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Seroprevalence of toxoplasmosis in high school girls in Bushehr city, South-west of Iran, 2009

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Toxoplasmosis is a parasitic disease prevalent all over the world and its causal agent is *Toxoplasma gondii*. Toxoplasmosis is very important in pregnant women. Seroepidemiological survey of toxoplasmosis in young girls before marriage for identifying non immune girls could be used to prevent congenital toxoplasmosis. A seroepidemiological study of toxoplasma IgG-antibody in 516 high school girls was conducted. Sample cases were chosen randomly from public high school girls. Blood samples were collected and the obtained sera were examined for anti toxoplasma IgG and IgM antibodies by using ELISA method. Prevalence of toxoplasmosis in high school girls in Bushehr city was 22.1%. There was significant correlation between seropositivity of toxoplasma antibodies and factors such as, contact with cat, raw milk consumption, raw vegetables consumption and food consumption habits, but there was no significant correlation between seropositivity and availability of drinking water, keeping pet and information about toxoplasmosis. About 78% of high school girls in Bushehr city were seronegative in toxoplasma IgG antibody. It is recommended to the health managers to design the educational measures and develop the serological tests for identifying the non immune girls before marriage to prevent congenital toxoplasmosis.

Key words: Elisa, toxoplasmosis, high school girls, seroprevalence.

INTRODUCTION

Toxoplasmosis is a zoonotic parasitic disease prevalent all over the world and its causal agent is an intra cellular protozoan, called *Toxoplasma gondii* (Barbosa et al., 2009). Most cases of toxoplasmosis in immunocompetent individuals are asymptomatic and clinical disease is uncommon (Kravetz and Federman, 2005), but in immune deficient persons it cause a large range of manifestations such as, fever, lymphadenitis and fatal encephalitis (Shimelis et al., 2009). Prevalence of toxoplasmosis varies in different parts of the world and this variation related to, life style, age, climatic conditions, nutritional habits and other sociocultural factors (Spalding et al., 2005). Toxoplasmosis is transmitted through oocysts shed in infected cat's faeces and also by consumption of contaminated unwashed/unpeeled vegetables, fruits, unpasteurized milk and raw or undercooked infected

meat, blood transfusion and organ transplantation (Hatam et al., 2005; Riemann et al., 1975; Sacks et al., 1982). Toxoplasmosis is very important in pregnant women, because it can lead to transplacental transmission and involvement of the fetus with pathological effects such as abortion, microcephaly, hydrocephaly, blindness, calcification of brain cells and even death in uterus (Hatam et al., 2005; Jones et al., 2003). Women who have already infected with toxoplasma parasites have immunity against toxoplasmosis in their pregnancy period and their fetus will be protected against congenital toxoplasmosis and women who do not have such immunity will be at risk of congenital toxoplasmosis (Cunningham et al., 1997).

Seroepidemiological survey of toxoplasmosis in young girls before marriage and pregnancy will be very useful to prevent congenital toxoplasmosis, because when we know the population of the girls who do not have immunity against toxoplasmosis we can design the preventive measures to prevent congenital toxoplasmosis (Odelis and Jesus, 2009). 15 - 18 year old girls are suitable

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Table 1. Distribution of anti toxoplasma IgG and IgM antibodies.

Antibody	Positive		Negative		Total	
	n	%	n	%	n	%
IgG	114	22.1	402	87.9	516	100
IgM	7	1.4	509	98.6	516	100

Table 2. Distribution of anti toxoplasma antibodies by educational level.

Level of education	Positive		Negative		Total	
	n	%	n	%	n	%
Class 10	26	15.8	139	84.2	165	49.5
Class 11	48	22.7	164	77.3	212	32.5
Class 12	40	30.8	99	69.2	139	11.6
Total	114	22.1	402	77.9	516	100

population for such study, so this survey was conducted to evaluate the anti toxoplasma IgG and IgM antibodies among high school girls in Bushehr city from September to May 2009.

MATERIALS AND METHODS

A descriptive-analytical study of toxoplasma IgG-antibodies in 516 high school girls was conducted. Sample groups were chosen from 12 public high school girls aged between 15 to 18 years. The sample size was calculated as 418 individuals on a prevalence of 26% $d = 0.05$ at a confidence level of 95% but to achieve more satisfactory results, 516 individuals were selected. Written agreements were obtained from all participants or their parents after explaining the purpose of the study. Questionnaire forms were filled out by all participants. Blood samples were collected and sera were separated by centrifugation at 3000 rpm for 5 min and then sera were aliquoted in several labeled vials and kept frozen at -20°C until being used.

The sera were examined for anti toxoplasma IgG and IgM antibody by using a commercial ELISA kit (Euro Immune Germany) and ELISA reader machine (Biotek- USA). Positive and negative controls and standard with three different concentrations were included per each batch of test run to ensure kits were working properly and technical procedures were carried out correctly. Tests were performed according to manufacturer instruction, briefly, serum samples were diluted 1:101 by using the sample buffer and mixed well by vortexing, transfer 100 μl of the serum sample, positive and negative controls and calibrators in to the individual microplate well and incubate for 30 min at room temperature then washing the microplate three times using 300 μl of working strength wash buffer, after the washing, 100 μl of enzyme conjugate (peroxidase-labeled anti-human IgG) was added to the wells and incubate for 30 min at room temperature, washing the micro plate wells as described above, pipette 100 μl of chromogen/substrate solution into each of the micro plate well, incubate for 15 min at room temperature, 100 μl of stop solution was added into each well and incubate for 15 min at room temperature, finally, the optical density was read at 450 nm by ELISA reader and converted into IU/mL of *T. gondii* IgG and IgM antibody through a standard curve.

The Sample that had toxoplasma IgG concentration less than 8 IU/ml were considered as negative and above 11 IU/ml considered as

positive. Data were recorded and analyzed using SPSS 15.0 software. The correlation between selected variables and seropositivity was analyzed by Chi square test. $P < 0.05$ was considered significant.

RESULTS

In this study 516 samples from high school girls were analyzed for anti toxoplasma IgG and IgM antibody by using ELISA method. 114 cases (22.1%) were seropositive and 402 cases (87.9%) were seronegative for IgG antibody and 7 cases (1.4%) were seropositive and 509 cases (98.6%) were seronegative for IgM antibody (Table 1).

A correlation between *T. gondii* seroprevalence and the educational level was also studied, 26 cases (15.8%) of the seropositive girls were in first level (class 10), 48 cases (22.7%) were in second level (class 11) and 40 cases (30.8%) were in third level (class 12). There was a significant correlation between seropositivity and the educational level and the seropositivity was increased with the educational level. Most likely this increase in toxoplasma seropositivity is related to increasing age (Table 2).

From 81 cases who had history of contact with cat, 28 individuals had IgG toxoplasma antibody and statistical analysis showed significant relationship between seropositivity of toxoplasma IgG antibody and history of contact with cat. There was no significant relationship between seropositivity and some factors such as keeping pet, having information about Toxoplasmosis transmission and the area of residency. Although, there was no significant correlation between seropositivity and the area of residency but seropositivity in urban residents (23.1%) is considerably higher than rural residents (11.6%) (Table 3). There was significant relationship between seropositivity and wearing the gloves while

Table 3. Distribution of anti toxoplasma antibodies by different sociocultural factors.

Risk factors	Bodies				P. value
	Negative		Positive		
	n	%	n	%	
Contact with cat					
Yes	28	34.6	53	65.4	< 0.05
No	86	19.8	348	80.2	
Animal mating					
Yes	7	18.4	31	81.6	0.367
No	107	22.4	370	77.6	
Wearing gloves when cut raw meat					
Yes	24	14.7	139	85.3	< 0.05
No	90	25.6	262	74.4	
Raw vegetables consumption					
Yes	102	23.1	340	76.9	0.131
No	12	16.4	61	83.6	
Taste food when cooking					
Yes	33	30.3	76	69.7	< 0.05
No	81	20	325	80	
Washing vegetables					
Antiseptics	44	16.5	222	83.5	< 0.05
Water and salt	14	23.3	46	76.7	
Only Water	56	29.6	133	70.4	
Residency					
Urban	109	23.1	363	76.9	0.083
Rural	5	11.6	39	88.4	
Drinking milk					
Pasteurized	66	17.6	308	82.4	< 0.05
Unpasteurized	16	51.6	15	48.4	
Not drinks milk	32	29.1	78	70.9	
Primary information about toxoplasmosis					
Yes	5	27.8	13	72.2	0.366
No	109	21.9	388	78.1	

cutting the meat (Table 3), 14.7% of cases who wear the gloves were seropositive and 25.6% of cases who did not have wear the gloves were seronegative (Table 3). Although there was no significant relationship between the toxoplasma IgG seropositivity and raw vegetables consumption but 23.1% of individuals who had raw vegetables consumption were seropositive and only 16.4% of individuals who did not have raw vegetables consumption were seronegative for toxoplasma IgG antibody. There was strong and significant relationship

between seropositivity and washing the vegetables, 16.4% of individuals who washed the vegetables by using the antiseptics had seropositive results, but 23.35% of individuals who washed the vegetables by using the sodium chloride solution had seropositive results and 29.6% of individuals who washed the vegetables by using the water had seropositive results. Statistical analysis showed the significant relationship between seropositivity and tasting the food while cooking and also raw milk drinking.

DISCUSSION

Toxoplasmosis is an infection caused by an intra cellular protozoan called *T. gondii*. Although most cases of this infection in immunocompetent individuals are asymptomatic but if it has done in pregnant women, it can cause congenital toxoplasmosis with severe pathological effects on fetus (Hatam et al., 2005; Jones et al., 2003). One of the useful ways to prevent congenital toxoplasmosis is to detect non immune girls before marriage. Girls aged 15 - 18 years are the best group to evaluate the immunity against toxoplasmosis. In this serosurvey 22.1% and 1.4% of high school girls in Bushehr city was seropositive for toxoplasma IgG and IgM antibody respectively. According to this results about 88% of young girls in Bushehr city did not have immunity against toxoplasmosis, therefore this group of population are at risk of acquired toxoplasmosis and if they acquire the infection during the pregnancy their fetus will be at risk of congenital toxoplasmosis (Mahmoodi et al., 2005; Yang et al., 2000). Results of this study is in accordance to several studies in different parts of Iran as follow: seropositivity in high school girl students aged 15 - 19 years in Isfahan province in central part of Iran, Fasa district in Fars province and Robatkarim district near Tehran were 18.4, 10 and 17.7% respectively, (Hatam et al., 2005; Mahmoodi et al., 2005). The highest seroprevalence of toxoplasma IgG antibody has been reported from North of Iran (Yang et al., 2000). In a serosurvey in Sari city 76.4% of premarital women were reported as toxoplasma IgG seropositive (Ajami et al., 2001) and in another survey in intellectual disability children in rehabilitation centers in North of Iran 77.4 of sample had toxoplasma IgG seropositive tests (Sharif et al., 2007).

According to our results, although there was no significant correlation between toxoplasma seropositivity and age, but seropositivity is increased with increase in the level of education that is indicated in the age, this results is in accordance with some studies (Daryani and Sagha, 2004; Rafiei et al., 2006). Results showed that about 35% of samples had history of contact with cat and a significant correlation between toxoplasma seropositivity and contact with cat was seen, this result is in accordance with several studies (Mahmoodi et al., 2005; Manouchehri-Naeini et al., 2007; Mohammadi et al., 2008). Seropositivity of toxoplasma antibody had a significant correlation with different high school in various parts of city. High schools sample are situated in areas with different socioeconomics conditions, Mohadaseh high school is situated in central part of the city, this parish is an ancient quarter and a lot of sandwich shop and old restaurants are there in this part of the city, this environmental condition has an attractive factors for cats. Therefore, transmission of toxoplasmosis can easily occur (Mahmoodi et al., 2005). Regarding the results there was no significant correlation between toxoplasma seropositivity and some factors such as availability of drinking water, keeping pet and area of residency, this

results was in accordance with several studies (Daryani et al., 2004; Kamyabi and Atapour, 1999; Ziaei et al., 2008; Ataeian et al., 2000). Toxoplasma seropositivity in persons who washed vegetables by detergents was significantly lower than individuals who washed vegetables by sodium chloride solution and water, but Daryani et al. (2004) could not show significant correlation between toxoplasma seropositivity and raw vegetables consumption (Daryani and Sagha, 2004). Based on our study, there was correlation between toxoplasma seropositivity and unpasteurized drinking milk and this result was in accordance with Saeedi et al. (2002) study. Although in this study 96.5 of girls did not have any information about toxoplasmosis but there was no correlation between toxoplasma seropositivity and primary information about toxoplasmosis, this result is in accordance with Mahmoudi et al. study (Mahmoodi et al., 2005). According to our results 78% of Bushehr high school girls do not have any information about toxoplasmosis, so, It is recommended to the health managers to design the educational measures and develop the serological tests for identifying the non immune girls before marriage to prevent congenital toxoplasmosis.

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