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Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtbEpidemiological investigation <http://dx.doi.org/10.1016/j.apjtb.2015.09.016>Seroepidemiology of *Toxoplasma* infection in blood donors in Jahrom District, Southern Iran

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ARTICLE INFO

Article history:

Received 9 Sep 2014

Received in revised form 24 Sep, 2nd
 revised form 26 Nov 2014

Accepted 30 May 2015

Available online 19 Oct 2015

Keywords:

Toxoplasma

Blood donors

ELISA

Iran

ABSTRACT

Objective: To identify the anti-*Toxoplasma* antibodies from blood donors who referred to blood transfusion bases of Jahrom County, using ELISA method.

Methods: Based on the prevalence and characteristics method, 400 serum samples were collected from blood donors referred to Jahrom blood transfusion bases, Southern Iran, during 2010–2011, designed at testing by ELISA. IgM and IgG antibodies against *Toxoplasma gondii* were tested using ELISA kits (Dia-Pro) on serums. The data were analysed by SPSS 19 software.

Results: Review of 400 cases, 54 of them were IgG positive for parasites (13.5%) and 346 of those with negative IgG (86.5%). In IgM examination, 1.75% of them have been positive IgM (7 cases) and 98.25% of them were IgM negative (393 cases). By comparing the different group ages, 40–50 year age group had the highest prevalence of IgG positive (17.9%) and the age group of 30–40 years had the highest incidence of IgM negative (2.5%).

Conclusions: Due to the serological infection rate of toxoplasmosis obtained from this study, toxoplasmosis should be considered as a significant transfusion risk factor in Jahrom and also in any region with similar situations.

1. Introduction

Toxoplasmosis is one of the most common parasitic infections between human and animals [1,2]. This infection has a worldwide distribution and cats, birds and domestic animals are the most important reservoirs of the parasite [3]. Eating of infected undercooked meat, vegetables and fruits, transmission

through the placenta, blood transfusion and transplantation are the main route of transmission to humans [4–6].

Most people infected with toxoplasmosis are asymptomatic and the infection can persist for years in the body [5]. The main symptoms of this disease include severe brain and ocular complications of abortion [2]. The highest prevalence has been reported among Paris residents (in France) (up to 93%) [2]. The lowest prevalence has been reported from Northern Mexico (7.4%) [7,8]. In Iran, the rate of infection is also remarkable (Tehran 82%, Shiraz 77%, and Isfahan 57%) [9–12]. However, in a recent study done by Sarkari *et al.*, it seems that the infection is reduced in Shiraz (19.3%) [13].

Latent *Toxoplasma gondii* (*T. gondii*) infection in blood donors could be one of the modes of transmission of infection to recipients of blood [7,14]. Due to the high risks of transfusion transmitted infection in healthy and asymptomatic donors, the

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Peer review under responsibility of Hainan Medical University.

Foundation Project: This research has been supported by Vice Chancellery of Shiraz University of Medical Sciences and Jahrom University of Medical Sciences (Grant No. 2.90).

use of these bloods in surgical transplants such as kidney, bone marrow, and blood bags, which require several different donors, is important to clean the screen for *Toxoplasma* infection in the donors. Moreover, all transplant recipients and children with different types of blood diseases such as leukemia, aplastic anaemia, and thalassemia will have a reduced immune system to prevent infection, and these blood transfusions will create a lot of problems to their safety [15].

Seroprevalence of *Toxoplasma* infection was studied in blood donors in Jahrom District, Southern Iran. This cross-sectional study was conducted to identify the anti-*Toxoplasma* antibodies from blood donors who referred to blood transfusion bases of Jahrom County, using ELISA method.

2. Materials and methods

2.1. Study area

Fars Province placed in south of Iran covers an area of about 122400 km². Jahrom County is located in the southeast of province and situated at 30°4'45" North, 51°43'29" East, and 1050 m above the sea level (Figure 1) [16].

2.2. Sampling

In this investigation, according to the prevalence and characteristics formula, sample size was calculated to be about 400 cases for the prevalence, reliability and error of 15%, 95%, and 0.04, respectively. Samples were prepared aimed at testing by ELISA for *Toxoplasma* from donors referred to donate blood transfusion bases of Jahrom County after filling out the questionnaire forms during 2010–2011. The blood donor questionnaire contains: 1) What were the age, gender and occupation of blood donors; 2) How was their level of exposure to cats? (high, to some extent, or rarely); 3) How was the washing hand and time? (with/without soap, before/after meal); 4) How to wash vegetable consumption? (with/without disinfectants); 5) How was the meat consumption? (using boiled broth/barbecued); 6) What was the amount of direct contact with the soil? (high, rare, or none); 7) Living place (town/village); 8) What were the results of each serology test? (IgM and IgG positive/negative); and 9) Which of the following clinical signs in the last three months had? (swollen lymph nodes, ocular discomfort, and/or troubled brain).



Figure 1. Map of Iran.

2.3. Serological assay

Prevalence of anti-*Toxoplasma* antibodies in blood donors was done according to the immunoglobulin class, sex, age and different locations of Jahrom County. After centrifugation, serum samples were separated. They were maintained at -70°C in the freezer temperature before assessment. After collecting all samples, IgM and IgG antibodies against *T. gondii* were tested using ELISA. Kits and materials used in this study were prepared from Italy (Dia-Pro, Milan, Italy). During the test, the whole kit was used according to the instructions given in the brochure ahead. In this assay, there was a 96-well (holes) plate, which made it possible to perform 96 tests simultaneously [17].

Samples were added to the plate and incubated for 30 min, and then plates were washed three times to remove excess molecules. After further washing and incubation, chromogen was added to the cavity containing the substrate material. After 5–10 min, a colorless substrate is converted to a colored end product in the presence of peroxidase. The color changes were measured and recorded by spectrophotometer at a wavelength of 450 nm [17].

2.4. Data analysis

Statistical analyses of tables and graphs were performed using SPSS 19 statistical software (SPSS Inc., Chicago, USA). Descriptive statistics were shown in either mean standard deviation note or as frequency tables. Relationships between the categorical variables were evaluated by *Chi-square* assay. *P*-values less than 0.05 were considered as statistical significant.

3. Results

Review of 400 cases, 13.5% of them were IgG positive for parasites that are equal to 54 and 86.5% of those with negative IgG were equivalent to 346 cases. In IgM examination, 1.75% of them have been positive IgM against 7 people and 98.25% of subjects were negative for IgM against 393 individuals. By grouping together people of different ages and comparing them, 40–50 year age group had the highest prevalence of positive IgG (equivalent to 17.9%). The highest incidence of IgM was in the age group 30–40 years (2.5%) (Table 1).

In terms of gender, the prevalence of IgM in men and women were 1.7% and 0%, respectively. Moreover, IgG prevalence in male and female were 13.6% and 9.1%, but these differences were not statistically significant ($P > 0.05$). Also, no significant correlation was found between positive serology and a chance encounter with a cat against parasites. In people who had washed their hands with soap, IgG and IgM positive rates were 13.9% and 1.8% separately. But these rates were zero percent in those who washed their hands without soap; consequently, the relationship was not significant according to the statistical test ($P > 0.05$). IgG and IgM-positive rates were 11.7% and 0.6% respectively in people who had washed their hands before meals. These rates were 12.3% and 0% among people who washed their hands after meals. These amounts were 15.3% and 2.2% in the people who washed their hands before and after meals. Statistically, results indicated that there was not statistically significant relationship between toxoplasmosis rates and washing hands before and/or after meals. In the people who washed the vegetables with disinfectant, IgG and IgM-positive rates were 12% and 1.7% respectively, but these amounts were 12.4% and 1.6% in those

Table 1Prevalence of anti-*Toxoplasma* IgG and IgM antibodies in the serum of blood donor in Jahrom County, Southern Iran [n (%)].

Age groups	IgM (-)	IgM (+)	Total	IgG (-)	IgG (+)	Total
≤ 30	173 (98.30)	3 (1.70)	176 (44.0)	158 (89.8)	18 (10.2)	176 (44.0)
31–40	119 (97.50)	3 (2.50)	122 (30.5)	103 (84.4)	19 (15.6)	122 (30.5)
41–50	77 (98.70)	1 (1.30)	78 (19.5)	64 (82.1)	14 (17.9)	78 (19.5)
≥ 51 ≥	24 (100.00)	0 (0.00)	24 (6.0)	21 (87.5)	3 (12.5)	24 (6.0)
Total	393 (98.25)	7 (1.75)	400 (100.0)	346 (86.5)	54 (13.5)	400 (100.0)

Table 2Statistical results of *Toxoplasma* risk factors among blood donors in Jahrom County, Southern Iran.

Risk factors	Blood donors (n = 400)	Statistics
Age (years)	31.30 ± 5.64	$P < 0.001$
Gender (male/female)	187/213 (46.75%/53.25%)	$\chi^2 = 0.672, P = 0.328$
Having contact with cat(s)	46 (11.5%)	$\chi^2 = 3.02, P = 0.104$
Washing hand and time (with/without soap)	312/88	$\chi^2 = 15.47, P = 0.061$
Washing vegetable (with/without disinfectants)	346/54	$\chi^2 = 8.86, P = 0.075$
Consumption of raw or uncooked meat	35 (8.75%)	$\chi^2 = 7.44, P = 0.122$
Contact with soil	67 (16.75%)	$\chi^2 = 23.05, P = 0.455$
Living place (town/village)	284/116	$\chi^2 = 0.455, P = 0.048$
Clinical signs*	3 (0.75%)	$\chi^2 = 10.35, P = 0.082$

*: Swollen lymph nodes, ocular discomfort, and/or troubled brain.

who only washed them with water. Moreover, these rates were 40% and 0% among people who washed them with water and disinfectant respectively. However, no significant association was found between vegetable consumption methods of washing and serological antibodies ($P > 0.05$) (Table 2).

Among those who used meat broth boil, these amounts were 19.4% and 1.3% in people who have eaten grilled meat. In people who used both types of meat, these rates were 9.1% and 3.2% respectively. Although the percentage difference could be seen between the different types of meat, this difference was not statistically significant. IgG and IgM-positive rates were 11% and 3.6% respectively in people who had high direct contact with the soil, these percentages were 17.2% and 0.7% in people who have had little contact with the soil. Moreover, 11.9% and 1.6% of IgG and IgM rates were positive in people who have had no direct contact with the ground at all. These differences were not statistically significant ($P > 0.05$). There was a little difference between the rates of IgG and IgM positivity among urban and rural residents ($P \leq 0.05$). Among people living in urban areas, these rates were 12.7% and 1.7% respectively. However, these rates were 13.2% and 1.5% among rural residents (Table 2).

4. Discussion

Toxoplasmosis is a common parasitic disease in the world that infects humans and a wide range of mammals and birds [12,18]. Several factors are known to be effective in increasing the prevalence of the disease. Some of them consist of age, climatic conditions, habits and behaviours related to nutrition, soil or cat exposure, occupation, gender and education level [19,20]. The factors that enter into the living human parasitic disease directly or facilitate it indirectly increase the incidence of toxoplasmosis [19].

Consuming unwashed agricultural crops, cats and contact with cat feces (oocyst), not frozen meat and poultry before eating animals, undercooked animal meat, and not using gloves when in contact with meat and viscera of animals are the most important factors that increase the incidence of toxoplasmosis [21,22]. Occupation, gender, and level of education may indirectly have effect on the prevalence of the disease [19].

ELISA assay, with a sensitivity of 100% and specificity of 97%, is considered as one of the best laboratory tool to detect the serum antibodies and immune levels against specific diseases [17]. In the present study, the serological infection rate of toxoplasmosis (13.5%) was closed to results obtained from Fasa County (10%) [23]. The rate was less than results obtained from Isfahan, Bushehr, Tehran and Shahre Rey [10,11,24], but more than results obtained from different parts of Iran, which probably due to different conditions in the same geographical regions of Jahrom and other studied regions [23].

The rate of infection in men and women was not significantly different. This result is similar to the report from Meshkinshar by Keshavarz *et al.* [25]. Keshavarz and colleagues in a study conducted in Islamshahr reported the infection in men (27.8%) and women (42.6%), where the difference was significant [26]. Moreover, another study was conducted in Tehran by Salahi Moghaddam and Hafizi that infection rates were 49.7% and 75.4% in men and women separately, which was significant [10].

There were no significant differences between age groups. IgG immunoglobulin is usually chronic and past infection is separated from the patient. Especially in toxoplasmosis which is contaminated for the first time and tissue cyst formation in the different tissues, most of the older adult population is positive for IgG. In a study conducted in Meshkinshar, the highest infection rate reported in those aged over 40 was 25.4%, and the infection rate increased with age [25]. In Isfahan, center of Iran, the highest rate of infection has been reported at the age of 40 (50%) [11]. In Islamshahr, the highest rate of infection was seen at the age of 30 years (52.8%) [26]. Mansouri and colleagues conducted a study in Kermanshah which obtained the highest infection rate in the age group of 30–40 years (48.3%) [27]. Moteallehi Ardakani and colleagues conducted a study in Yazd reported the highest infection rate in the age group of 30–40 years [28].

In this study, no significant relationship was found between the frequency of toxoplasmosis and cat contact, which may be due to lack of awareness of participants about ways of infection with parasites excreted by cats or other pets when filling out the questionnaire. In other studies like Keshavarz *et al.* in Meshkinshahr, no relationship was seen in this group as well [25], but in

Fasa, the relationship between contact with the cats and the increasing percentage of *Toxoplasma* infected was significant [23].

Use of raw and cooked meats listed as the risk factors for these parasites. The method of cook meat consumption introduced as the most common cause of toxoplasmosis infection in Sanliurfa Province (Turkey) by Tekay and Ozbek [29]. In the present study, the prevalence of *T. gondii* in individuals who consumed grilled meat was higher than those who ate beef broth or poached meat (12.6%). However, the differences were not statistically significant. In the study conducted in Fasa, no correlation was seen between type of cooking and *Toxoplasma* infection [23].

In Iran, the prevalence of toxoplasmosis were reported higher in the rural areas than in urban areas from Kermanshah, Islamshahr, and Chaharmahal and Bakhtyari [26,27,30]. These differences were not significant in any of these studies. Contact with soil, arable land, rural people can be different cited as the causes of contamination in urban and rural areas. In the studied area of Isfahan, Saveh, and Bushehr, contamination in urban areas has been reported more than in rural areas and only the difference in Saveh was significant [10,31,32]. In the present study, the infection rates in rural areas (13.2%) were more than in urban areas (12.7%), which was not statistically significant. In other study done by Sarkari et al. in Shiraz, 12.3% and 5.47% of the blood donors were seropositive for only IgG and IgM, respectively. Moreover, 1.6% were positive for both IgG and IgM [13].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are grateful for the logistical and financial support given by the Vice-Chancellor for Research and Technology, Shiraz University of Medical Sciences, Shiraz, Iran.

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