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Original Article

Seroprevalence and risk factors of *Toxoplasma gondii* infection among pregnant women

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Abstract

Introduction: *Toxoplasma gondii* is an obligate intracellular parasite affecting a broad range of warm-blooded animals, including humans. Infection acquired during pregnancy can be transmitted to the fetus and leading to serious problems such as spontaneous abortion, stillbirth, or severe mental and/or physical handicaps in the child. The purpose of this study was to investigate the seroprevalence of *Toxoplasma* infection and related risk factors in pregnant woman.

Methodology: The study enrolled 1200 serum samples of pregnant women from February-November 2017. Then the samples were tested for the presence of anti-*T. gondii* antibodies (Ab) using enzyme-linked immunosorbent assay.

Results: Out of the 1200 samples, 381 (31.7%) and 41 (3.4%) subjects were positive for IgG and IgM Ab, respectively. Among the evaluated risk factors, the seroprevalence of *Toxoplasma* infection was not related to the occupation in a significant way. However significant relationship was observed with factors such as; contact with soil, cats, consumption of raw washed vegetables, and washed hands before meals.

Conclusions: According to the results, more than two-thirds of pregnant women are susceptible to *Toxoplasma* infection, hence training health care programs should be provided to prevent infection.

Key words: *Toxoplasma gondii*; seroprevalence; congenital; ELISA.

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Introduction

Toxoplasmosis is a zoonotic disease caused by the obligate intracellular parasite *Toxoplasma gondii* (*T. gondii*) [1]. Members of the feline family are considered final hosts, while other vertebrates, such as birds and livestock, serve as intermediate hosts [2-3]. The disease can be acquired through various routes, including ingestion of uncooked meat containing tissue cysts, ingestion of oocysts, congenital transmission, and organ transplants [2,4,5]. Toxoplasma infection is usually asymptomatic; however, it can develop into acute, chronic, or symptomatic forms depending on the individual's immune status [6-7]. Since infection acquired during pregnancy can be transmitted to the fetus, sometimes the disease displays serious symptoms. Clinical symptoms of congenital infection include stillbirth, microcephaly, hydrocephalus, miscarriage, and severe fetal neurological disorders [8-10]. Studies have estimated that at least one-third of the world's population has a positive immune response to

T. gondii [11]. The prevalence rates of infection vary in different parts of the world and depend on a variety of factors, such as dietary habits, host susceptibility, geographic location, and culture [12-13]. It is indeed important to recognize Toxoplasma infection before or in the early stage of conception to prevent or decrease the risk of congenital infection. Serological tests are routinely used for determining Toxoplasma-specific IgG and IgM antibodies (Ab) [14-16]. Due to the worldwide distribution of *T. gondii* and its potential for opportunistic infection, it is necessary to assess the prevalence of the infection and its associated risk factors. Therefore, we investigated the rate of anti-Toxoplasma seropositivity and the related risk factors in pregnant women.

Methodology

Study design and data collection

The present cross-sectional study aimed to determine the seroprevalence of *T. gondii* in pregnant women. A total of 1200 pregnant women in their first trimester were randomly selected for the study. Structured questionnaires, consisting of simple closed questions, were used to collect demographic information and assess risk factors such as age, educational level, contact with cats, and consumption of unwashed raw vegetables.

Sample collection and questionnaire

The samples were collected from pregnant women (without any other manifestations of infectious or non-infectious diseases) between February and November 2017. Informed consent was obtained from all individuals participating in the study. Approximately 3 mL of blood sample was collected from the brachial vein of each participant (aged 18-42 years old) under sterile conditions using a venipuncture, disposable syringe, and needle. The blood samples were transferred to the parasitology department, where sera were separated and stored at -20 °C until analysis (Figure 1). Serological tests for *T. gondii* were performed by enzyme-linked immunosorbent assay (ELISA) with the Pishtaz Teb Commercial Kit (Pishtaz Teb Diagnostics, Tehran, Iran) (cut-off > 0.15) following the manufacturer's instructions.

Data analysis

Data analysis was performed using SPSS software (Version 11.0, Chicago, IL, USA) to assess the relationship between infection and potential risk factors. Bivariate analysis by chi-square test and Fisher test was used to estimate the association between seropositivity and risk factors. A *p*-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

Ethics approval

Ethical approval for this study was obtained from Bam University of Medical Sciences, Bam, Iran by number (IR.MUBAM.REC.1399.015) in 2020.4.25.

Results

Out of the 1200 serum samples (mean age: 30 ± 12 years old), 381 (31.7%) tested positive for anti-*T. gondii* IgG Ab, and 41 (3.4%) samples were positive for anti-*T. gondii* IgM Ab (Table 1). The highest seroprevalence (38.73%), was found in women aged between 28 and 32 while those under 22 exhibited the lowest seroprevalence (16.35%).

Figure 1. Toxoplasma gondii tramsmission pathways and serological based detection.

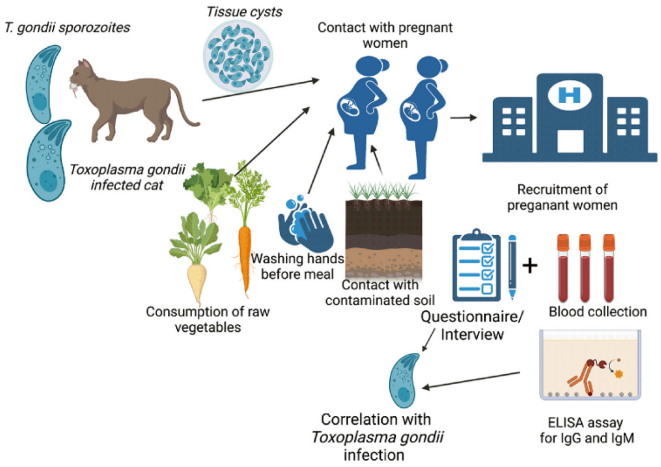

Table 1. Seropositivity of Anti- *Toxoplasma* IgG and IgM antibodies from ELISA in different age group.

Age (years)	Number	IgG positive	IgG negative	IgM Positive	IgM negative
	N (%)	N (%)	N (%)	N (%)	N (%)
18-22	214 (17.8)	35 (16.35)	179 (83.65)	5 (2.33)	209 (97.66)
23-27	274 (22.8)	83 (30.29)	191 (69.71)	9 (3.28)	265 (96.72)
28-32	284 (23.7)	110 (38.73)	174 (61.27)	16 (5.63)	268 (94.37)
33-37	207 (17.2)	75 (36.23)	132 (63.78)	5 (2.41)	202 (97.58)
38-42	221 (18.4)	78 (35.29)	143 (64.71)	6 (2.71)	215 (97.29)
Total	1200 (100)	381 (31.75)	819 (68.25)	41(3.41)	1159 (96.59)

The result of current study showed that *T. gondii* seroprevalence was not age-dependent ($p > 0.05$). Among the factors studied, hand washing showed a statistically significant association with positive anti-*Toxoplasma* IgG Ab in the study group ($p < 0.001$) (Table 2). Additionally, the consumption of raw vegetables, contact with soil, and cats showed significantly higher prevalence in exposed women ($p < 0.001$). Other demographic factors such as "education level" and "occupation" showed a $p > 0.05$ (Table 2).

Discussion

An epidemiological study was conducted to determine the seroprevalence of *T. gondii* infection and associated risk factors of pregnant women. The findings of the study showed that the seropositivity of anti-*Toxoplasma* IgG and IgM Ab was 31.7% and 3.41%, respectively. These results align with those reported by Soltani *et al.,* who found a prevalence of 32.95% for the seropositivity of anti-Toxoplasma IgG antibodies among women referred to clinics in southwest Iran [17]. Recent studies have reported infection rates of 74.5%, 58.3%, and 35.1% among reproductive-age women in Brazil, Turkey, and Sudan, respectively [18-20]. In Iran, the estimated prevalence of *Toxoplasma* infection in pregnant women is 41%, determined using a randomized model. Furthermore, IgG and IgM antibodies were obtained at rates of 38% and 4%, respectively [10]. In Iran, the highest and lowest incidences of *T. gondii* were reported in the Southern and Eastern parts, respectively [21]. The results of the current study compared to other studies conducted in Iran showed the prevalence of IgG and IgM Ab in this study were 6.3% and 0.59% lower than the average prevalence in Iran [10].

Anti-*Toxoplasma* IgG and IgM Ab are routinely screened using serological methods, particularly ELISA [21]. An increased anti-*Toxoplasma* IgG Ab can indicate either a primary infection or a reactivation of chronic toxoplasmosis, which is more common in immunocompromised patients (e.g., HIV patients, organ transplant recipients, and individuals with chronic diseases) and is associated with severe manifestations. On the other hand, Anti-*Toxoplasma* IgM antibodies, are a specific indicator of newly acquired infection [22-23]. As a result, anti-*Toxoplasma* IgG and IgM Ab testing is a common method for detecting chronic (latent) or acute (recently acquired) infection phases. Seronegative pregnant women who have not been exposed to Toxoplasma infection are also at risk, as antibodies are protective against congenital toxoplasmosis. Therefore, screening for anti-*Toxoplasma* Abs before pregnancy is required for disease prevention and control [10,23]. The age of women is an important factor in the consequences of infection. Therefore, the study groups consisted of women aged between 18 to 42 years, as this period corresponds to the highest pregnancy rate. The results of the present study showed the highest prevalence of anti-*Toxoplasma* IgG and IgM Ab in the 28-32 age groups. Previous Similar studies have also demonstrated correlation between higher seroprevalence and increasing, likely due to greater exposure as individuals grow older [15,22]. A study was conducted in Norway to update the prevalence of *Toxoplasma* IgG Ab among pregnant women. The result of the study showed a low prevalence of

Table 2. Potential risk factor for *Toxoplasma* seropositivity in pregnant women in Tabriz.

Characteristic	No. Persons	No. Positive (%)	OR	95% CI	*p*
Educational level					
High school or less	778	238 (30.5)	0.77	0.60-0.98	0.03
College studies (ref.)	422	143 (33.8)			
Occupation					
Laborer	465	162 (34.8)	1.26	0.98-1.61	0.07
Non laborer (ref.)	735	219 (29.7)			
Cats in home or neighborhood					
Yes	405	150 (37)	1.52	1.18-1.97	0.001
No (ref.)	795	221 (27.7)			
Contact with soil					
Yes	280	116 (41.4)	1.74	1.32-2.31	< 0.001
No (ref.)	920	265 (28.8)			
Consumption of raw washed vegetables with disinfectant					
Yes	201	88 (43.7)	1.87	1.37-2.57	< 0.001
No (ref.)	999	293 (29.3)			
Hand washing before meals					
Yes	921	249 (27)	0.41	0.31-0.54	< 0.001
No (ref.)	279	132 (47.3)			

toxoplasmosis, and a higher prevalence has been reported among women ≥ 40 years [23].

Accordingly, the need for a global effort is necessary to educate women during child-bearing age about the potential of *T. gondii* transmission and the adverse effects of this parasitic disease. Washing hands before meals is an important factor that increases the risk of *Toxoplasma* contamination. The percentage of contaminated hand washers (27%) was much lower than that of non-washers (47.3%). According to previous studies, the seroprevalence of infection in Tabriz was lower than the average infection rate in Iran (39.3%) [21]. A study conducted in 2014 in east Iran revealed a 29.35% prevalence of toxoplasmosis in pregnant women [24].

The climate parameter is another important factor in *Toxoplasma* prevalence. The appropriate condition for oocyst sporulation and survival in the environment plays an important role in the infection rate and disease burden. Iran has seasonal variation so the weather condition varies in the provinces of this country. Studies have shown that a low prevalence of *Toxoplasma* infection belongs to cold and hot climates and a high prevalence belongs to wet and mild climates. Tabriz province is located northwest of Iran with cold climates which might be one of the reasons contributing to the low prevalence *Toxoplasma* infection [21,25].

Furthermore, our findings revealed that the proportion of *Toxoplasma* in women exposed to contaminated vegetables was higher (43.7%) than in women who were not exposed (29.3%). A study conducted in Mexico City found a significant increase in the number of women who consumed half-cooked lamb and raw vegetables compared to those who consumed it fully cooked [26]. Mizani *et al.* found the same results in Iranian women's systematic review and meta-analysis [25]. Another study was conducted in northern Ethiopia to evaluate seroprevalence and risk factors of *Toxoplasma gondii* among pregnant women. Results of the study showed 35.6% of pregnant women were found to be positive for Ab specific to *T. gondii*. The most significant risk factors associated with *T. gondii* were age, educational level, the habit of hand washing after contact with garden soil or domestic animals, presence of domestic cats, history of contact with domestic dogs, and consumption of raw vegetables [27].

Also, the study surveyed Tanzania to evaluate risk factors of toxoplasmosis. Overall *T. gondii* seroprevalence was reported at 44.5%. Two risk factors including consumption of raw and having regular contact with soil were both associated with *T. gondii* Ab status [28]. *Toxoplasma* infection can also be contracted by eating unwashed vegetables or drinking water that has been contaminated with soil or exposed to cat oocysts. Variations in *Toxoplasma* seroprevalence are related to the dietary habits of any given population [25]. Indeed, seronegative women during pregnancy are vulnerable to toxoplasmosis infection, which can be fatal if raw vegetables are consumed. As a result, some demographic factors such as climate, general knowledge, and eating habits are linked to *T. gondii* seroprevalence [15]. More research is required to complete the information flow, and a larger sample size should be implemented in multiple organizations. One-third of the pregnant women in Tabriz tested positive for *T. gondii* infection. Despite this, the absence of Ab in the sera of 68.3% of study participants raised concerns about congenital toxoplasmosis during pregnancy. Given the low seroprevalence of IgG and IgM Ab in the area, pregnant women must be aware of the disease's risk factors in order to reduce their risk of infection.

Conclusions

The findings suggest that soil exposure, cats, and poor personal hygiene may significantly increase the risk of infection. Understanding *Toxoplasma* risk factors may be beneficial for the prevention and control of the infection.

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Authors' contributions

The study was designed and directed by Ehsan Ahmadpour, Aleksandra Barac, Veeranoot Nissapatorn and Mohammad Hasan Kohansal. Material preparation and sample collection were performed by Sirous Mehrani Moghaddam, Gholamreza Barzgar and Mohammad Hasan Kohansal. Data analysis was performed by Tohid Jafari Koshki, Alok K. Paul and Jelena Micić. The first draft of the manuscript was written by Mohammad Hasan Kohansal and Ehsan Ahmadpour and reviewed by, Aleksandra Barac, Veeranoot Nissapatorn, Alok K. Paul and Jelena Micić. All authors read and approved the final manuscript.

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