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Short Communication

Seroprevalence and Risk Factors of Toxoplasmosis in Female Students from Northwest Iran

Mehrdad Rostami[1], Sanam Rezaei Benam[2], Elham Sadat Roointan[3], Arezoo Bozorgomid[4], Saber Raeghi[5], Reza Shafiei[6*]

[1]Student Research Committee, Iran University of Medical Sciences, Tehran, Iran
[2]Danesh Pathobiology Laboratory, Maragheh, Iran
[3]Department of Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4]Infectious Diseases Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran
[5]Department of Parasitology & Mycology, Urmia University of Medical Sciences, Urmia, Iran
[6]Vector-borne Diseases Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran

*Corresponding Author: Saber Raeghi, Email: saberraeghi@gmail.com

Abstract

Background: Toxoplasmosis is a significant zoonotic disease caused by the protozoan parasite *Toxoplasma gondii*. This infection can have dire consequences if it occurs during pregnancy, as it may result in spontaneous abortion or congenital disorders. Using serological methods, we investigated the seroprevalence of *Toxoplasma* infection in female students of childbearing age and also determined the association between *Toxoplasma* infection and pregnancy.

Methods: In this cross-sectional study, blood samples were collected from female students of childbearing age from three provinces in northwest Iran who studied at Maragheh University of Medical Sciences. Anti-*Toxoplasma* IgG and IgM antibodies were identified by ELISA and ELFA methods, and the relationship between *Toxoplasma* infection, demographic characteristics, and risk factors was evaluated.

Results: The ELISA and ELFA serology test results for measuring IgG and IgM, were similar. One hundred cases (28.73%) were positive for Toxo IgG, and 6 cases (1.72%) were positive for Toxo IgM. The results showed that some risk factors, such as contact with cats and activities, such as agriculture or contact with soil, were significantly ($P<0.05$) related to *T. gondii* seropositivity.

Conclusion: More than 70% of the childbearing-age female students studied in this investigation were susceptible to infection during pregnancy, and the performance of regular serological testing during pregnancy and health education about toxoplasmosis before and after gestation is essential to prevent congenital toxoplasmosis.

Keywords: toxoplasmosis, GIRLS, Iran, ELISA, ELFA

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Introduction

Toxoplasmosis is a global zoonotic disease caused by an intracellular coccidian parasite, *Toxoplasma gondii*. This parasite is estimated to affect about one-third of the world's human population (1). Transmission of *T. gondii* occurs by eating tissue cysts in raw or undercooked meat or consuming food sources and water contaminated by sporulated oocytes shed in the feces of cats (2). Toxoplasmosis is usually asymptomatic in healthy people (70%–80% cases) but can cause severe problems in immune-suppressed individuals such as HIV-positive individuals and cancer patients or fetuses of seronegative mothers (3,4).

The risk of parasite transmission to the fetus during pregnancy increases with increasing gestational age. However, infection during early pregnancy brings about more harm, including abortion, mental or physical retardation, stillbirth, intracranial calcification, hydrocephalus, hepatosplenomegaly, and chorioretinitis for the fetus (5,6).

Several serological methods, such as enzyme-linked fluorescent assay (ELFA), enzyme-linked immunosorbent assay (ELISA), and indirect fluorescent antibodies (IFA), have been used for the detection of antibodies against *T. gondii* (7-9). Additionally, the IgG avidity test has been used to discriminate between acute and chronic phases of toxoplasmosis. IgG avidity is low in the acute phase and high in the chronic phase of infection (10). Social and cultural habits, lifestyle habits, ecological and geographical factors, transmission form, and climate can be effective in the prevalence of *Toxoplasma* infection. The prevalence varies widely between different countries (10%–80%); the prevalence is 58.5% in Indonesia (11), 58.5% in Japan (12), and 58.5% in Brazil (12,13).

Primarily, to avoid the consequences and disorders of the disease, counseling before marriage and informing people about routine tests at research centers can benefit women of childbearing age. Regardless of whether mothers are vulnerable to acute or chronic disease, remedial measures can be taken so that high-risk individuals can benefit from medicinal service offices after pregnancy (14).

There are many investigations on the seroprevalence of toxoplasmosis among Iranian women. However, there is minimal information about the rate of toxoplasmosis in childbearing-age girls in northwest Iran.

This study was designed to investigate the seroprevalence of *Toxoplasma* infection in female students of childbearing age using ELISA and ELFA methods and determine the association between *Toxoplasma* infection and some risk factors.

Materials and Methods

Study design

This cross-sectional study included 348 female students of childbearing age from three provinces of northwest Iran (West Azerbaijan, East Azerbaijan, and Kurdistan) who studied at Maragheh University of Medical Sciences f by rom October 2017 to September 2019. These areas are generally considered mountainous, but due to their topographic diversity, they have different climates.

Sampling

Blood samples (2 mL without anticlotting agent) were taken and transferred to the parasitological lab. Sera were separated and stored at -20 °C until used.

Also, a demographic questionnaire was completed before sampling by the researchers. This questionnaire included age, awareness of toxoplasmosis and infection transmission paths, life conditions, cat contact, and raw/half-cooked meat consumption.

Serological (ELISA & ELFA) tests

All serum samples were tested for anti-*Toxoplasma* IgM and IgG antibodies using *Toxoplasma* ELISA and ELFA methods. All the serum samples were tested using a commercially available ELISA kit (Acon, China) with sensitivity and specificity of 99.9% and 99%, respectively. The results were considered positive when the OD450 index was equal to or higher than the cut-off value. IgG and IgM anti-*Toxoplasma* levels > 9 UI/ml were considered negative, and they were considered positive > 11 UI/ml and equivocal if they were between 9 and 11 UI/ml, according to the manufacturer's instructions.

The ELFA method is an enzymatic sandwich that generates a fluorescent product. The assays were performed by IgG (sensitivity: 99.65%, specificity: 99.92%) and IgM (sensitivity: 96%, specificity: 99.6%) ELFA kits (VIDAS, Biomerieux, France). According to the manufacturer's instructions, IgG ≤ 4 UI/mL was unfavorable, IgG ≥ 8 UI/mL was positive, and values between 4 and 8 UI/ml were equivocal. For IgM, IgG < 0.55 UI/mL was negative, IgG ≥ 0.65 UI/mL was positive, and values between 0.55 and 0.65 UI/mL were equivocal.

Statistical analysis

Data were analyzed using SPSS version 17 to perform paired and Spearman's tests. *P* values less than 0.05 were considered statistically significant.

Results

A total of 348 female students of childbearing age participated in the study. They were, on average, 20.5 years old (18–22 years old). Of these participants, 252 cases (72.4%) lived in urban areas, and 96 cases (27.6 %) were from rural areas.

The serology test results for measuring IgG and IgM by the ELISA and ELFA methods were not statistically different: 100 cases (28.73%) were positive for Toxo IgG, and 6 cases (1.72%) were positive for Toxo IgM.

The results, including seroprevalence data with demographic variables, are detailed in Table 1. The results showed that some risk factors, such as cat contact and activities like agriculture or contact with soil, were significantly ($P < 0.05$) related to *T. gondii* seropositivity. Consumption of raw or half-cooked meat and vegetables did not correlate with seropositivity for *T. gondii* infection (Table 2).

Using the ELISA and ELFA methods, there was no statistical difference between serological results in the anti-Toxo IgG and IgM tests. There was also no statistical difference between the three provinces and their seroprevalence results.

Discussion

This study investigated the seroprevalence of toxoplasmosis among female students of childbearing age from three provinces in northwest Iran who were studying at Maragheh University of Medical Sciences. One hundred individuals (28.73%) were positive for Toxo IgG according to ELFA and ELISA methods. However, it is essential to note that the other 71.27 are not immune to *Toxoplasma*. There is a possibility and risk of congenital transmission to the fetus in the future.

Toxoplasmosis is one of the most widespread infections in humans and warm-blooded animals. Prevention of congenital toxoplasmosis is recommended before pregnancy, and it is essential to increase the awareness of girls of childbearing age and use screening tests (9,15,16). However, Mumcuoglu et al believe it is also necessary to implement a diagnostic model in pregnant mothers for this infection and facilitate access to it. Without doubt, the most cost-effective way to diagnose and prevent the disease will be to raise awareness and inform girls about to get married (17). Training girls and assessing the

Table 1. Seroprevalence of *T. gondii* among female students in northwest Iran based on demographic information

Variables	IgG (ELISA, ELFA)	IgM (ELISA, ELFA)	Total (*n*/%)	*P* value (IgG)	
	Positive [*n* (%)]	Positive [*n* (%)]		ELISA	ELFA
Age group, years					
18–18.9	2 (2)	1 (0.29)	2 (0.57)		
19–19.1	26 (26)	4 (1.17)	26 (7.47)	-	-
20–20.9	28 (28)	0	28 (8)		
21–22	44 (44)	1 (0.29)	44 (12.64)		
Residence area					
Urban	88 (88)	-	252 (72.4)	0.02	0.03
Rural	12 (12)	-	96 (27.6)		
Awareness					
No	24 (24)	2 (0.58)	26 (6.9)		
Somewhat	48 (48)	0	90 (25.9)	0.01	0.01
Relatively	20 (20)	4 (1.17%)	204 (58.6)		
Complete	8 (60)		30 (8.6)		
Occupation					
Only student	94 (94)	6 (1.72%)	336 (96.6)	0.9	0.8
Others	6 (4)	0	12 (3.4)		
Province					
West Azerbaijan	83 (83)	5 (1.43)	(23.8)	-	-
East Azerbaijan	15 (15)	1 (0.29)	(4.31)		
Kurdistan	2 (2)	0	(0.57)		

Table 2. Risk factors and *T. gondii* seroprevalence among female students of childbearing age in northwest Iran (*n* = 348)

Variables	IgG positive (ELISA, ELFA)	*P* value	
	Positive [*n* (%)]	ELISA	ELFA
Contact with cat			
Yes	68 (19.54%)	0.001	0.001
No	280 (80.46%)		
Agriculture or contact with soil			
Yes	320 (92%)	0.01	0.01
No	28 (8%)		
Raw/half-cooked meat Consumption			
Yes	336 (96.5)	-	-
No	12 (3.44%)		
Raw vegetable consumption			
Yes	336 (96.5)	-	-
No	12 (3.44%)		

prevalence of this disease in different regions can increase the scope of health development.

In a systematic review, the minimum and maximum prevalence rates of this infection in Iranian childbearing-age women were estimated as 23% and 64%, respectively, using the ELISA method. The overall estimate was 37.6% based on the ELISA method (18). It is emphasized that the general estimation for this prevalence is 39.9% (95% CI: 26.1–53.7). Our seropositive results for Toxo IgG using both ELFA and ELISA methods were lower than those of the above-mentioned study, which can be due to the difference in age range and education levels.

In other studies, the prevalence of anti-*Toxoplasma* was reported in northwest Iran (Jolfa) as 21.8%. Borna et al. reported seroprevalence rates between 4.6% and 74.6% in childbearing-age women (19). The results of our study are close to those of this research. It seems that the weather, habits, and behaviors can be effective. On the other hand, the highest prevalence rate is found in northeast Iran (Mashhad), with 54% (9,20).

Some studies from Iran have been done on pregnant women. For example, in Chalus and Ilam, researchers found a prevalence of 52% and 44.8% in pregnant women, respectively (21,22). It seems necessary to have a coherent system throughout Iran for diagnosing this infection among women of reproductive age. The impact of aging on the risk of *Toxoplasma* has already been shown (23). These factors are attributed to occupational activities and lower socioeconomic and hygienic levels, which are not included in our study.

Of course, the difference in results can be related to climate conditions, humidity, temperature, nutritional behavior, geographic region, and contact with cats, but diagnostic methods may also affect the reported seroprevalence in many parts of the world.

The ELFA method is an enzymatic sandwich that

generates a fluorescent product. The ELISA method tracks any complex, including antigen and antibody couples. Although the results of these methods are close to each other, the automatic methods (ELFA) are preferred because of high reproducibility, lower personnel budget, etc. Therefore, Gharavi *et al* suggested using automatic methods to diagnose toxoplasmosis (24). In addition, the confirmatory diagnostic method in *Toxoplasma* serological profile with a positive or equal IgM titer has been emphasized before, which showed the superiority of the ELFA method with the VIDAS system over other methods (25).

Serological methods like ELFA adequately identify residual IgM anti-*Toxoplasma gondii* and have been proven to have an acceptable detection role in pregnant women (26,27). This study showed no difference between the ELFA and ELISA methods results.

Results show that contact with cats and engagement in agriculture or contact with soil are significant risk factors for *T. gondii* seropositivity. However, in other studies, consuming raw or undercooked meat are effectively transmitted these parasites (14,28,29). In addition, occupation did not show a significant association with seroprevalence of *T. gondii* infection. These findings are consistent with studies reported from Ethiopia (30).

Conclusion

More than 70% of the females of childbearing age studied in this investigation were susceptible to infection during pregnancy. Therefore, regular serological testing during pregnancy and health education about toxoplasmosis before and after gestation are essential to prevent congenital toxoplasmosis. Further studies are recommended in this area to consider other possible infection risk factors in pregnant women and neonates to reduce the effects of congenital toxoplasmosis.

Authors' Contribution

Conceptualization: Mehrdad Rostami.
Data curation: Sanam Rezaei Benam.
Formal analysis: Reza Shafiei.
Investigation: Arezoo Bozorgomid, Saber Raeghi.
Methodology: Elham Sadat Roointan.
Project administration: Saber Raeghi.
Writing–original draft: Saber Raeghi, Arezoo Bozorgomid.
Writing–review & editing: Saber Raeghi.

Competing Interests

The authors declare they have no conflict of interest.

Ethical Approval

The study was approved by the Ethics Committee of Maragheh University of Medical Sciences. Before blood sampling, written informed consent was obtained from all the participants.

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