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Full Length Research Paper

Seroepidemiological study of toxoplasmosis in childbearing age women in Bushehr City, south west of Iran in 2009

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Toxoplasmosis is a parasitic disease caused by an intracellular protozoan, *Toxoplasma gondii* and congenital form is one of the most important clinical aspects of this disease. Seroepidemiological studies among women of childbearing age could provide appropriate approaches to design prevention measures to prevent congenital Toxoplasmosis. In this study, 303 blood samples were collected from women of childbearing age who referred to the health center clinics for premarital examinations in Bushehr city. Sera were tested for anti-Toxoplasma IgG and IgM antibodies by using ELISA method. 71 cases (23.4%) were seropositive and 232 (76.6%) serum samples were seronegative for anti-Toxoplasma IgG antibody, 10 samples (3.3%) were seropositive and 293 samples (96.7%) were seronegative for anti-Toxoplasma IgM antibody. According to the results, there was no significant association between seropositivity and age groups, area of residency, history of contact with cat, educational levels and nutritional behaviors. Most women of childbearing age in Bushehr City do not have immunity against Toxoplasmosis. These women are at risk of acquired Toxoplasmosis in their pregnancy period. It is recommended to health managers to design the educational measures to prevent congenital Toxoplasmosis.

Key words: *Toxoplasma gondii*, premarital women, seroepidemiolog, ELISA.

INTRODUCTION

Toxoplasmosis is a parasitic disease caused by an intracellular protozoan parasite, *Toxoplasma gondii*. This parasite can infect almost all mammals and human infection results from ingestion of foods, vegetables and water contaminated with oocyst from cat faeces and raw or insufficiently cooked meat contained tissue cysts. Transmission from infected mothers to their fetuses or by blood transfusion and organ transplantation can also occur (Sharif et al., 2006; Ajami et al., 2001).

Toxoplasmosis has a wide range of prevalence and this variability is related to various factors such as, age, sociocultural and nutritional habits, contact with domestic cats, climatic and geographical conditions (Barbosa et al., 2009). Most cases of primary infection in immuno-

competent individuals are asymptomatic, but in pregnant women and immunocompromised individuals may lead to severe complications and even death (Fallah et al., 2005).

One-third of primary Toxoplasmosis cases occurring during pregnancy lead to congenital Toxoplasmosis with consequent pathological effects such as, microcephaly, hydrocephaly, blindness, calcification of brain and abortion (Dubey et al., 1998; Tenter et al., 2000). Existence of IgM antibody titer indicates the acute infection while increasing IgG antibody titer indicates the recent and active Toxoplasma infection (Toply and Wilson, 1998). Finding of anti-Toxoplasma IgG antibody in women of childbearing age indicates that these women already have been infected (Toply and Wilson, 1998), so, they have immunity against Toxoplasma infection in their future pregnancies. Because of the peculiarity of congenital Toxoplasmosis and its severe effects on fetus, seroepidemiological studies among women of child bearing age

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Table 1. Distribution of anti-Toxoplasma IgG and IgM antibodies.

Antibody	Positive		Negative		Total	
	Number	%	Number	%	Number	%
IgG	71	23.4	232	76.6	303	100
IgM	10	3.3	293	96.7	303	100

Table 2. Distribution of anti-Toxoplasma IgG antibody in relation to age groups.

Age (year)	Positive		Negative		Total	
	Number	%	Number	%	Number	%
14-20	27	18	123	82	150	49.5
21-25	29	29.6	69	70.4	98	32.5
26-30	8	22.8	27	77.2	35	11.6
31-40	7	35	13	65	20	6.4
Total	71	23.4	232	76.6	303	100

$p = 0.25$.

could provide appropriate information to design the preventive measures. Several studies about seroepidemiology of Toxoplasmosis during pregnancy and in the women of childbearing age have been conducted in Iran (Rabiee et al., 2003; Sharifi Mood et al., 2006; Fallah et al., 2004; Abdi et al., 2008), and Seroprevalence of anti-Toxoplasma IgG antibody in these studies have been reported as follows: Kerman province 22.4%, Rafsanjan 48.3%, Shiraz 77.3%, Isfahan 57% and in Sari 71%. Due to paucity of data about the seroepidemiology of Toxoplasma infection in women of childbearing age in Bushehr, this survey was conducted.

MATERIALS AND METHODS

This study was conducted in 303 childbearing age women who referred to the health centers for premarital examinations from January to September 2009 in Bushehr City. Both written and oral informed consent was sought, then questionnaire forms were filled out by all participants. Blood samples were collected and transferred to parasitology laboratory. Sera were separated by centrifugation at 3000 rpm for 5 min and then aliquoted into several labeled vials and kept frozen at -20°C until use. The sera were examined for anti-Toxoplasma IgG and IgM antibodies by using a commercial ELISA kit (Euro Immune Germany) and ELISA reader machine (Biotek, USA). Positive and negative controls and standards with three different concentrations were included per batch of test 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 1:100 using sample buffer and mixed by vortexing. 100 μl of the sample, positive and negative control and calibrators were dispensed into individual microplate well and incubated for 30 min at room temperature. The microplate was then washed three times with 300 μl working strength wash buffer, 100 μl of enzyme conjugate (peroxidase-labeled anti-human-IgG) was added to the wells and incubated for 30 min at room temperature. The microplate was again washed three times with working strength wash buffer to

remove unbound enzyme conjugate to the antigen. Finally 100 μl of chromogen/substrate solution was added into each of the microplate well and then incubated for 15 min at room temperature. 100 μl stop solution was added and incubated for 15 min at room temperature. The optical density was read at 450 nm by ELISA reader and converted into IU/mL of *T. gondii* antibody. The sample that had Toxoplasma IgG concentration less than 8 IU/mL was considered as negative and above 11 IU/mL as positive. Data were recorded and analyzed using SPSS 15.0 software. The association between selected variables and seropositivity was analyzed by Chi square test. $P < 0.05$ was considered significant.

RESULTS

In this study 303 serum samples from premarital women referred to health centers were evaluated for presence of anti-Toxoplasma IgG and IgM antibodies by using ELISA method. 71 samples (23.4%) were seropositive and 232 cases (76.6%) were seronegative for Toxoplasma specific IgG antibody and 10 samples (3.3%) were seropositive and 293 samples (96.7%) were seronegative for Toxoplasma specific IgM antibody (Table 1). The mean age of the samples was 21 years (range from 14 - 40). There were no significant differences in seroprevalence of Toxoplasma antibody between different age groups ($p > 0.2$) (Table 2).

Regarding to the data of Table 3 we found that the highest prevalence rate is observed in women who were illiterate (36.8%) but there was no significant association between educational status and seroprevalence of Toxoplasma antibody ($p = 0.45$). 29 (75.6%) women of our studied population were resident in rural areas and 74 (24.4%) of them were resident in urban areas. There was no significant association between the area of residency and seropositivity of Toxoplasma (Table 4), ($p = 0.40$). 18 cases had history of contact with cat which 6 cases of

Table 3. Distribution of anti-Toxoplasma IgG antibody in relation to educational status.

Educational status	Positive		Negative		Total	
	Number	%	Number	%	Number	%
Illiterate	7	36.8	12	63.2	19	6.3
Primary school	11	22	39	78	50	16.5
High school	32	21.6	116	78.4	148	48.8
Academic	22	25.6	64	74.4	86	28.4
Total	71	23.5	231	76.5	303	100

$p = 0.25$.

Table 4. Distribution of anti-Toxoplasma IgG antibodies in relation to residency.

Habitat	Positive		Negative		Total	
	Number	%	Number	%	Number	%
Urban area	55	24	174	76	229	75.6
Rural area	16	21.6	58	78.4	74	24.4
Total	71	23.4	232	76.6	303	100

$p = 0.25$.

Table 5. Distribution of anti-Toxoplasma IgG antibodies by different sociocultural factors.

Variable	Positive		Negative		Total
	Number	%	Number	%	Number
Contact with Cat	6	33.3	12	66.7	18
Raw meat consumption	31	27.4	82	72.6	113
Availability of drinking water	42	25	126	75	168

P values based on table rows from top to bottom are: ($p = 0.30$), ($p = 0.13$), and ($p = 0.78$).

them were detected as IgG positive and there was no significant association between the history of contact with cat or presence of cat in /or around the house and seropositivity of anti-Toxoplasma antibody ($p = 0.30$), also statistical analysis demonstrate that no association could be found between raw meat consumption ($p = 0.13$), drinking water habits ($p = 0.78$) and *T. gondii* IgG-seropositivity (Table 5).

DISCUSSION

Toxoplasmosis is a zoonotic infection with world wide distribution and exhibits the wide range of prevalence based on sociocultural and climatic conditions (Barbosa et al., 2009; Hatam et al., 2005). Toxoplasmosis is an important disease in pregnant women and organ transplant and immunodeficient patients (Fallah et al., 2004). Congenital Toxoplasmosis is a major problem in most communities with a high prevalence of *T. gondii* infection and study of the seroepidemiology of this infection among

women of childbearing age could provide appropriate approaches to design the preventive measures (Neto et al., 2004; Breugelmans et al., 2004).

Most studies in Iran have been focused on childbearing age and pregnant women and wide range of prevalence rate of Toxoplasma antibodies have been reported as follows: Sari 74.6%, Tehran 31%, Kerman 29.4% (Fallah et al., 2005; Daryani and Sagha, 2004), Sanandaj 28.2%, Hamedan 33.5%, Roudsar 86.3% (Mohammadi et al., 2008; Dubey et al., 1998), Gorgan 48.3%, and Kermanshah 23.7% (Saeedi et al., 2002; Tenter et al., 2000). Several studies in different countries and cities around the world have indicated that seroprevalence rate of Toxoplasma antibodies have a wide range as follow: India 36.8%, Krowasi 36.4%, Ireland 12.8%, Thailand 21.7%, Turkey 49.4% (Daryani and Sagha, 2004), Paris 83%, and USA 31.7% (Saeedi et al., 2002).

This study was performed on premarital women to distinguish previously infected women from women who had not been previously infected. In this study, 71 out of 303 cases (23.4%) were positive for specific IgG antibody

and 10 cases (3.3%) were positive for specific IgM antibody, therefore, this study showed a moderate seroprevalence (23.4%) of *T. gondii* IgG antibody compared to other parts of the Islamic Republic of Iran (Hatam et al., 2005; Sharif et al., 2006; Sharifi et al., 2006; Fallah et al., 2004). Results of this study indicate that the prevalence of Toxoplasma antibodies in Bushehr is lower than some other parts of Iran. Although, Bushehr climate is warm and humid but the mean temperature of Bushehr during the year is higher than other area such as the central, north and the west parts of Iran and this temperature is not optimum for sporulation of Toxoplasma oocysts. Except the high temperature, other reasons for lower seroprevalence of Toxoplasma antibody could be related to the nutritional behaviors such as deep cooking and baking of foods, freezing the meat for long time and also the habit of sea food consumption instead of lamb or beef meat (Daryani and Sagha, 2004). Results from some studies have indicated that the risk of *T. gondii* infection increased with age (Dubey, 2004). Although, there was no association between seropositivity of Toxoplasma antibody and different age groups in our study, but the seropositivity rate in 20 - 25 age group was obviously higher than other groups. This result is in accordance with the results of some studies (Saeedi et al., 2002; Fallah et al., 2005; Daryani and Sagha, 2004; Alvarado-Esquivel et al., 2006; Trikha and Wig, 2001). Although, some studies have indicated the association between seropositivity of Toxoplasma and contact with cat, yet in this study this association was not established. This result is in accordance with the results of several studies in Shiraz, Ardabil and Ahwaz cities (Daryani and Sagha, 2004; Alvarado-Esquivel et al., 2006). Statistical analysis also did not show a significant association between seropositivity of Toxoplasma and area of residency, educational status, availability of drinking water and raw meat consumption habit. It seems that most probable factors which affect the Toxoplasma infection are unwashed/unpeeled vegetables and contaminated water. Bushehr is a tropical region in southwest of Iran and the drinking water is supplied from 200 kilometer away from Bushehr City by pipelines. Almost all households in Bushehr City have a metallic reservoir for reserving the water, which can increase the chance of contamination of drinking water. Vegetables are also provided from other province and can be contaminated during transportation. Altogether, results of this study indicates that 76.6% of premarital women do not have immunity against Toxoplasmosis, therefore they are at risk of congenital Toxoplasmosis for their fetuses and it is necessary for health policy makers to design the preventive measures against congenital Toxoplasmosis.

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