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Seroepidemiological Study of *Toxoplasma gondii* in Women Referred to Khorramabad Laboratory of Health Center for Medical Examination before Marriage, Lorestan Province, Iran, 2008

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Abstract: Infection by *Toxoplasma gondii* is widely prevalent in humans and other warm-blooded animals. Symptomatic disease is usually uncommon and most of the infections are asymptomatic. The important aspect of this parasitic infection is the probable danger of congenital transmission and its severing effects on the fetus. This cross-sectional study was conducted to determine the seroprevalence of *Toxoplasma gondii* IgG antibodies among women referred to Khorramabad central laboratory of health center for pre-marriage medical examinations in 2008. A total of 465 serum samples were examined for detection of specific *Toxoplasma gondii* IgG antibodies by Enzyme-linked immunosorbent assay (ELISA). Various information about participants was collected via., questionnaires. The SPSS 15.0 software was utilized to analyze the data from experiments. In order to check for statistical differences, Chi-square test and Fisher's exact test were used. The results indicated that 97.2% (452 out of 465) of the women's sera had anti-*Toxoplasma gondii* IgG antibodies. However, no statistically significant differences were observed between age group, level of education, rural or urban residence and job in the seroprevalence of *Toxoplasma gondii* IgG antibodies. Regarding the results of this study, Khorramabad city has relatively hyperseropositivity for *Toxoplasma gondii* antibodies in women who intend to get married. However, a low number of these women were seronegative for Toxoplasmosis and susceptible to infection with *Toxoplasma gondii* and after it to congenital Toxoplasmosis in their pregnancy. Women infected with *Toxoplasma gondii* in pregnancy period have an abortion or baby's burn with mental retardation, hydrocephaly and macrocephaly, joundice and blindness symptoms; therefore, health education especially in women who are going to marry and also during pregnancy is necessary.

Key words: *Toxoplasma gondii*, women, Lorestan, Iran

INTRODUCTION

Infections by the obligate intra-cellular protozoan parasite *Toxoplasma gondii* are widely prevalent in humans and other warm-blooded animals in all continents (Dubey, 2007). It causes a large range of clinical manifestations in humans, from fever, lymphadenitis,

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abortion and congenital infection to eye diseases and fatal encephalitis (Dubey, 2007; Diab and El-Bahy, 2008). Whilst the sexual life cycle of the parasite is confined to cats (the definitive host), the asexual cycle occurs in many warm-blooded animals (Diab and El-Bahy, 2008). Consumption of undercooked meat was identified as the principle risk factor for *Toxoplasma gondii* infection in humans (Haddadzadeh *et al*., 2006). However, the importance of oocysts, blood transmission, organ transplantation and also in congenital period from placenta in the transmission of *Toxoplasma gondii* infection to human have been identified (Saeedi *et al*., 2007). *Toxoplasma gondii* is an opportunistic parasite that is infective for two groups; immunocompromised patients and fetus, which has a weak immunity system (Luk *et al*., 2008; Gatkowska *et al*., 2008). The frequency of Toxoplasmosis acquisition during pregnancy ranges from 1 to 4 per 1000 pregnancies in several countries and the congenital infection has a prevalence of 0.2-2 per 1000 births (Williams *et al*., 1981). Toxoplasmosis during pregnancy can cause congenital infection and manifestation as mental retardation, blindness and low IQ in the infant (Sever and Ellenberg, 1988). It has been known that 15-85% of humans are infected with *Toxoplasma gondii* but the rate of infection varies widely by location, age and other factors (Walzer and Genta, 1989). Reports of epidemiological studies indicate that prevalence of *Toxoplasma gondii* infection in women (pre-marriage or pregnant) varies substantially among countries. For instance in the American continent countries such as in the USA 31.7% and in south Brazil 74.5% of the pregnant women had anti-*Toxoplasma gondii* IgG antibodies (Jonse *et al*., 2003; Spalding *et al*., 2005). In a Cuban study, 70.9% of the women studied had anti-*Toxoplasma gondii* antibodies 12 weeks before pregnancy (Gonzalez-Morales *et al*., 1995). In European countries, prevalence of *Toxoplasma gondii* infection in women varies from 9 to 67% (Alvarado-Esquivel *et al*., 2006). The prevalence of infection in other countries includes Korea 79% (Song *et al*., 2005), Turkey 30.1% (Ertugl *et al*., 2005), Jordan 31.7% (Jumaian, 2005), Venezuela 49.7% (Leonor *et al*., 2001), Kuwait 58.2% (Nakib *et al*., 1983), Sweden 12% (Petersson *et al*., 2000), Mexico 18.2% (Alvarado-Esquivel *et al*., 2006) and Norway 10.9% (Jenum *et al*., 1998). This coccidian protozoan parasite is widely prevalent in Iran as follows: Tehran 84% (Medghalchi, 1991), Karaj 45.5% (Keshavarz-Valian *et al*., 1998), Ilam 44.8% (Abdi *et al*., 2008), Zahedan 27% (Sharifi-Mood *et al*., 2006), Ardabil 34.7% (Daryani *et al*., 2006) and Gorgan 48.3% (Saeedi *et al*., 2007). Since, there is little information on the prevalence of infection in the women intending to marry in Khorramabad City of Lorestan Province, West of Iran, this cross-sectional study was undertaken to determine the frequency of anti-*Toxoplasma gondii* IgG antibodies among women referred to Khorramabd central laboratory of health center for medical examinations before marriage in this area.

MATERIALS AND METHODS

This study was performed on 465 sera collected from women referred to a central laboratory for medical examinations before marriage in Khorramabad city, West Iran, in 2008. Various information about participants such as age group, level of education, type of Occupation and Rural or urban residence was collected through questionnaires. Blood samples were collected between August 2008 and November 2008 and transferred to the research laboratory and the sera samples were separated by blood centrifugation at 3000 rpm for 5 min and frozen at -20°C until being used. The sera were examined for immunoglobulin G antibodies by using a commercial Elisa kit (Diaplus Toxo-IgG, USA). The test procedure was carried out like as procedure described by Voller *et al*. (1976). After all the reagents and samples were brought to room temperature (20-25°C) and mixed gently, 1:101 dilution of test

samples were prepared by adding 5 μL of samples to 0.5 mL sample diluents in the separate glass tubes. Then 100 μL of calibrators and samples were dispensed into the assigned wells and incubated for 30 min at room temperature. Then after the wells were washed 5 times with the washing buffer, 100 μL of enzyme conjugate were dispensed into each well except the blank well and incubated for 30 mins at room temperature. Then all the wells were washed five times with the washing buffer. One hundred microliter of solution A (containing peroxide) and 100 μL of solution B (Tetramethylbenzidine) were dispensed into each well and then micro-wells were incubated for 15 min at room temperature. Reactions were stopped by adding 50 μL of stop solution to each well and read at 450 nm with micro-well reader against the blank well. The samples that had Toxoplasma IgG concentration less than 10 IU mL^{-1} were considered as Negative and those with Toxoplasma IgG concentration between 10-20 IU mL^{-1} were considered as equivocal. The suspected samples were reexamined and when the results were equivocal once more they would be considered as negative. Toxoplasma IgG concentration above 20 IU mL^{-1} was considered as positive and evidence of previous exposure to *Toxoplasma gondii*. To analyze the data from this experiment, the SPSS 15.0 software was utilized. In order to check for statistical differences, Chi-square test and Fisher's exact test were adopted (Agresti, 2007). Moreover, the results were presented as means. The difference between the 2 groups was considered to be significant when p-value was <0.05.

RESULTS

Sociodemographic Description of the Study Population

General Sociodemographic characteristics of the 465 pre-marriage women studied including age group, educational status, occupational group and place of residence, are shown in Table 1-4. The mean age of the samples was 22 years (range: 13 to 42). The majority of the studied women had high school educational status and most of them were homemakers and were residents of urban regions. 92.9% of the studied women resided in Khorramabad City at the period of the study. As shown in Table 1, the seropositivity rates in 20-25 and 25-30 age groups are obviously higher than those in other groups (98.3 and 98.0%, respectively). Table 2 shows that the highest seropositivity rate for

Table 1: Distribution of IgG antibody to Toxoplasma gondii by age, in 13-42 years old women referring to Laboratory of Health Center, Khorranabad, Iran, 2008

		Seropositive		Seronegative	
Age groups	No tested	No.	%	No.	%
<20	162	155	95.7	7	4.3
20-25	172	169	98.3	3	1.7
25-30	98	96	98.0	2	2.0
≥30	33	32	97.0	1	3.0
Total	465	452	97.2	13	2.8

Table 2: Distribution of IgG antibody to Toxoplasma gondii by educational status in women referring to Laboratory of Health Center, Khorranabad, Iran, 2008

		Seropositive		Seronegative	
Educational status	No tested	No.	%	No.	%
Uneducated	17	17	100.0	0	0.0
Primary and Junior high school	92	87	94.6	5	5.4
High school	241	233	96.7	8	3.3
Academic	115	115	100.0	0	0.0
Total	465	452	97.2	13	2.8

Table 3: Distribution of IgG antibody to Toxoplasma gondii by occupation group in women referring to Laboratory of Health Center, Khorranabad, Iran, 2008

		Seropositive		Seronegative	
Occupation group	No tested	No.	%	No.	%
Teachers and employees	45	42	93.3	3	6.7
Homemakers	394	385	97.7	9	2.3
Workers and other job categories	26	25	96.2	1	3.8
Total	465	452	97.2	13	2.8

Table 4: Distribution of IgG antibody to Toxoplasma gondii by rural or urban residence in women referring to Laboratory of Health Center, Khorranabad, Iran, 2008

		Seropositive		Seronegative	
Rural or urban residence	No tested	No.	%	No.	%
Rural residence	108	104	96.3	4	3.7
Urban residence	357	348	97.5	9	2.5
Total	465	452	97.2	13	2.8

Toxoplasma gondii IgG antibodies was observed in the uneducated and academic women (100%) while the lowest seropositivity rate was observed in primary and junior high school women (94.6%). The maximum seroprevalence of *Toxoplasma gondii* antibodies was within the homemakers (97.7%) and the minimum was within teachers and employees (93.3%) as shown in Table 3. Table 4 shows that the maximum rate of seropositivity was observed in the women who resided in urban regions (97.5%).

Serology and Prevalence

Out of the 465 women intending to marry in Lorestan province, four hundred and fifty two (97.2%) were positive for anti-*Toxoplasma gondii* IgG antibodies. Therefore, a 97.2% prevalence of latent *Toxoplasma gondii* infection was found. Statistical analysis did not show a significant difference between distribution of Toxoplasmosis and age group, educational status, occupational group, rural or urban residence ($p>0.05$). Nevertheless, most of the seropositivity rates were observed within the 20-25 age groups (98.3%). Also, the highest infection rate was observed within the uneducated and academic women (100%) as well as in the homemakers (97.7%) and the urban residing women (97.5%).

DISCUSSION

The main goal of this study was to evaluate the seroepidemiology of *Toxoplasma gondii* IgG antibodies in women intending to marry in Khorramabad city. The findings showed that 452 out of 465 pre-marriage women (97.2%) in Khorramabad City were seropositive for *Toxoplasma gondii* IgG antibodies; therefore, these women would not be at risk for congenital Toxoplasmosis during their pregnancy. The positive rate of anti-*Toxoplasma gondii* antibodies in different parts of Iran varies as follows: Sari 77.6% (Sharif *et al*., 2007), Esfahan 20.1% (Jalayer and Alame, 1997), Sabzevar 19.2% (Moalaee *et al*., 1999), Rafsanjan 48.3% (Keshavarz-Valian and Zare-Ranjbar, 1992) and Bushehr 37.8% (Foladvand and Jaafary, 1998). In a similar research on matrimonial women in Babol, 63.9% of studied women had anti-*Toxoplasma gondii* IgG antibody in their serum (Youssefi *et al*., 2007). The prevalence of Toxoplasmosis in pre-marriage women in Khorramabad city was higher than in other regions of Iran. This is presumably due to the high presence of cats, climatic, hygienic and socioeconomic conditions and consumption of raw or improperly cooked meat in the region. In this area of Iran with a mild climate, transmission of Toxoplasmosis by

infective cat feces might play a predominant role in the spread of human infection in the population. This study was performed on 13-42 year old women and after statistical analyses, no statistically significant difference was observed in various age groups although the seropositivity rates in 20-25 and 25-30 age groups were obviously higher than those in other groups 98.3 and 98%, respectively (Table 1). The only presumable reason for this high seropositivity rate could be a result of more contact with cats or infected materials and vegetables in these age groups. Most of the seropositivity rate for *Toxoplasma gondii* antibodies was observed in the uneducated and academic women (100%) and the reason for this highseropositivity is not clear (Table 2). The majority of seroprevalence rates of *Toxoplasma gondii* antibodies were within the women homemakers (97.7%) and it can be a result of more consumption of unwashed or unpeeled vegetables or uncooked meat or presence of cats in or around the house (Table 3). Although no statistically significant differences in the seropositivity of *Toxoplasma gondii* antibodies were observed in relation to rural or urban residence, the maximum rate of seropositivity was observed in the women, who resided in urban regions (97.5%). It must be a result of high presence of cats and more consumption of raw meat or vegetables in these regions (Table 4).

Present results are similar to the results of Sharif *et al.* (2007) and Youssef *et al.* (2007) researches that reached 77.6 and 63.9% seropositivity rates in the studied women, respectively. But present results are higher than the results achieved by Jalayer and Alame (1997), Moalaee *et al.* (1999), Keshavarz-Valian and Zare-Ranjbar (1992) and Foladvand and Jaafary (1998). In this study, a 97.2% prevalence of latent *Toxoplasma gondii* infection was found in women intending to marry in Khorramabad City which is much higher than those reported in the previous studies of pre-marriage or pregnant women in other regions of Iran. It may be speculated that the high prevalence found could be also explained by a number of environmental characteristics. First, Khorramabad city has a mild climate and the prevalence of *Toxoplasma gondii* infection in mild climates has been reported to be higher than that in other climates (Jenum *et al.*, 1998; Montoya and Prof. Liesenfeld, 2004). Second, it can be due to low hygienic and socioeconomic conditions and more consumption of raw or improperly cooked meat and vegetables in this region. Regarding the results of this study, the majority of women in this region of Iran are infected with *Toxoplasma gondii* before pregnancy and have anti *Toxoplasma gondii* antibodies in their serum; therefore, most of these women will have a safe pregnancy to Toxoplasmosis. Nevertheless, a low percentage of these women who were seronegative for *Toxoplasma gondii* IgG antibodies (2.8%) should be aware of the routes of transmission of infection and prevention methods must be taught. This survey is meaningful as a community and general population based study to reveal the seroprevalence of toxoplasmosis in Iran, but further surveys from various areas will be necessary to clarify the seroepidemiological status of toxoplasmosis in the Iranian population.

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