



Toxoplasmosis-related risk factors in pregnant women in the North Khorasan province, Iran

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ABSTRACT

Congenital toxoplasmosis is a disease with severe clinical manifestations in newborns so screening of pregnant women is crucial. This study was aimed at evaluating the latest status of toxoplasmosis in pregnant women and its respective risk factors in the northeast of Iran. This survey was conducted between 2013 and 2015 on 350 pregnant women. Blood samples were taken from the participants and their serums were separated. Anti-Toxoplasma IgG and IgM were assessed in the serum samples using ELISA method. Moreover, a questionnaire about toxoplasmosis-related risk factors and other information was completed by each participant. Of the 350 pregnant women studied, 110 (31.42%) were positive for IgG and 12 (3.42%) for IgM. The IgM-positive subjects were also positive for IgG. Age of pregnancy, residence area, contact with cat, degree of meat cooking, unwashed raw vegetable or fruit consumption, raw milk consumption, and history of miscarriage were toxoplasmosis-related risk factors considered in this study, the results showed that all these factors were statistically significant in IgG-positive subjects. In IgM-positive subjects, contact with cat, degree of meat cooking, unwashed raw vegetables, fruits consumption, and history of miscarriage were among the more important risk factors. The new cases of toxoplasmosis are being occurring in the pregnant women in the region under the study and therefore these pregnancies are uncertain.

Keywords: Toxoplasmosis, Risk factors, Pregnant women, Iran

INTRODUCTION

Toxoplasma (*T.*) *gondii*, a parasitic protozoan with worldwide prevalence, is able to infect wide-ranging warm-blooded vertebrates such as human [1]. With a prevalence of about one-third of population all over the world, toxoplasmosis, the disease caused by this parasite, is mostly transmitted to human via consumption of *T. gondii* oocyst-contaminated food and water as well as raw meat, especially lamb, infected with *T. gondii* tissue cyst; rarely, it can also be transmitted via contaminated egg or raw milk [2]. About 80% of toxoplasmosis cases were related to the acquired toxoplasmosis, mostly with a mild fever and ephemeral lymphadenopathy symptoms in immunocompetent individuals [1]. Given that these patients do not usually visit physicians, the risk of silent epidemics for this parasite arises. Although benign in healthy people, toxoplasmosis is a life-threatening disease in patients infected with AIDS/HIV⁺ as well as those taking immunosuppressant's [1]. More importantly, congenital

toxoplasmosis is one kind of this disease affecting the fetus and depending on the time at which pregnant women are infected, its clinical manifestation is different [3]. Accordingly, infection of pregnant women is divided into three trimesters in which the risk of vertical transmission of the parasite increases over time but the disease severity decreases [3]. Hydrocephaly, chorioretinitis, microcephaly and other CNS-related manifestations in newborns are of diagnostic importance in congenital toxoplasmosis [3]. The most common method for diagnosis of congenital toxoplasmosis is detection of IgG and IgM in pregnant women sera using various diagnostic tests, including enzyme-linked immunosorbent assay (ELISA) which enjoys higher validity. In addition to serodiagnostic tests such as ELISA, some types of polymerase chain reaction (PCR) methods, especially nested-PCR, are useful to find *T. gondii* parasite in the tissues of miscarried fetus [4].

As noted earlier, taking into consideration that toxoplasmosis is far more important in pregnant women and regarding the fact that the incidence of this disease is often silently; this study was aimed at evaluating the latest status of toxoplasmosis in pregnant women and its respective risk factors in the northeast of Iran.

MATERIALS AND METHODS

Subjects

This study was verified by the university research ethics committee (UREC) of the North Khorasan University of Medical Sciences. This survey was conducted between 2013 and 2015 on pregnant women referred to the Imam Khomeini Hospital in the Shirvan city located in the North Khorasan province, the northeast of Iran. In this study, 350 pregnant women natives of the province at the age range of 18 to 45 enrolled and completed informed consent form. A questionnaire about toxoplasmosis-related risk factors and other information was given to each participant to be completed.

Sampling

From each participant a blood sample of 3 ml was taken, the serum of which was separated by centrifugation at 3000 ×g, 4 °C, for 10 min. Subsequently, the harvested sera were poured into sterile microtubes separately and stored at -20 °C until use.

Anti-Toxoplasma antibodies measurement

Anti-*Toxoplasma* antibodies were measured in pregnant women for detection of infection with *T. gondii*. This was performed by ELISA method and the use of ELISA kit (Trinity Biotech Inc.) based on manufacturer instruction. For this objective, anti-*Toxoplasma* IgG and IgM were assessed as the new infections to be distinguished from the old ones.

Data analysis

The data were evaluated by Kolmogorov-Smirnov statistical test in order to check whether the data are normally distributed or not. Chi-square and Fisher's exact test were used to investigate the relationship between categories in the questionnaire and risk-related factors. The researchers found a statistically significant difference between the variables with the P-value being less than 0.05. The statistical tests were performed using PASW Statistics v.18 software (IBM-SPSS Inc.).

RESULTS

Out of 350 pregnant women under the study, 110 (31.42%) were positive for IgG and 12 (3.42%) for IgM. The IgM-positive subjects were also positive for IgG. Therefore, the overall prevalence of toxoplasmosis in pregnant women was the same (31.42%). Regarding the IgG-positive cases, the statistical analysis showed a significant difference for all of the risk factors considered in this study (Table 1).

In IgM-positive subjects, it showed a significant difference for the risk factors including contact with cat, degree of meat cooking, unwashed raw vegetables and fruits consumption, and history of miscarriage. All of the risk factors related to the IgM-positive cases are shown in Table 2.

Table 1. Risk factors and their relation to IgG positive pregnant women

Risk factors	Number of subjects	Positive cases (%)	P-value ^a
Age of pregnancy			
18-25	140	34 (24.3)	< 0.001
25-35	157	66 (42)	
35-45	53	10 (18.9)	
Residence area			
Urban	229	56 (24.5)	< 0.001
Rural	121	54 (44.6)	
Contact with cat			
Yes	196	97 (49.5)	< 0.001
No	154	13 (8.4)	
Degree of meat cooking			
Extra-rare or rare	17	14 (82.4)	< 0.001
Well-done	333	96 (28.8)	
Unwashed raw vegetable or fruit consumption			
Yes	30	26 (86.7)	< 0.001
No	320	84 (26.3)	
Raw milk consumption			
Yes	198	80 (40.4)	< 0.001
No	152	30 (19.7)	
History of miscarriage			
Yes	35	35 (100)	< 0.001
No	315	75 (23.8)	

^aP-value was calculated by chi-square statistical test**Table 2. Risk factors and their relation to IgM positive pregnant women**

Risk factors	Number of subjects	Positive cases (%)	P-value
Age of pregnancy			
18-25	140	5 (3.6)	0.302 ^a
25-35	157	7 (4.5)	
35-45	53	0	
Residence area			
Urban	229	6 (2.6)	0.354 ^b
Rural	121	6 (5)	
Contact with cats			
Yes	196	12 (6.1)	0.002 ^b
No	154	0	
Degree of meat cooking			
Extra-rare or rare	17	0	< 0.001 ^b
Well-done	333	12 (3.6)	
Unwashed raw vegetable or fruit consumption			
Yes	30	6 (20)	< 0.001 ^b
No	320	6 (1.9)	
Raw milk consumption			
Yes	198	4 (2)	0.138 ^b
No	152	8 (5.3)	
History of miscarriage			
Yes	35	5 (14.3)	0.004 ^b
No	315	7 (2.2)	

^aP-value was calculated by chi-square statistical test^bP-value was calculated by Fisher's exact test

DISCUSSION

It has been demonstrated that *T. gondii* had three genotypes including genotypes I, II, III [5]. Moreover, mixed genotypes including I/II, I/III, and II/III were also reported for this parasite [6]. The most prevalent genotype isolated from human is genotype II which includes congenital toxoplasmosis as well [7]. Studies showed that toxoplasmic miscarriage in murine models can happen several times by a given *Toxoplasma* strain while it can cause miscarriage in human only once [8]. Nevertheless, the results of one study showed that *T.gondii* infection can cause repeated miscarriages in a pregnant woman [9], raising the hypothesis that it may occur due to the infection with different strains of *T. gondii*. This issue should be clarified in further studies. The prevalence of this parasite in different countries is dissimilar depending on people life style such as dietary habits, keeping cat as well as host geographical distribution in a region, parasite strains and so on. One example in this matter is the fact that the

transmission pattern of toxoplasmosis in India is usually by way of oocyst ingestion and this is different from the rest of the world [10]. It seems that in the United States about 85% of women at childbearing age are at risk of infection with *T. gondii* with the incidence of congenital toxoplasmosis in this region being between 400-4000 cases annually [11]. The toxoplasmosis prevalence in pregnant women of the European countries is estimated to be between 2-70% with the lowest prevalence in Italy and the highest in France [12, 13]. Studies in the African countries showed the prevalence of toxoplasmosis in pregnant women was approximately between 20-60% [14-16]. Based on the studies conducted in Asia, it has prevalence between 3-35% in different countries [17-19]. In Iran, many studies have been conducted on this issue in different provinces and the result showed that the prevalence of toxoplasmosis in women at childbearing age is between 23-64% depending on IgG antibody [20]. In addition, in pregnant women, the highest prevalence rate of IgG and IgM was related to the Khuzestan (70.65%) and Mazandaran province (12.4%), respectively [20, 21]. Moreover, the lowest prevalence of both IgG (17.2%) and IgM (0%) were related to Tehran province [22]. One study in the north of Iran showed that about more than half of the pregnant women in the region had anti-*T. gondii* antibodies in their serum [23]. The closest study to the current one in terms of geographical location is the one conducted in the Mashhad city (i.e. distance from Mashhad to Shirvan, 208 km) which showed the prevalence rate of toxoplasmosis was about 35% in pregnant women [24]. The result of the present study is consistent with theirs; however, in the current study, anti-*T. gondii* IgM was also detected in 12 subjects (Table 2) in whom anti-*T. gondii* IgG was considerably high as well. This issue strengthens the hypothesis that the increase in IgM in these individuals may be due to the infection with new strain(s) of this parasite. Interestingly, the more significant risk factors observed in this study were contact with cat, and consumption of unwashed raw vegetable or fruit (Table 1 and 2), suggesting that the predominant transmission pattern of toxoplasmosis in the region under study is most likely by way of oocyst ingestion. In addition, the findings showed that the age group 25-35 years old had the highest positive cases.

As a final point, as discussed elsewhere [25], the authors of the present study believe that health education plays an important role in reducing infection with this parasite in the studied region and other areas where infection with this parasite is high. Moreover, this study showed that new cases of toxoplasmosis are being occurring in pregnant women in the region under the study and therefore these pregnancies are uncertain. Moreover, it is essential to evaluate whether or not different strains of this parasite can cause repeated miscarriages or congenital toxoplasmosis in a woman in different pregnancies.

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