



Assessment of Pregnant Women Toxoplasmosis by ELISA Method in Miandoab City, Iran

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Abstract

Objectives: Immunity to *Toxoplasma gondii* infection is known to be a critical point for women during pregnancy. Non immune pregnant women may at risk to be infected with the parasite. The parasite can be transmitted via placenta and causes adverse effects in fetus. The main objective of the present work was to study sero-prevalence of *Toxoplasma* infection in pregnant women referred to Health Center in Miandoab.

Materials and Methods: Totally 200 blood samples were collected from 18-40 years pregnant women referred to Health Center in Miandoab during one year. The sera were tested for IgG and IgM titration with enzyme-linked immunosorbent assay (ELISA) kits. In addition, demographic characteristics of the women were obtained through appropriate questionnaires.

Results: Positive IgG and IgM titers were identified in the sera of (39.5%) and (2.5%), of the pregnant women, respectively. The higher frequency of positive titers was associated with older age. In addition, 94.42% of the women with positive titer had a history of contact with cats.

Conclusion: Regarding the prevalence rate of 60% in non-immune pregnant women in Miandoab, the preventive measurements in nutrition and contact with cats should be considered by the women. We recommend the sera of the pregnant women should be monitored for *Toxoplasma* infection at least once a pregnancy period, particularly during the first trimesters of pregnancy.

Keywords: *Toxoplasma gondii*, Pregnant women, ELISA method, Iran

Introduction

The intracellular protozoan parasite, *Toxoplasma gondii*, causes infection in men and animals. The primary hosts which harbour the intestinal, sexual stage are cats (1,2). Transmission to humans happens mainly by eating raw or undercooked contaminated meat (3,4), raw cow's milk and birds eggs, swallowing oocysts discharged in faeces of infected cats, inoculation of trophozoites through the skin, or by inhalation (5-7). Transmission from a mother infected during pregnancy, to the fetus causes congenital toxoplasmosis (8-10). Human toxoplasmosis is worldwide. In adolescence and adulthood, most infections are subclinical or run a very mild clinical course (11). Toxoplasmosis is a systemic infection, always accompanied by the production of serum anti-bodies at high titre. After the acute stage antibodies persist at lower titre, usually throughout life. The number of seropositive persons in a population, therefore, increases with age. Although antibodies for *T. gondii* have been found in the sera of humans and animals throughout the world, the proportion of subjects with positive reactions varies considerably by geographic area, age and test method used (11-13). The risk, by age, of acquiring infection is not uniform throughout the world. It has been reported that prevalence of seropositivity among Eskimos is zero, among Brazilians, 72%

(14). Frequency among the population of the United States ranges from 10% to 20% in young adults and from 35% to 70% in older persons (11). Little is known about prevalence of *T. gondii* infection in Iran. The present study was undertaken to determine the prevalence of toxoplasmosis in pregnant women in Miandoab city, Iran for *Toxoplasma* IgG antibodies. Since very high IgG antibody levels may correlate with current infection, IgM testing may be used for differential diagnosis. Detection of IgM antibodies establishes the diagnosis of recently acquired or reactivated infection, but these antibodies soon disappear or decrease to very low levels followed by the appearance of IgG which stays longer. It was also planned to determine *T. gondii* IgM antibodies in pregnant women who were positive for toxoplasma IgG antibodies.

Materials and Methods

This cross-sectional study was performed from September to October 2014 in Miandoab city, north-west Iran. The city has a moderate and semi-humid climate. In the current survey, sample size was calculated considering a prevalence of 35%, a degree of precision of 4 (d=0.04) and 95% CI. Consequently, the sample size was calculated as 200 pregnant women.

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Collecting Samples

The objects were women referred to Health Center in Miandoab for routine examinations of pregnancy. A questionnaire containing sociodemographic and behavioral habits was designed and completed for individuals. Overall, 200 blood samples were collected and sera separated by blood centrifugation at 3000 rpm for 5 minutes. Serum samples were transferred to the laboratory and stored at -20°C until use.

Analyzing Samples

The anti-*T. gondii* IgG and IgM antibodies were tested with commercial enzyme-linked immunosorbent assay (ELISA) kit (Pishtaz Teb Zaman, Tehran, Iran) according to manufacturer instructions and results read by an automated ELISA reader machine (Avecina, Pishtaz Teb Zaman, Tehran, Iran). All samples were conducted as a single test. Standards with three different concentrations were employed to ensure kits were working properly and technical procedures were performed correctly.

Statistical Analysis

ELISA results and data from questionnaires were analyzed employing chi-square statistical test with 95% CI using SPSS version 16. The correlation between *T. gondii* infection with some variables such as age, living place (urban/rural), education, occupation, cat or other animals ownership, soil contact, consumption of raw/undercooked meat

or egg, consumption of raw/unpasteurized milk, vegetables washing method, frequency of consuming vegetables was estimated.

Results

The overall seroprevalence of *T. gondii* infections among pregnant women referred to Health Center in Miandoab was 41% (82/200). The IgG and IgM antibodies against *T. gondii* were positive in 79/200 cases (39.5%) and 5/200 cases (2.5%), respectively. Two pregnant women (1%) indicated both IgG and IgM antibodies against *T. gondii*. The results of seroprevalence along with personal and sociodemographic data are indicated in Table 1.

The correlation between age ($P=0.042$) and soil contact ($P=0.002$) with the *T. gondii* infection was statistically significant. No significant relationship was seen between toxoplasmosis and other tested variables.

Discussion

This study revealed a seroprevalence of 39.5% (79/200) and 2.5% (5/200) for IgG and IgM antibodies against *T. gondii* in pregnant women in Miandoab city, respectively. Congenital toxoplasmosis can lead to a wide variety of manifestations from spontaneous abortion and still-birth to hydrocephalus or microcephalus, cerebral calcifications and retinochoroiditis in the fetus and infant (11,12). Studies had been performed to evaluate the *T. gondii* infection in pregnant women or child bearing age in some countries

Table 1. Prevalence of Specific anti-*Toxoplasma* IgG and IgM Antibodies and Sociodemographic Data in Pregnant Women in Miandoab, Iran

Sociodemographic Characteristics	No. of Tested Women	Prevalence of <i>Toxoplasma gondii</i> IgG		Prevalence of <i>Toxoplasma gondii</i> IgM	
		No.	(%)	No.	(%)
Age group (y)					
≤20	22	6	27.3	1	4.5
21-30	141	49	34.7	4	2.8
>30	37	19	51.35	1	2.7
Total	200	79	39.5	5	2.5
Location					
Urban	115	44	38.2	7	6.08
Rural	85	37	43.5	2	2.3
Education					
None	10	4	40	1	10
Elementary school	38	20	52.6	2	5.2
Guidance school	48	21	43.75	2	4.1
High school	83	31	37.3	4	4.8
University	21	7	33.3	1	4.7
Occupation					
Employed	16	5	31.2	1	6.25
Unemployed	184	75	40.76	12	6.52
Gestational age					
First trimester	92	37	40.21	3	3.26
Second trimester	25	10	40	1	4
Third trimester	83	32	38.5	1	1.2

and different seroprevalences were estimated. The reported seroprevalences of *T. gondii* infection were 51.4% in Saudi Arabia (13), 59% in Argentina (14), 43% in Austria (15), 30% in Spain (16), 22.1% in Slovakia (17), 24.6% in Turkey (18) and 92.5% in Ghana (19). The prevalence rate of 29.1% and 0.8% for anti-IgG and IgM antibodies in pregnant women was estimated in Zair, Nigeria (20). Among pregnant women tested in rural Durango State, Mexico, IgG antibodies against *T. gondii* infection varied from 0% to 20% in different communities. Overall, 8.2% had IgG and 2.3% had IgM antibodies, too (21).

In Iran, the prevalence rates of 22.7% and 31% were estimated in pregnant women from Kermanshah (22) and Khorram-Abad (23), whereas the rate of *T. gondii* infection was 20.1% and 19.2% in pregnant women of Isfahan (24) and Sabzavar (25), respectively. Abdi et al found the prevalence rate of 44.8% of infection in Ilam province (26). A study in Kerman, South eastern Iran, reported a prevalence of 46.9% in pregnant women (27). In Zanjan city, located in northwest of Iran, 1.4% and 37.2% of tested pregnant women had IgG and IgM antibodies against *T. gondii*, respectively (28-31).

North of Iran has suitable climate for oocyst sporulation of *T. gondii*, so high prevalence of infection is expected there. The present study showed a high rate of IgG anti-*T. gondii* anti-body (39.5%) positive along with a relatively low prevalence rate for IgM (2.5%) in pregnant women in Miandoab city.

However, in the present study the relation of the *Toxoplasma* infection with age was statistically significant that is in concordance with results of some previous studies in other parts of Iran such as Bandar Abbas (32), Hamadan (34), Khorram-Aabad (23,36,37) and Alashtar (35,38,39). In Turkey the percentages of seropositivity of IgG antibody for *T. gondii* and Rubella and Cytomegalovirus are 31.5%, 90% and 73.3% respectively. The percentages of seropositivity of IgM antibody for *T. gondii* and Rubella and Cytomegalovirus are 2.0%, 0.6% and 3.7% respectively (40).

Also, our results indicated a significant correlation between *T. gondii* infection and soil contact that is not surprising since north-west of Iran has appropriate climate for oocyst sporulation and contacting with oocyst infected soil is one of the common routes of human infection. In the current study there was no statistically significant relationship between toxoplasmosis and some tested criteria such as living place (urban/rural), education, occupation, cat or other animals ownership, or egg, consumption of raw/unpasteurized milk, vegetables washing method, frequency of consuming vegetables. Whereas significant correlation was reported between the infection with education, consumption of raw/undercooked meat and frequency of consuming vegetables (41,42) in some previous studies in Iran.

Conclusion

The results of this study indicate that about 60% of pregnant women in this city had no contact with the parasite

and are at risk for congenital toxoplasmosis, so, preventive measures and establishing diagnostic toxoplasmosis tests during pregnancy are warranted.

Ethical Issues

We have no ethical issues to declare.

Conflict of Interests

The authors declare no conflict of interests.

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