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Original article

Evaluation of seroprevalence and seroconversion rates of Toxoplasma gondii infection in female students at Babol University of Medical Sciences, Babol, Iran

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Abstract

Background: The primary infection of pregnant women by Toxoplasma gondii can cause severe complications in the fetus, and can also lead to neurological complications. The aim of the present study was to determine the seroprevalence and seroconversion rates of Toxoplasma gondii in female students who were at childbearing age in Iran.

Methods: This cross sectional study was performed on female students at Babol University of Medical Sciences (Babol, Iran) over a three-year period between 2012 and 2014. Two ml blood sample was obtained from volunteers, and the specific antibodies (IgG) to T. gondii were detected by Enzyme-Linked Immunosorbent Assay. Second, the blood samples obtained from seronegative cases were used to evaluate the seroconversion during a year.

Results: The mean age of the subjects was 21±2.2, ranging from18 to 35 years. Overall, 28% of the participants were positive and 232 (72%) were negative for anti-Toxoplasma antibodies (IgG). The rate of this infection was higher among female students from Mazandaran province (31.7% vs 12.5%) (P=0.031). Based on the results of Enzyme-Linked Immunosorbent Assay, the seroconversion rate of Toxoplasma infection among female students was estimated to be 2.5%.

Conclusion: This study found a noticeable rate of seroconversion in female students. Therefore, there is an urgent need for a national screening project to determine the seroconversion rate of Toxoplasma infection in women at childbearing age, particularly pregnant women from all over Iran.

Keywords: Anti-Toxoplasma antibodies, Prevalence, Seroconversion, Toxoplasma gondii, Women

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite widely distributed throughout the world. Human and other warm-blooded vertebrates have been known as intermediate hosts and cats are the definitive host (1). Commonly, Toxoplasma-infection in human occurs by the ingestion of oocysts released

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from cats' feces or Toxoplasma-tissue cysts via eating raw and under-cooked meat. Toxoplasma is classified as a "Category B pathogen" because the infection can remain in the host for life in a cyst format in various organs such as muscles and the brain. These cysts may be reactivated, which could have life-threatening results (2). However, the course of infection could be generally benign in immunocompetent persons, but it may be life-threatening in immunocompromised individuals (3, 4). Furthermore, T. gondii can be transmitted from a mother to her fetus through the placenta and cause severe complications such as miscarriage, stillbirth, chorioretinitis, cerebral calcifications, and hydrocephalus (5, 6).

The prevalence of congenital toxoplasmosis varies in different epidemic areas ranging from 0.1 to 0.3 per 1000 live births (6). The burden of congenital toxoplasmosis in the United States showed one case for every 10,000 live births, and about 400 newborns have, as a result, acquired congenital toxoplasmosis (2, 7). In France, the overall prevalence and the incidence of congenital toxoplasmosis were 3.3 and 2.9 per 10,000 live births, respectively (8). The prevalence of congenital toxoplasmosis in other European countries, e.g. in Poland, Denmark, Sweden and Switzerland were reported 5.5, 2.1, 0.73 and 4.3 per 10,000 live births, respectively (9-12). The prevalence rate of congenital toxoplasmosis is extensively higher in developing countries such as Argentina and Brazil, for which the rates have been reported to be 5.8 and 5-23 per 10, 000 live births (13, 14).

However, several studies on the seroprevalence of toxoplasmosis in adults, pregnant women in particular, have already been performed in Iran. The results of these studies demonstrated that the rate of toxoplasmosis was between 23.7-71.3% (15, 16). According to one study performed on pregnant women and their offspring in Iran, it was found that the risk of infection during pregnancy in seronegative individuals was 14 per 1000 in Tehran. It was also found that the risk of congenital toxoplasmosis was 6.1 per1000 live births (17). Currently, there is no screening program for pregnant women in Iran, and the incidence and the prevalence of Toxoplasma infection are mainly unclear. In order to plan a strategic approach for the prevention of congenital toxoplasmosis, it is necessary to know the seroconversion rate of Toxoplasma infection in general population, particularly in women at childbearing age. To this end, this study tried to investigate the rates of seroprevalence and the seroconversion rate of Toxoplasma gondii in female students at childbearing age in Iran.

Materials and Methods

This cross sectional study was performed on female students at Babol University of Medical Sciences (Babol, Iran) over a three-year period between 2012 and 2014. The study was approved by the Ethics Committee of Babol University of Medical Sciences, for ethics in medical research (No. MUBABOL. REC.1393.10). This university is located in Babol, Mazandaran Province. It comprises five schools with annual admission of approximately 1000 students. Most of the students there come from different parts of the Mazandaran province. This province, located in north of Iran, has an area about 23,831.64 Km2, which is close to the Caspian Sea, and is at the interface of Golestan, Gilan and Tehran provinces from the northeast, northwest and south, respectively. The mean annual temperature varies between 13 to 23.3°C, and the annual rainfall ranges are from 59.1 in the spring to 367.2 mm in autumn. The mean relative humidity is 80%, ranging from 56% in summer to 92% in the autumn (18). The minimum sample size was based on the estimated prevalence of 52% with a standard score of (Z) 95%, and α=0.06 was calculated to be 266. The power estimation was not calculated for the seroconversion rate in the current study. Informed consent forms were given to all participants in this study. Two ml blood samples were taken from each participant, and the serum was separated. The samples were centrifuged and then stored at -20°C until use. All samples were tested in the microbiology department at Babol University of Medical Sciences. After collecting the samples, the frozen sera were thawed in room temperature and were evaluated for Toxoplasma IgG antibodies using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit (EUROIMMUNE, Toxoplasma IgG, UK). Based on the manufacturers' instruction, the IgG antibody titers were read at optical density (OD) of 450 nm using an automatic ELISA reader. The samples with sera IgG titer < 8 IU/mL were considered to be negative for anti-Toxoplasma gondii IgG antibodies and11≥ IU/mL positive. The sensitivity and specificity of the assay are reported 100% by the manufacturer. To evaluate the seroconversion rate during one year, all seronegative cases were called back after one year from the first

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Table 1. Association of certain socio-demographic variables with prevalence of anti- Toxoplasma gondii antibodies (IgG) in female students, Babol University of Medical Sciences, Babol, Iran.

Variables	N	(%)	Anti-Toxoplasma (IgG)				*P*-Value
			Positive		Negative		
			N	(%)	N	(%)	
Overall	322	100	90	(28)	232	(72)	
Age	313	100					
<21	228	72.8	62	(27.2)	166	(72.8)	0.698
>21	85	27.2	25	(29.4)	60	(70.6)	
Locality*	308	100					
Yes	252	78.3	80	(31.7)	172	(68.3)	0.004
No	56	21.7	7	(12.5)	49	(87.5)	
Soil Contact	191	100					
Yes	68	35.6	21	(30.9)	47	(69.1)	0.334
No	123	64.4	30	(24.4)	93	(75.6)	
Cat owner	194	100					
Yes	34	17.5	13	(38.2)	21	(61.8)	0.098
No	160	82.5	39	(24.4)	121	(75.6)	

* Yes: From Mazandaran; No: From other provinces

blood sampling. Then, two ml blood samples were taken from each seronegative participant, and the ELISA test was repeated.

Statistical analysis

The data were analyzed by SPSS version 19.0. Chi-square was used to analyze the data at 95% confidence level. The P-value of less than 0.05 was considered significant.

Results

A total of 322 female students, with the mean age of 21±2.2 ranging from18 to 35, were participated for the purpose of this study. The majority of the participants, 252 (78.3%), were from Mazandaran province, where the present study was performed (Fig 1).

Overall, 90 (28%) cases were proved positive and 232 (72%) were negative for anti-Toxoplasma antibodies (IgG). The rate of infection was higher among female students from Mazandaran province (31.7 %) compared with those who were from other provinces (12.5%) (P=0.031) (Table1).

The highest prevalence in Mazandaran province was observed in participants from Gallogah (66.7%), which was followed by participants from Noor (50%), Jouibar (45.5%) and Mahmoodabad (37.5%), respectively (Fig. 1). Furthermore, we found that the prevalence of anti-T. gondii antibodies (IgG) was higher in individuals who had contact with cats and soil compared with cases who had no contact with cats and soil (Table 1).

118 out of 232 seronegative subjects participated in the second part of the current study. The second blood samples were obtained after one year from the first blood sampling, and the presence of anti-Toxoplasma antibodies were evaluated. The Toxoplasma infection was found in 2.5% cases. All participants who were seroconverted for Toxoplasma infection were from the Mazandaran province. Also, they did not mention any symptoms and signs related to acute toxoplasmosis.

Discussion

The results revealed that 28% of female students were positive for anti- Toxoplasma gondii antibodies (IgG). This seropositivity rate is significantly lower than the rates reported in north of Iran (15, 19). Higher rates were reported for East Azarbaijan province (20) and Qazvin (21). However, the prevalence rates of Toxoplasma infection are varied in different parts of the world.

Reports, for instance, show that the prevalence rate in child bearing population is 48.6% in France (22), 51.4% in Saudi Arabia (23), 30.7% in Turkey (24), 92.5% in Ghana (25) and 88.6% in Ethiopia (26). In Iran, the prevalence rates of Toxoplasma infection in women at reproductive age were estimated to be

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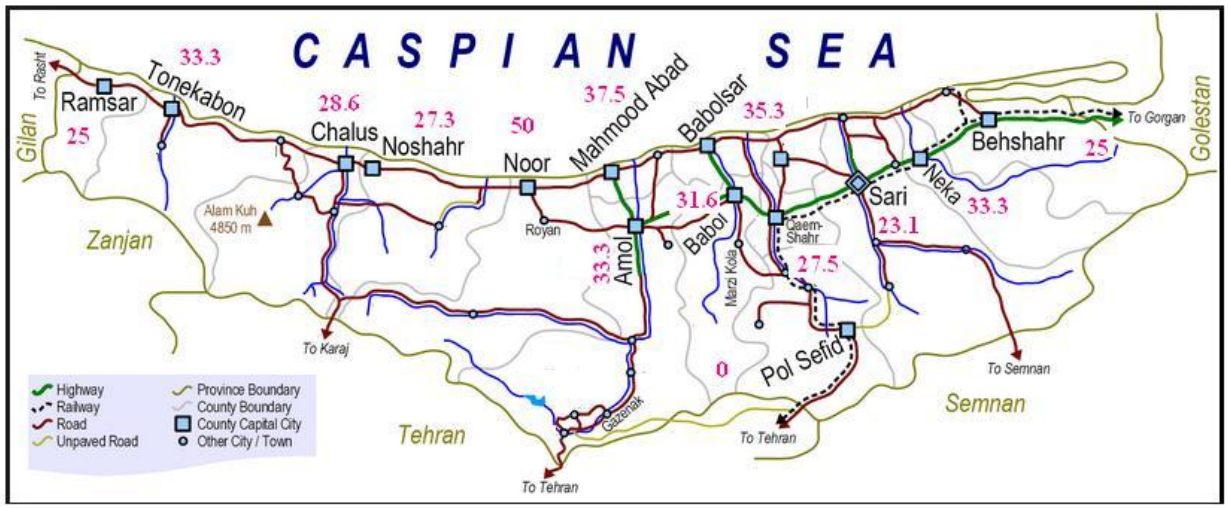

Fig. 1: Distribution of anti- Toxoplasma gondii antibodies (IgG) prevalence among female students came from different parts of the Mazandaran province. The prevalence rates were expressed as a percentage of the population came from each city (%).

54.13% in northwest provinces (16), 33.5% in Hamedan (27), 16% in Ajabshear (20), and 60.6% in Babol (15).

Many factors can affect the seroprevalence of toxoplasmosis in humans. These include climatic factors, anthropogenic reasons, underestimated cats, and the quality of water and sanitation coverage. Anthropogenic factors include dietary habits, economic and social factors, which could explain a large part of the variations in human seroprevalence (28). It is suggested that dietary habits such as the method of cooking meat, hygiene, the type of meat or vegetables consumed, and the washing of vegetables can play an important role in the rate of Toxoplasma infection. It should be mentioned that there is a noticeable change in the rate of Toxoplasma infection between our previous study and the present one (60.6% vs.28%) (15). This finding is in line with the result of another study which found that married women have a higher seroprevalence rate of toxoplasmosis than unmarried women (29).

The mean age of seropositive individuals (21.03±2.22) was approximately the same as the mean age of seronegative cases (20.97± 2.23). There was also a slight increase in the prevalence rate among female students over 21 compared with those under 21 (29.4% vs 27.2%). There are many studies reporting that the seroprevalence of Toxoplasma infection can increase with age (1, 30, 31). With regard to age, the rate of infection can vary according to the country and socioeconomic level (28).

In the present study, we found a statistically significant difference in Toxoplasma infection between Mazandaran province and other parts of the country, which is consistent with the results of other studies. Several reports from different parts of Iran, with the population similar to ours, found lower seroprevalence rates of toxoplasmosis than those found in north of Iran (1, 16, 32). A possible explanation for that is the climate in Mazandaran province, which is very humid and relatively warm. It is worth mentioning that toxoplasmosis is more common in humid and warmer climates than in more arid climates, mountainous, and colder regions.

The findings of this study revealed that the seroconversion rate among susceptible women at childbearing age during one year, 2.5% (3 out of 118), was relatively high. The seroconversion rate of Toxoplasma infection can vary significantly from country to country. For example, seroconversion was obtained as zero and 2.4/1000 in pregnant women in Turkey (33) and in women aged 30 in France, respectively (34). In Iran (2002), the seroconversion rate of Toxoplasma gondii infection in pregnant women was 1.4% (17), and there have been no recent studies to evaluate the incidence of toxoplasmosis in susceptible populations.

We assume that soil contact may be the main risk factor for Toxoplasma gondii infection in our region. The finding, accordingly, showed that two out of three (66. 7%) seroconverted cases had soil contact history and experience in gardening. This result is consistent

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with the life cycle of the parasite. Many studies indicated that contact with the contaminated soil and the sporulated oocysts are the main cause of contamination (28).

The current study had some limitations such as a high level of missing cases for soil contact and cat ownership, and the small sample size of the seronegative population attending the evaluation of the seroconversion rate. Another limitation of this study was the evaluation of seroconversion of toxoplasmosis by IgG screening test.

In conclusion, this study demonstrated that more than two-third of female students were seronegative, and were, as a result, at risk of Toxoplasma gondii infection on contact. It also found a noticeable rate of seroconversion in female students. There is an urgent need for a national screening project to determine the seroconversion rate of Toxoplasma infection in women at childbearing age in Iran.

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Conflict of interest

The authors have no conflicts of interest.

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