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Full Length Research Paper

Evaluation of IgM against *Toxoplasma gondii* in under marriage women and its pathogenicity relation with demographic factors

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Toxoplasmosis is an acute infectious disease with worldwide spread between human societies and domestic animals. This study has been carried out to determine the immunity against toxoplasma infection in women at the time of marriage counseling in Zahedan and its relation with conducted demographic factors. This descriptive - analytical study was conducted on 280 women referred to marriage counseling of central laboratory of Zahedan city from January 2010 to May 2010. Immunoglobulin M (IgM) antibody levels were measured by enzyme-linked immunosorbent assay (ELISA) method and disease relationship was examined with place of residence, educational status, keeping cats at home, and keeping domestic animals and way of meat consumption (cooked or half-cooked) based on statistical tests. In this study the rate of positive cases of IgM was obtained as 5/4%. The results of this study showed no significant relationship between IgM positive cases and education, place of residency and maintenance of domestic animals at home, but significant relationship between IgM positive cases and maintenance of cats at home was observed. Thus, among those who kept cats at home, the rate of IgM antibody positive cases had increased. On the other hand those who had consumed half-cooked or raw meat, positive cases of antibodies increased and significant relationship between the two cases was observed. The results of this study indicated that 94/6% of referred women are negative for toxoplasma antibody and they are at risk of acquiring toxoplasmosis especially during pregnancy and its complications of congenital infections for their babies.

Key words: Antibodies, *Toxoplasma gondii*, ELISA.

INTRODUCTION

Toxoplasma gondii is an intracellular compulsory protozoan of blood and tissue cells with acute infection, asymptomatic and it is one of the common parasitic diseases of human and animals (Montoya and Liesenfeld, 2004). The parasite exists in various acute forms in the inner and outer parts of intestines, and as active forms of tachyzoite, oocyst with cat stool disposed and as resistant form and chronic disease form as tissue cysts (Duby et al., 1998). Cats are the main source of disease and other infected animals, including birds and domestic animals which are considered as secondary hosts (Tenter et al., 2000). Humans get infection through food consumption, including usage of contaminated raw vegetables, half cooked or raw infected animals that have been in contact with oocysts excreted by infected cats,

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and also through the placenta and fetus during pregnancy, tissue graft, blood transfusion, scratches and skin wounds. Transmission also occurs to the laboratory workers randomly (Put authors and year as mentioned in the instructions (Munoz-Zanzi et al., 2010; Hegab et al., 2003; Liu et al., 2009). This parasite enters the different body tissues via the blood circulation, and then after proliferation inside the cell, the cells will be destroyed and cysts are formed in skeletal muscle and brain tissues (Jenum et al., 1998).

In congenital condition, tachyzoites enter the fetus via mother infected placenta and all tissues including skeletal muscle and myocardium, lungs, liver, and central nervous system are infected (Wong and Remington 1994; Jones et al., 2009). The most common lesion due to congenital toxo-plasmosis is chorioretinitis that results in inflamed and necrotic retina (Lopez et al., 2009). Parasite transmission to the fetus is greater when mother gets infection at the end of her pregnancy period but infant usually show disease with subclinical symptoms and if mother gets infection at the first months of pregnancy, neonatal transmission is less and disease in infant is more severe creating complications as blindness and mental retardation (Nowakowska et al., 2006; Zangi et al., 2002).

Accurate diagnosis of toxoplasmosis on time and appropriate treatment are important in reducing the disease complications. Based on disease progress is slow and benign in healthy individual, thus in sero-epidemiological survey, the study of their serum components, including increased serum Immunoglobulin M (IgG) indicates previous exposure to the parasite and if IgM is increased that may indicate the disease is in acute phase process (Jenum et al., 1998; Dzitko et al., 2006).

According to the many studies, high titer of anti-toxoplasma immunoglobulins level in women who are under marriage, is problematic and in relation to direct contact with infected cats at home. Thus it is important to pay attention to the immune system of them for diagnosis of acute status, by measuring IgM anti-toxoplasma antibody before they decide to become pregnant and if they get infection and having symptoms, treat them properly and for diagnosis of congenital toxoplasmosis besides, serum, infant CSF has to be examined (Tenter et al., 2000).

Prevalence of toxoplasma antibody in under marriage women differs by age, how to keep cats at home, education level, meat consumption, the geography and place of residency (Alvarado-Esquivel et al., 2007). Based on the disease often asymptomatic with slow progress, and according to the prevalence of the disease in the region, the trend is likely, that no study has been done in Zahedan , Diagnosis of toxoplasmosis in under marriage women may not be taken seriously, thus this study has been carried out to determine the level of IgM anti-toxoplasma antibody in them and if the disease pathogenicity were associated with demographic variables such as place of residency, education level, the process of keeping pets at home, and consumption of meat (cooked or half cooked), trying to decrease its complications and be a good guidance for their health care with the aim of preventing congenital toxoplasmosis in this region.

MATERIALS AND METHODS

This descriptive analytical study was conducted from January to May 2010 on under marriage women who were referred for counseling at Central Laboratory of Zahedan city, the only laboratory counseling center in the city. Based on the ratio of prevalence of disease as 35%, and regarding to the studies conducted in different parts of the country with accuracy 0.07 and 95% confidence and according to the statistical formula, the number of study population was calculated as 280 individuals. The data was collected through a questionnaire form containing demographic information that included age, educational level, residency status, keeping cats at home, and keeping domestic animals and way of meat consumption (cooked or half-cooked). Two cc of heparinized peripheral blood was taken from each individual and serum was separated by centrifugation at 2000 rpm for 10 min and transferred to the test tubes for measuring IgM by ELISA method (Zangi et al., 2002) using commercial available kits (Dia Sorin Inc, USA) and results were read using the Enzyme-linked immunosorbent assay (ELISA) reader at 450 to 620 wave length and relevant concentrations squares were drawn by densitometer apparatus and of specific antibodies lesser than 10 IU/ml as negative, between 10 to 12.5 IU/ml suspicious and more than 12,5 IU/ml as positive were included. The results were analyzed using SPSS 17 statistical software and for finding significance of data, Chi square test was used.

RESULTS

In this study, 280 women aged 13 t0 43 years old with an average age 21.73 and the 20 years were most common age enrolled. The most common education level was diplomas (41.1%). No significant difference between antibody positive and educational statuses was found. Two hundreds and two (72.1%) persons were city resident and 78 (27.9%) were living in marginal urban and rural areas. Sixty six (23.6%) of them kept cats in their home and 55 (19.6%) of them kept pets (except a cat) such as cattle, sheep, dog, chicken, at home.

ELISA results showed that 5.4%had positive IgM antibody with a range of 95% confidence range (3 to 8.7), and 7.1% had questionable IgM antibodies with 95% confidence range (83 to 91.1) and 87.5% were negative for this antibody with 95% confidence range (4.4 to 10.8). Concerning relation between positive IgM levels and place of residency, 9 women (4.45%) were living in the city, and six (7.69%) were living in marginal urban and rural areas. 15 women (7.4%) of city resident and 5(6.41%Revise) of people who were living in marginal urban and rural areas, had suspected IgM that was determined that this relationship did not prove statistically (P. Value = 0. 54).

Seven (10.6%) women who had kept cat in their home were IgM positive and 8 (12.1%) women had suspected IgM this relationship was statistically significant (P. Value

Table 1. Relationship between keeping cats at home and IgM positive and negative women.

Total	IgM						Keeping cat
	Suspected		Negative		Positive		
	Percentage	Frequency	Percentage	Frequency	Percentage	Frequency	
66	12.1	8	77.3	51	10.6	7	Yes
214	5.6	12	90.7	194	3.73	8	No
280	7.1	20	87.5	245	5.4	15	Total

P Value = 0.014

Table 2. Relationship between keeping pets at home and IgM positive and negative women.

Total	IgM						Keeping pets
	Suspected		Negative		Positive		
	Percentage	Frequency	Percentage	Frequency	Percentage	Frequency	
55	10.9	6	85.4	47	3.7	2	Yes
225	6.2	14	88	198	5.8	13	No
280	7.1	20	87.5	245	5.4	15	Total

P Value = 0.41

Table 3. Relationship between meat consumption and IgM positive and negative women.

Total	IgM						Meat consumption
	Suspected		Negative		Positive		
	Percentage	Frequency	Percentage	Frequency	Percentage	Frequency	
217	6.9	15	89.4	194	3.7	8	Cooked
63	7.9	5	81	51	11.1	7	Half-cooked
280	7.1	20	87.5	245	5.4	15	Total

P Value = 0.046

=0.014) (Table 1). Two (3.6%) women who had kept pets in their home were IgM positive and 6 (10.9%) women had suspected IgM that due to statistical tests, this relationship was statistically non-significant (P. Value =0.
41) (Table 2). 8 (3/7%) women who had cooked meat consumption were positive IgM and 7 (11/1%) women who had half cooked meat consumption were positive IgM that due to statistical tests, this relationship was statistically significant (P. Value =0/046) (Table 3).

DISCUSSION

With regard to the results of this study, 5/4% of women had positive IgM that means that 15 out of 280 women lived under acute phase of disease.

The result of this study also revealed that 94/6% of women in Zahedan city who were under marriage, are susceptible to toxoplasma infection during the pregnancy. But, since the environmental conditions have effect on spreading and prevalence of *T. gondii* infection in hot climate (Taravatee et al., 2003) and because Zahedan city is located in warm region and appropriate conditions are suitable for growing and survival of oocysts, in comparison with the prevalence obtained in different parts therefore this result is acceptable in this area.

Several studies across different areas of country have reported various results of high and medium levels of infection. As in Babol during the study among women under marriage, antibody positive rate for IgM, was 12.4% (Youssefi et al., 2007), in women referred to Avicenna Infertility Center at Tehran, was 4/2% (Chamani et al., 2006). There was no statistical relationship between antibody positive cases and age observed.

Our findings are similar to that of Taravatee et al. (2003) who showed no difference with regard to prevalence of toxoplasma antibody between urban women and border towns. In this study prevalence of toxoplasma antibody existed in10.6% of village residents who had a history of contact with cats, and were diagnosed as infected women also, data analysis confirmed a positive correlation between IgM reactivity

and contact with cat (P. Value =0.014). In the study done by Saeedi et al. (2002) on women referred for marriage counseling in Gorgan, showed that 29.4% of them has the risk of exposure to cats and the amount of this pollution, 16% has been reported by Mahmoudi et al. (2004) on high school age girls in Isfahan and 37/5% of this group of people in the town of Jolfa were identified as infected and had a history of contact with cats (Falah et al., 2005) and these results are consistent with our results. According to some studies, storage conditions for keeping cat at home and cat house pollution and their owners are important (Haddadzadeh et al., 2006). Considering the importance of acute infection of this disease and results of this study and others, it is recommended to pay keen attention to consider these points in order to prevent cat owners from getting this disease. One of the other variables investigated in this study was maintenance of other domestic animals except cats at home showed no significant relationship with rate of infection.

In this study, no statistically significant relationship among with education, incidence of disease, Job, way of washing and consumption of raw vegetables was observed. Similarly, significant relationship between con-sumption of half-cooked or uncooked meat and disease wasn't found and these findings are similar with the findings of Saeedi and his colleagues (Saedi et al., 2002) that confirmed meat is cooked completely in this region.

In this study, we came to the conclusion that 94.6% of Zahedani women had negative IgM antibodies to toxoplasma that are potentially at risk of acquiring acute toxoplasma infection during pregnancy and when they become pregnant, they are able to transfer the disease to their feti. Similar sero-epidemiological disease studies were carried out in other areas on pregnant women referred to health centers such as Khorramabad city, this rate was observed as 69% (CheraghiPoor et al., 2010), in Brazilian pregnant women 99% (Barbosa et al., 2009) and in Norwegian pregnant women it was found as 93.2% (Jenum et al.,1998) and in Mexican pregnant women 100% (Alvaredo-Esquivel et al., 2006) and these findings will issue insight to understand better the reason for changing among different study groups.

It is necessary that pregnant women antibody screening programs with aim of recognition and identi-fication of infection at the beginning of disease should be carried out to determine the infections occurring during pregnancy or earlier. Thus the exact time of occurrence of infection is very important. For this purpose, the mea-surement of the specific anti-toxoplasma antibodies such as IgA, IgG and IgM at the beginning time of infection is necessary.

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