minutes) and maintained at -20 °C until assessment.

ELISA assay

Serological evidence of toxoplasmosis was assessed through the identification of *T. gondii*-specific IgG and IgM antibodies. Serum specimens were isolated from peripheral blood and kept in aliquots at −20°C until subsequent assessments. Samples were analyzed for the existence of IgG and IgM antibodies using a commercially available ELISA-based NovaLisa test kit (NovaTec GmbH, Germany). Additionally, a *Toxoplasma*-specific IgG avidity assay was done utilizing an avidity *T. gondii* IgG NovaTec GmbH ELISA kit (Germany) to discriminate between chronic and acute infection phases. The laboratory procedures were conducted strictly following the producer's protocols and guidelines. Optical density readings were obtained using a Bio-Rad ELISA microplate reader (USA) and subsequently compared against standardized calibrators and positive/negative controls for result interpretation.

Molecular Methodology for Real-Time PCR

DNA extraction was done using a DNA extraction kit as instructed (Bioneer, South Korea). The extraction process included cell lysis, DNA purification, and final DNA concentration steps. Real-Time PCR was conducted to amplify a 194 base pair segment of the *T. gondii* B1 gene (Table 1). The PCR reaction was done in a final volume of 25 µL including 1.5 units of AmpliTaq Gold DNA polymerase, 200 µM dNTPs, 0.5 µM primers, 1.5 mM MgCl2, and DNA template (4 µL).

The thermal cycling protocol consisted of a primary denaturation (95°C / 10 min), followed by 40 cycles including denaturation (95°C / 15 s), annealing (60°C / 30 s), and extension (72°C / 30 s).

The Real-Time PCR products underwent electrophoresis on a 2% agarose gel to confirm the target DNA segment amplification and verify the accuracy of the amplification process. The gel electrophoresis enabled visualization and confirmation of the 194 bp amplicon corresponding to the B1 gene of *T. gondii*.

Statistical Analysis

To characterize the quantitative variables, the standard deviation and mean were applied if the distribution was normal; if it was not normal, the interquartile range (IQR) and median were reported. For qualitative variables, the counts and percentages were provided. Comparison of the frequency of different variables according to PCR results was done using Chi-Square test. The Mann-Whitney test and T-test compared the mean of IgG and IgM based on different variables. False positive (FP), true negative (TN), true positive (TP), and false negative (FN) values were determined according to the study data and using PCR findings as the gold standard. Then the accuracy indices including specificity, negative predictive value, sensitivity, positive predictive

Table 1. Primers employed for real-time PCR.

Gene	Primer	Sequence	Amplicon
B1	Forward	5′-GTT GGG AAG CGA CGA GAG TC-3′	194 bp
B1	Revers	5′-ATT CTC TCC GCC ATC ACC AC-3′	
B-actin (housekeeping)	Forward	5’- CATCCGTAAAGACCTCTATGCCAAC -3’	619 Bp
B-actin (housekeepin)	Revers	3’- ATGGAGCCACCGATCCACA -5’	

Table 2. Baseline Characteristics and Clinical Manifestations of Women with Abortion.

Different variables		Positive n=2	Negative n=118	Different variables
		Number (%)	Number (%)	
Fever				
	Yes	2 (100)	0(0)	0.001<
	No	0(0)	118(100)	
Inflammation of lymph nodes and sore throat				
	Yes	1(50)	3(2.54)	0.001<
	No	1(50)	115(97.46)	
A history of more than one abortion				
	Yes	2 (100)	27 (22.88)	0.01
	No	0(0)	91 (77.12)	
History of contact with animals				
	Yes	2 (100)	14 (11.86)	0.001<
	No	0(0)	104(88.14)	

Table 3. Sensitivity, Specificity, and Predictive Values of IgG Antibody Testing for *Toxoplasma gondii* Detection.

Accuracy indicators	Estimate	confidence interval 95%
(%) Sensitivity	100	100-80.100
(%) Specificity	83.90	76.90-0.002
PPV(%) (Positive predictive value)	9.52	1.30-17.38
NPV(%) (Negative predictive value)	100	96.34-100
LR^+	6.21	-
LR^-	0	-

Table 4. Sensitivity, Specificity, and Predictive Values of IgM Antibody Testing for *Toxoplasma gondii* Detection.

Accuracy indicators	Estimate	confidence interval 95%
(%) Sensitivity	100	100-81.15
(%) Specificity	100	92-96.100
PPV(%) (Positive predictive value)	100	15-81.100
NPV(%) (Negative predictive value)	100	92-96.100
LR^+	0	-
LR^-	0	-

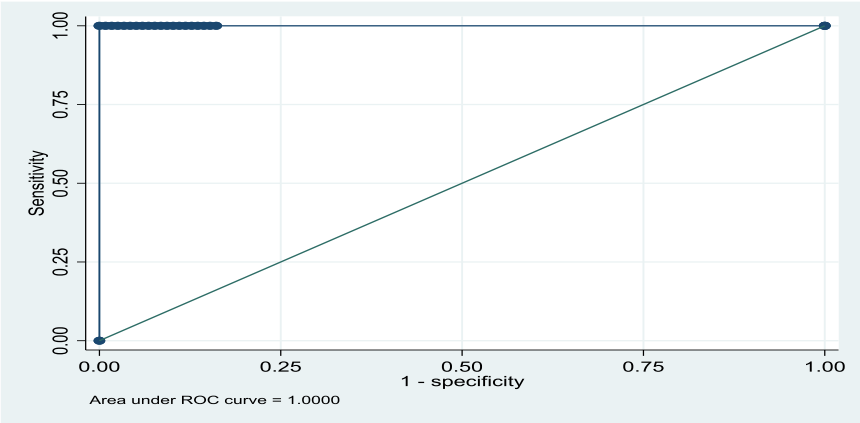

Figure 1. IgG rock curve for *Toxoplasma gondii* infection.

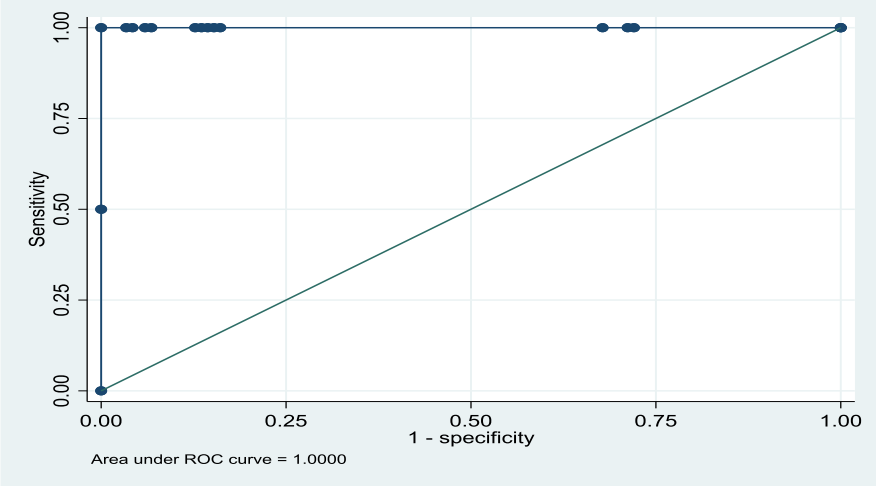

Figure 2. IgM rock curve for *Toxoplasma gondii* infection.

value, LR+ and LR- as well as ROC curve and area under the curve (AUC) with 95% confidence interval were calculated for ELISA tests (IgG and IgM). Data analysis was done using Stata 14. A $p<0.05$ was considered significant.

Results

A total of 120 women experiencing abortion were investigated. The participants' mean age was 31.42 ± 6.52 years (range: 14 to 53 years). Also, 44.17% were under 30 years of age, while 55.83% were over 30 years old. Clinical symptoms were reported as follows: fever in 1.67% (n=2), lymphadenopathy and sore throat in 3.33% (n=4), and headache accompanied by shortness of breath in 6.67% (n=8) of the women. Additionally, 24.17% (n=29) of participants reported a history of recurrent abortions. Animal contact was documented in 13.33% (n=16) of cases (Table 2). PCR analysis revealed a *Toxoplasma gondii* infection prevalence of 1.67% (n=2) among women with miscarriage. The mean serum concentrations of Toxo IgG and Toxo IgM antibodies were 9.82±30.68 mg/dL and 0.57±1.38 mg/dL, respectively. Age-stratified analysis demonstrated that *T. gondii* prevalence was 0% in females < 30 years compared to 2.99% in females older than 30 years.

The mean age of infected individuals was higher than non-infected participants, although the difference was not significant (P=0.15). Clinical symptom prevalence was significantly higher among *T. gondii*-positive women compared to seronegative individuals: fever (100% vs. 0%), lymphadenopathy (50.00% vs. 2.54%), and headache with shortness of breath (100% vs. 5.08%). Additionally, a history of recurrent abortions and animal contact exposure were more frequently reported among infected women, with all these findings demonstrating statistical significance (P<0.05)..Antibody titers and advancing age showed no significant correlation (P>0.05). However, mean IgG and IgM levels were significantly increased in PCR-positive women versus seronegative controls (P<0.001).

When PCR results were considered as the gold standard and IgG titers were categorized as negative (<7.2 mg/dL) and positive (>8.2 mg/dL), the diagnostic performance for *Toxoplasma gondii* infection demonstrated 100% sensitivity and 83.90% specificity. The positive and negative predictive values were respectively 9.52% and 100% (Table 3). Notably, no false-negative cases (positive PCR with negative IgG) were observed in this study, confirming 100% sensitivity.

Similarly, when IgM titers were stratified as negative (<5 mg/dL) and positive (>7 mg/dL) using PCR as the reference standard, the diagnostic accuracy for *Toxoplasma gondii* detection yielded 100% sensitivity and 100% specificity. Both negative and positive predictive values were 100% (Table 4). Consistent with IgG findings, zero false-negative cases (positive PCR with negative IgM) were identified, maintaining 100% sensitivity for IgM detection.

Regarding PCR findings as the gold standard, the optimal cut-point of IgG for *Toxoplasma gondii* infection was 146.5 mg/dL. The specificity and sensitivity for this cut point were also respectively 100% and 100% ,. Also, the AUC was determined as 1.00 (Figure 1).

Considering the PCR findings as the gold standard, based on the data of this study, the optimal cut-point of IgM for *Toxoplasma gondii* infection was 5 mg/dL. The specificity and sensitivity for this cut point were also respectively 100% and 100%. Also, the AUC was determined as 1.00 (Figure 2).

Discussion

Toxoplasmosis as a parasitic infection, is caused by *T. gondii*, presenting with many clinical symptoms (21). Infection occurs through the consumption of undercooked food, as well as vegetables and water contaminated with cat feces (22). As soon as a woman ingests tissue cysts or *Toxoplasma* oocysts for the first time throughout pregnancy, tachyzoites spread throughout her body via the bloodstream (23). The *Toxoplasma* parasite can then enter the fetus's circulation through the placenta. Maternal infection prior to pregnancy

seldom poses a risk to the fetus, except in individuals with compromised immune systems (24). Maternal infection throughout the 1st or 2nd trimester is linked to stillbirth frequencies of respectively 5% and 2% (25). Research in New York, USA, found that 6% of pregnant women contracted toxoplasmosis during their pregnancy, and 13% of their newborns are diagnosed with congenital toxoplasmosis. Overall, the congenital toxoplasmosis prevalence was 7 cases per 10,000 live births (26). The incidence of toxoplasmosis among Iranian pregnant females is relatively high, with estimates of 27% in Zahedan in southeastern Iran (27), 60.6% in northern Iran (28), and 34.09% in Abadan in southwestern Iran (29).

To enhance the accuracy of identifying infections during pregnancy, DNA-oriented molecular techniques have been utilized in recent years, offering greater sensitivity and specificity compared to serological methods, with various regions of the *T. gondii* genome being explored. The B1 gene is considered the most significant target for detecting toxoplasmosis (30). PCR test results indicated a prevalence of *T. gondii* of 1.67% (2 individuals) among women who experienced miscarriage, with the prevalence among women under 30 years old and those over 30 years old being [data missing] and 2.99%, respectively. Saki et al.(2021) conducted a study on blood samples from 480 women who experienced abortions, reporting 20.27% of samples as positive for *Toxoplasma gondii* presence using PCR methodology(31). Abdoli et al.'s(2017) study reported a prevalence of *Toxoplasma gondii* in women with abortion as 3.8%(32).

Multiple studies have indicated that *T. gondii* may play a role in causing abortions. Different serological tests have identified immunoglobulin (IgG and IgM) antibodies against *T. gondii* in serum samples (33, 34). The average levels of Toxo IgG and Toxo IgM in patients were 9.82±30.68 mg/dL and 0.57±1.38 mg/dL, respectively. Saki et al. conducted a study on 130 serum samples from women with abortions to investigate toxoplasmosis prevalence, reporting IgG antibody positivity in 24.6% of samples using ELISA methodology (35). Mohammed et al.'s study, conducted on 75 serum samples from women with abortions to investigate toxoplasmosis prevalence, reported IgM in 4% and IgG in 22.6% of positive cases by ELISA method(36). Mihu et al.'s study, conducted on 1081 women, reported *Toxoplasma gondii* IgG antibody ELISA test positivity in 41% of cases, with prevalence increasing with age (37).

Toxoplasmosis can present as either an acute or chronic infection, with or without symptoms. The symptoms and complications primarily arise during the infection acute stage. After the host's immune system is activated, the proliferation of the parasite is managed, leading to the formation of tissue cysts in the neuromuscular tissues of the host (38, 39). While acquired toxoplasmosis typically results in asymptomatic or mild infections in immunocompetent individuals, it can cause severe clinical outcomes and even death in cases having weakened immune systems. Additionally, for those with immunodeficiency or those on immunosuppressive medications, a chronic infection may reactivate, leading to serious and potentially life-threatening complications such as encephalitis, myocarditis, and pneumonia (40). Transplacental transmission of *T. gondii* mainly occurs during a woman's first pregnancy (41). Congenital toxoplasmosis that develops during pregnancy can lead to spontaneous abortion, stillbirth, and a range of developmental issues including mental or physical disabilities, hydrocephalus, blindness, and deafness (40, 41).

In our study, the prevalence of fever, lymphadenitis, sore throat, headache, and dyspnea was higher in women with *Toxoplasma gondii* infection compared to non-infected women. A history of multiple miscarriages was also more prevalent in women with *Toxoplasma gondii* infection. Additionally, animal contact frequency was reported higher in affected women, and these findings were all statistically significant. Several studies have reported associations between *Toxoplasma gondii* infection and complications in

the digestive system, inflammatory conditions, and cardiovascular problems (42-44). Nayeri et al.'s review study, conducted to investigate the relationship between *Toxoplasma gondii* infection and headache, found that 17.6% of infected individuals suffered from headache and migraine (45). Garrido et al.'s study, aimed at establishing a relationship between *Toxoplasma* infection and miscarriage in women, found that 35.8% of 218 pregnant women were infected with *Toxoplasma*, with miscarriage reported in 14.7% of cases (46). Kalantari et al. (47) and Elaadli et al. (48) emphasized the relationship between *Toxoplasma* infection and miscarriage in pregnant women. Almeida et al.'s study reported *Toxoplasma* infection rates in individuals who were in contact with animals (49). Sink et al.'s study found higher *Toxoplasma* infection rates in people who had contact with animals, blood, or their secretions (50).

While serological testing is an important diagnostic approach used to identify toxoplasmosis, it is associated with several limitations. It may not identify specific anti-*Toxoplasma* antibodies throughout the active infection stage, as these antibodies may not be generated until weeks following parasitemia begins. Additionally, it may be less effective in identifying *T. gondii* infections in individuals with compromised immune systems due to insufficient antibody generation. Therefore, employing PCR methodology for *T. gondii* infection detection overcomes the challenges encountered with serological methods, particularly when dealing with immunocompromised patients (51,52). Considering PCR results as the gold standard, the IgG ELISA test demonstrated sensitivity and specificity of 100% and 83.90%, respectively, for *Toxoplasma gondii* diagnosis, while the IgM ELISA test showed 100% sensitivity. Matin et al.'s study, which aimed to compare molecular and serological methods for toxoplasmosis detection in 200 women, reported 4% of cases positive for IgM and 43% for IgG antibodies by ELISA method, with 53.5% of samples positive for toxoplasmosis by PCR method. This study highlighted the high sensitivity of PCR methodology in diagnosing this infection (53). Aly et al. (2023) reported that PCR demonstrated superior diagnostic value compared to ELISA in terms of sensitivity, specificity, and diagnostic accuracy (97.3% vs. 89.2% sensitivity, 100% vs. 94% specificity, and 98.9% vs. 91.95% diagnostic accuracy). In this study, PCR was identified as the most sensitive and accurate method (54). El-Sayad et al.'s study reported *Toxoplasma* prevalence by ELISA as 23% and by PCR as 20%. In this study, PCR sensitivity and specificity were declared as 58.90% and 88.89%, respectively, with the PCR method being characterized as a sensitive and accurate diagnostic approach (55).

Conclusion

As a conclusion, our findings emphasize the continued significance of toxoplasmosis as a potential contributor to adverse pregnancy outcomes, particularly in regions with high seroprevalence rates.

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Ethics approval and consent to participate

No formal ethical code was applicable to this retrospective analysis, as it utilized anonymized laboratory data without direct human subject involvement.

Conflict of interest

The authors declared no conflicts of interest.

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