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ORIGINAL ARTICLE

Seroepidemiology of *Toxoplasma gondii* infection in pregnant women in west Iran: determined by ELISA and PCR analysis

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Abstract Congenital toxoplasmosis can lead to severe damage for the fetus and newborn. Considering that the seroepidemiology of *Toxoplasma* infection in the pregnant women is poorly studied in west of Iran, the main objective of this study was to estimate the seroprevalence and potential risk factors for congenital toxoplasmosis in Delfan, Iran. In this cross-sectional study, the serum samples obtained from pregnant women who were referred to health centers for routine monitoring of the pregnancy. Totally, 264 sera were screened for IgG and IgM anti-*T. gondii* antibodies by enzyme linked immunosorbent assay method. All women with IgM anti-*T. gondii* positive checked by RT-PCR and confirmed. In addition, structured questionnaires were used to obtain information on risk factors for *T. gondii* infection. Anti-*Toxoplasma* IgG and IgM were positive in 66 (25 %) and 15 (5.7 %) respectively. Seropositive subjects were more frequently seen in those with >30 years old compared to younger women (<25 years old) ($p < 0.001$). No significant relationship was found between the seroprevalence of *T. gondii* infection and level of education, and gestational age ($p > 0.05$), while there was statistical difference between the infection with cat exposure, consumption of raw/undercooked meat, eating raw or uncooked eggs, consumption of unwashed vegetables and drinking unpasteurized milk ($p < 0.001$). In the present study, it was found that *T. gondii* infection was present among pregnant women in west of Iran. Therefore, it is suggested to provide health education for preventing primary infection during pregnancy and subsequently congenital toxoplasmosis in the pregnant women.

Keywords *Toxoplasma gondii* · IgM · IgG · ELISA · PCR

Introduction

Toxoplasmosis is a zoonosis disease caused by a protozoan apicomplexan parasite called *Toxoplasma gondii* that can infect all warm-blooded vertebrates, including mammals and birds throughout the world. Almost 1/3 of the world's population has been exposed to this parasite worldwide. Although feline species are the definitive host, a wide variety of warm-blooded animals and birds can act as a intermediate host (Alsammani 2014).

Toxoplasma gondii infection may occur vertically by cross-placental transmission and infect the fetus (congenital transmission), or horizontally by oral ingestion of infectious oocysts from the environment, oral ingestion of tissue cysts contained in raw or undercooked meat or primary offal (viscera) of intermediate hosts, unpasteurized milk and tissue transplants (Mahmoudvand et al. 2015). Whereas infection of healthy adult humans is generally mild to asymptomatic, severe disease can outcome in utero or in Immunodeficiency host. The fetus is only endangered of congenital disease when acute

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infection happens in pregnancy (Shaapan 2015). Congenital infection has also been reported from a chronically infected immunocompromised mother with a reactivation of toxoplasmosis (Goldstein et al. 2008). Economical damages of toxoplasmosis are of medical and veterinary importance, in humans is due to abortion, embryonic dissonance, mortality and morbidity in congenitally infected and immunocompromised patients (Dubey 2009; Ezatpour et al. 2009). A significant reduce in the prevalence has been observed during the last few decades. As raw meat is probably one of the main sources of *T. gondii*, reduce in frequency is may be related to betterment in health education and meat processing. Prevention of congenital toxoplasmosis in pregnant women has been based on serological test for *Toxoplasma* antibodies. Several studies were conducted about the screening for *T. gondii* antibodies among Iranians peoples. The overall seroprevalence rate of *T. gondii* is among the general population in Iran was 39.3 % (Daryani et al. 2014). In recent years, several studies reported the rate of toxoplasmosis in Iranian cities such as; 30.8 % for Zahedan (Ebrahimzadeh et al. 2013), 27.3 % for Khuzestan (Yad et al. 2014), 37.8 % for Zanjan (Hajsoleimani et al. 2012), 44.8 % for Ilam (Abdi et al. 2008), 33.5 % for Hamadan (Fallah et al. 2008), and 30.4 % for Khorramabad (Ezatpour et al. 2013). In order to design a strategic approach for the prevention of congenital toxoplasmosis, it seems necessary to know the prevalence of infection amongst pregnant women. Anyway, toxoplasmosis should be diagnosed at the initial acute stage, when treatment is more helpful.

To the best of our knowledge, there is infrequent data about the epidemiology of this zoonotic parasite in pregnant women in Lorestan. This study aims to determine the prevalence of IgM and IgG anti-*T. gondii* antibodies and the associated risk factors among pregnant women in Delfan city (Lorestan, Iran) using enzyme linked immunosorbent assay (ELISA) method, and confirmed IgM anti-*T. gondii* positive by RT-PCR.

Materials and methods

Study area

The study was carried out in Delfan, a mountainous region in Lorestan province, west of Iran with a latitudinal position of latitude 34°04′N and longitude 47°58′E. Delfan is one of the five cities with more than 2000 m heights from the sea level in Iran and has very cold winters. The city has a population of 137,385 based on 2006 census ("Statistics in Iran, I.B.O. population census", 2009).

Study design

This cross-sectional study was conducted from April 2012 to March 2013. The sample size was calculated using an expected IgG prevalence of 35 %, a desired precision of 0.05 with 95 % level of confidence. Therefore, the sample size was calculated as 264 pregnant women.

Ethics

This study was approved by Ethics Committee of Iran University of Medical Sciences (Tehran, Iran). In addition, a written informed consent was obtained from all the participants before blood sampling.

Participants and sample collection

The study populations were pregnant women aged between 16 and 45 years. Blood samples were collected from women visiting four urban health centers, ten rural health centers and eighteen seven city-wide health houses for antenatal follow up or medication. Blood samples (5 ml) were collected from the participants by aseptic technique. The collected blood samples were centrifuged at 3000 rpm for 10 min and the sera/plasma were separated. Sera were aliquoted and stored at −20 °C until the time of use.

Questionnaire

After taking the informed consents, a trained person distributed the questionnaires. A questionnaire sheet was designed to evaluate several main risk factors that may influence the prevalence of toxoplasmosis. These information were intended to be completed by interviewing each participant during health centers visit. The important risk factors considered in our study include: age, educational levels, stage of pregnancy, contact with cats, consumption of raw/undercooked meat, eating raw or uncooked eggs, consumption of unwashed vegetables and drinking unpasteurized milk.

Serologic tests

All the samples were tested by ELISA for assessment of *T. gondii* IgG and IgM antibodies using ELISA kit (VIRO-IMMUN Labor-Diagnostika, Germany). The cut-off value of the test was computed according to the manufacturer's instructions and results were expressed in an index by dividing sample absorbance by the cut-off value. The result was considered positive if the index was $\geq$1.1, the result was equivocal when index was from 0.9 to <1.1, while the negative result was indexed <0.9. A negative reaction demonstrates non attendance of *Toxoplasma* antibodies.

Real-time PCR

To confirm the presence of *T. gondii* in the IgM-positive cases, sera was collected 4 weeks after the first sampling and analyzed for DNA detection of *T. gondii* with PCR. Isolation of DNA from blood samples was performed through a high Pure PCR Template Preparation Kit (Roche Applied Sciences, Germany) according to the manufacture's protocol. At the end of the procedure, 200 μL of eluted buffer yielded approximately 0.5–25 ng/μL purified DNA. SYBR® Green was used to detect fluorescence in real-time PCR using the StepOne™ Real-Time PCR System (Applied Biosystems). The primer used (Invitrogen) amplified a 126 bp B1 gene region. The forward and reverse sequences were 5′-GGAGGACTGGCAACCTGG TGTCG-3′ and 5′-TTGTTTCACCCGGACCGTTTAGC AG-3′, respectively. As positive control, *T. gondii* genomic DNA, serially tenfold diluted and ranging from 5000 to 0.5 parasites per microliter (TIB MolBiol), and 1 negative control, prepared by the substitution of template DNA with distilled water, were used in each Real-Time PCR run. Melting curve analysis was performed for the Real-Time PCR positive samples after quantification analysis.

Statistics

The SPSS 16.0 software for Windows (SPSS Inc., Chicago, Illinois) was used to record data and for analyses. Chi Square and logestic regression were used for comparison and appropriate p value of <0.05 was considered significant.

Results

Totally, 264 pregnant women with an average age 22.37 years consented to be included in this study. The overall prevalence of *T. gondii* antibodies was 30.7 %. Among this participants, 15 (5.7 %) and 66 (25 %) were seropositive for IgM and IgG antibodies, respectively. There was no statistical relationship between IgG and IgM antibodies with educational levels and gestational age ($p > 0.05$), while the significant difference was found between serum anti-*Toxplasma* antibody and other variables of this study (Table 1).

Real-time PCR

In order to detect the presence of *T. gondii* in the IgM-positive cases molecular assays has been done. Real-Time PCR demonstrated the no DNA of *T. gondii* was detected in any sera positive for anti-*Toxoplasma* IgM antibody.

Discussion

Congenital toxoplasmosis eventually has devastating clinical consequences for nearly all infected infants and survey of the seroepidemiology of this infection amongst women of childbearing age will help to design the preventive measures. Most epidemiologic studies about toxoplasmosis in Iran have been centralize on pregnant and childbearing age women, and broad range of prevalence rates of *Toxoplasma* antibodies have been publish as follows: Isfahan 45.7 %, Kerman 46.9 %, Ilam 44.8 %, and Hamadan 33.5 % (Keshavarz et al. 2000; Abdi et al. 2008; Fallah et al. 2008; Mostafavi et al. 2012; Ezatpour et al. 2013). Many investigation in various countries around the world have demonstrated that seroprevalence rate of *Toxoplasma* antibodies have a wide range as follow: Bangladesh 38.5 %, Turkey 49.4 % and India 49.52 % (Khatun et al. 1998; Al-Mendalawi 2010; Sarkar et al. 2012). Our study was carrying out on pregnant women to differentiate previously infected women from women who had not been previously infected, and to know the prevalence rate of pregnant women at risk of toxoplasmosis. Results of this investigation show that the prevalence of *Toxoplasma* antibodies in Delfan is less than some other regions of Iran. A moderate prevalence of chronic toxoplasmosis (IgG positive and IgM negative) during pregnancy was found in this study (25 %) compared to other areas of Iran that is likeness with weather and geological conditions of our area (Daryani and Sagha 2004; Fallah et al. 2005). At least 30 % of Iranian people have been found to seropositive for IgG antibody against T. *gondii* (Hashemi and Saraei 2010). The present study showed a seroprevalence of 30.7 % among 264 pregnant women. Thus, these pregnant women were not at risk for toxoplasmosis. Also in this study, the prevalence of recently acquired toxoplasmosis (IgM positive) was relatively little (5.7 %). This survey also showed the effect of age on seroprevalence of *T. gondii* among pregnant women. There was significant association between age and presence of IgM and IgG anti-*T. gondii* antibodies ($p < 0.001$). Women older than 30 years had a significantly higher seroprevalence (52.6 %) and 10.5 % compared to other age groups ($p < 0.001$). In addition, the significant relationship were demonstrated between *T. gondii* prevalence rate and the mother's age affirms the fact that seroprevalence of *T. gondii* is well known to increase with age; the greater the prevalence, the earlier the rise. This association does not mean that older age is a risk factor predisposing to toxoplasmosis but might be explained by the older the person the longer time being exposed to the causative agent and may retain a fixed level of IgG anti-*T. gondii* antibodies in serum for years (Al-Harthi et al. 2006). A paradoxical result was observed in

Table 1 Factors associated with *T. gondii* infection among pregnant women (N = 264) among pregnant women, Delfan, Iran

	IgG		IgM		*p* value
	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)	
Age groups					
14–19	13 (17.8)	60 (82.2)	2 (2.7)	71 (97.3)	
20–24	22 (19.3)	92 (80.7)	7 (6.1)	107 (93.9)	0.001
25–30	21 (36.2)	37 (63.8)	2 (6.9)	54 (93.1)	
≥30	10 (52.6)	9 (47.4)	4 (10.5)	17 (94.3)	
Educational levels					
No educated	2 (40)	3 (60)	0 (0)	5 (100)	IgM: 0.369
Elementary school	15 (35.7)	27 (64.3)	4 (9.5)	38 (90.5)	IgG: 0.555
Guidance school	14 (25.9)	40 (74.1)	1 (1.9)	53 (98.1)	
High school	25 (21.4)	92 (78.6)	7 (6)	110 (94)	
University graduated	10 (21.7)	36 (78.3)	3 (6.5)	43 (93.5)	
Stage of pregnancy					
1st trimester	14 (29.2)	34 (70.8)	2 (4.2)	46 (95.8)	IgM: 0.365
2nd trimester	33 (29.5)	79 (70.5)	9 (8)	103 (92)	IgG: 0.126
3th trimester	19 (18.3)	85 (81.7)	4 (3.8)	100 (96.2)	
Cat exposure history					
Yes	43 (33.1)	87 (66.9)	10 (7.7)	120 (92.3)	0.001
No	23 (17.2)	111 (82.8)	5 (3.7)	129 (96.3)	
Unpasteurized milk consumption					
Yes	50 (83.3)	10 (16.7)	14 (23.3)	46 (76.7)	0.001
No	16 (7.8)	188 (92.2)	1 (0.5)	203 (99.5)	
Raw or undercooked eggs consumption					
Yes	53 (88.3)	7 (11.7)	15 (25)	45 (75)	0.001
No	13 (6.4)	191 (93.6)	0 (0)	204 (100)	
Raw or unwashed vegetables consumption					
Yes	54 (50.9)	52 (49.1)	14 (13.2)	92 (86.8)	0.001
No	12 (7.6)	146 (92.4)	1 (0.6)	157 (99.4)	
Raw or undercooked meat consumption					
Yes	53 (51)	51 (49)	14 (13.5)	90 (86.5)	0.001
No	13 (8.1)	147 (91.9)	1 (0.6)	159 (99.4)	

the eastern region where seropositivity diminished with age (Shin et al. 2009). Hung et al. (2007) reported that older age group of ≥35 years had a significantly higher seroprevalence than that of the younger age group of 15–25 years. This may be because of decrease in the immune system with advanced in age. On the other hand, Fallah et al. (2008) reported that age was statistically significantly associated with higher infection rates.

Despite some researchers have stated that low level of education was associated with higher rate of toxoplasmosis (Varella et al. 2003; Nash et al. 2005), we did not find a significant relationship between the seroprevalence of *T. gondii* infection and the level of education. The results of similar reports in Turkey and Hamadan are comparable with our study (Ertug et al. 2005; Fallah et al. 2008). However, some other studies showed a significance decrease in seropositivity as the level of the education increases (Hashemi and Saraei 2010; Mostafavi et al. 2012). High level of education may reduce risk exposure and increase awareness to adopt suitable hygienic measures regarding food and cooking behavior such using different chopping board for meats and vegetables, washing chopping boards with soap or bleach the frequent washing of knifes and hands while cooking and avoiding contamination of food by protecting it from dust and flies (Al-Harthi et al. 2006).

Previous epidemiological studies have been reported that in most areas of the world, presence of cats is the most important reason for transmission of toxoplasmosis. Unlike our study, other studies found no relationship between cat exposure and presence of anti-*T. gondii* antibodies in various regions of Iran, e.g. Ardebil, Kamyaran and

Khorramabad (Daryani and Sagha 2004; Cheraghipour et al. 2010; Parvizpour et al. 2010).

The surveyed pregnant women at their first, second and third gestational trimesters showed in different rates of the *Toxoplasma* infection that is similar to other report (Hajsoleimani et al. 2012). In our study, the highest prevalence rate was in the pregnant women who consumed undercooked/raw eggs and meat, or unwashed vegetables and unpasteurized milk consumption. Therefore, pregnant women should be aware of the route of transmission of infection. Those should be made aware through seminars focusing on medical practices to successfully protect their fetus against toxoplasmosis. Confirmed positive maternal serological toxoplasmosis screening should be accompanied by fetal diagnosis. Prenatal diagnosis of congenital toxoplasmosis is primarily based on ultrasonography and polymerase chain reaction (PCR) with amniotic fluid (Lappalainen et al. 1995; Montoya 2002; Tekkesin 2012).

The PCR amplification of toxoplasmosis DNA from amniotic fluid has been deemed the most reliable and safe method of prenatal diagnosis and has basically replaced direct sampling of fetal blood (Lappalainen et al. 1995; Romand et al. 2001; Montoya 2002). However, this method is very expensive and difficult in medical labs. Therefore, use of the PCR techniques on the sera of pregnant women and detection of *T. gondii* antigens could be replacement in the medical laboratories. RT-PCR is an easy and inexpensive method that could be used for detection of *T. gondii* antigens in sera of pregnant women. In addition, we could use of this method for confirmation of acute toxoplasmosis (patients with IgM positive).

T. gondii antibody screening tests in some developed countries include France, Australia and Belgium is obligatory in prenatal care (Edelhofer and Prossinger 2010). The French national program to recognize and treat cases of acute toxoplasmosis in pregnant women has reduced the rate and severity of congenital toxoplasmosis (Mandell et al. 2009). National standards specific to prenatal care for pregnant women have not yet been developed in Iran and so in Lorestan province.

Our investigation showed that there were high percentages *T. gondii*-seronegative pregnant women. They were at considerable risk to acquire acute toxoplasmosis and the future congenital toxoplasmosis. Hence, before birth screening tests are recommended in Lorestan province.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to disclose.

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