Original Article

Seroprevalence and molecular diagnosis of *Toxoplasma gondii* infection among blood donors in southern Iran

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Abstract

Introduction: *Toxoplasma gondii* is a protozoan parasite which can be transmitted to human through a variety of routes including blood transfusion. This cross sectional study aimed to evaluate the seroprevalence of *Toxoplasma* infection and related epidemiological features among healthy blood donors.

Methodology: A total of 1,480 healthy blood donors from five blood service centers in Fars province were analyzed for anti-*Toxoplasma* antibodies. Blood samples were tested for anti-*T. gondii* IgG and IgM antibodies by enzyme immunoassay. IgM-positive samples were also tested for the presence of *Toxoplasma* DNA by Polymerase Chain Reaction (PCR). Demographic characteristics of participants were also recorded during samples collection.

Results: Anti *T. gondii* antibodies were detected in sera of 286 out of 1,480 blood donors corresponding to an overall seroprevalence of 19.3% in this population. From these, 182 (12.3%) were seropositive only for IgG, 81 (5.47%) were seropositive only for IgM and 23 (1.6%) were positive for both IgG and IgM. PCR detected active parasitemia in two (1.9%) of the IgM-positive subjects. Age, place of residence and level of education were statistically significant ($p < 0.05$) with seropositivity to *Toxoplasma*.

Conclusions: Our results highlighted that asymptomatic blood donors, especially those with active parasitemia, may constitute a significant risk of transmitting toxoplasmosis to susceptible recipients.

Key words: *Toxoplasma gondii*; blood donors; seroprevalence

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Introduction

Toxoplasma gondii is a protozoan which infects almost one third of world's population [1]. The seroprevalence of *Toxoplasma* varies in different countries or even in different areas of a given country. Although toxoplasmosis is a mild diseases in people with competent immune system, the disease is severe and life threatening in immunocompromised individuals [2]. Moreover, the consequences of congenital transmission of the parasite to a fetus are devastating.

The infection is usually transmitted through ingestion of oocyst-contaminated food or water, consumption of undercooked meat and vertical transmission during pregnancy [3]. Furthermore, *Toxoplasma* infection can be transmitted through organ transplantation and whole blood or white blood cell transfusion from a seropositive donor to a seronegative recipient [4].

The infection in immunocompromised individuals such as transplant recipients and HIV-positive patients can result in severe consequences including encephalitis, chorioretinitis and myocarditis [5].

Toxoplasmosis is a common infection in human and animals in all areas of Iran, including Fars province [6-7].In a recent study in this region, prevalence of *Toxoplasma* infection among animals (sheep and goats) was found to be 33.3% [7].

It has been demonstrated that *Toxoplasma* can transmit through blood transfusion [8]. Healthy seropositive blood donors, especially those who are in the acute phase of the infection, may play a major role in this case [9].

The rate of *Toxoplasma* infection in healthy blood donors varies in different areas of the world and this mainly depends on the rate of infection in the community [10-15]. In some areas, such as northeast Brazil, north India and Egypt, more than 50% of blood donors have been seropositive for *Toxoplasma*

infection [11, 14, 16]. Lower rate of infection in blood donors have been reported from Taiwan (9.3%), Thailand (9.6%), Mexico (7.4%) or Turkey (19.5%) [10, 15, 17-18].

Lack of information about the status of *Toxoplasma* infection in healthy blood donors in Iran justified this study which aimed to evaluate the seroprevalence rate of *Toxoplasma* and its relative epidemiological factors in asymptomatic healthy blood donors in southern Iran. Moreover, the study aimed to assess the possible presence of parasite DNA in healthy donors.

Methodology

Study population

The current study was conducted between July 2012 and March 2013 in Fars province. Fars is one of the 31 provinces in Iran and known as the Cultural Capital of Iran. It is situated in the south of the country and its capital is Shiraz. It has an area of 122,400 km². In 2006, this province had a population of 4.57 million people. After getting approval from the ethics committee of Shiraz University of Medical Sciences, blood samples were taken from 1,480 healthy volunteer blood donors from five branches of blood service centers in the province. These centers were in Kazeroun, Jahrom, Darab, Firouzabad and Nourabad counties. The sample size was estimated based on the population of the area. Demographicfeatures of participants were recorded during sample collection.

Serological test

Sera were obtained from the fresh whole blood of the blood donors. Moreover, buffy coat was obtained from each samples for subsequent DNA extraction. Samples were transferred from each blood service centers in each county to the serology laboratory at department of parasitology and mycology in Shiraz (Shiraz University of Medical Sciences, Shiraz, Iran). Samples were kept at -20 °C until use. Sera samples were tested for anti-*Toxoplasma* IgG and IgM, using a commercial enzyme immunoassay kit (PishtazTeb Diagnostics, Tehran, Iran).

DNA Extraction and nested PCR

DNA was extracted from the buffy coat of all of the IgM positive samples. DNA of each sample was extracted, using proteinase K and lysis buffer followed by phenol/chloroform/isoamyl extraction. Absolute ethanol was used to precipitate the DNA. Precipitated DNA was resuspended in 100 µL of double distilled water and stored at 4°C until use. Nested PCR was performed as described by Asgari *et al.* [7]. Nested primer sets were used for amplifying fragments of the B1 gene of *Toxoplasma gondii*. The outer primers which produced an amplified product of 432 bp were from bases 171 to 190 (5′-CCG TTGGTT CCG CCT CCT TC-3′) and from bases 602 to 583 (5′-GCA AAA CAG CGG CAGCGT CT-3′). Inner primers were from bases 180 to 196 (5′-CCG CCT CCT TCG TCCGTC GT-3′) and from bases 392 to 372 (5′-GTG GGG GCG GAC CTC TCT TG-3′) producing an amplified product of 213 bp.

Analysis of data

Results were analyzed by SPSS software (version 17), with a p-value<0.05 taken as statistically significant. Chi-squared and Fisher exact tests were used to compare the seroprevalence values related to the characteristics of the subjects.

Results

The mean age of participant was 39.1 (range: 20-68 years of age). Most of the subjects were aged 31-40 years. Male constituted 94.3% of participants and female were 5.7% of the subjects. Anti *T. gondii* antibodies was detected in sera of 286 out of 1,480 blood donors corresponding to an overall seroprevalence of 19.3% in this population. Of these, 182 (12.3%) were seropositive for only IgG, 81 (5.47%) were seropositive for only IgM and 23 (1.6%) were positive for both IgG and IgM. Demographic features of the blood donors are shown in Table 1.

Considering the residence of the blood donors, Kazeroun had the highest seroprevalence rate of *Toxoplasma* (25.5%) followed by Nourabad (22.7%), Darab (18.3), Firouzabad (16.3%) and Jahroum (14.7%) counties. The differences in *Toxoplasma* seropositivity and residence of participant was statistically significant ($p<0.05$). The highest seroprevalence rate of *Toxoplasma* (31.2%) was found in the age group of 41-50 years while the lowest seropositivity (16.2%) was seen in the age group of 20-30 years. The differences between age and presence of anti-*Toxoplasma* antibodies was statistically significant ($p < 0.05$).

Seroprevalenc erate of *Toxoplasma* was lower in educated people and this difference was statistically significant ($p < 0.05$). The seroprevalence rate was higher in married subjects than in unmarried ones and the differences were significant ($p < 0.05$).

Table 1. Demographic characteristics of blood donors and relative seropositivity to *T. gondii* in Fars province, southern Iran

Characteristics	Frequency (No.)	Percent (%)	Positive for anti-*Toxoplasma* antibodies (either IgG or IgM)		P value
			No.	%	
Gender					
Male	1396	94.3	264	19	> 0.05
Female	84	5.7	22	26.2	
Age group					
20-30	364	24.6	59	16.2	< 0.05
31-40	455	30.7	74	16.3	
41-50	383	25.9	87	22.7	
51 through higher	262	17.7	59	22.7	
Residence					
Kazeroun	297	20.1	73	24.6	< 0.05
Jahrom	285	19.3	42	14.7	
Darab	306	20.7	56	18.3	
Firouzabad	296	20	48	16.3	
Nourabad	296	20	67	22.7	
Marital status					
Married	1175	79.4	242	20.6	< 0.05
Unmarried	305	20.6	44	14.4	
Educational level					
Uneducated	38	2.6	8	21.1	< 0.05
Primary and secondary level	603	40.9	139	23.1	
Post-secondary level	498	33.6	81	16.3	
University level	342	23.1	56	16.4	
Occupation					
Employee	398	26.9	76	19.2	> 0.05
Business	719	48.6	142	19.8	
Housewives	77	5.2	21	27.3	
Student	113	7.6	13	11.5	
Laborer	65	4.4	13	20	
Farmer and stockbreeder	73	4.9	13	17.8	
Unemployed	35	2.4	8	22.9	
Blood group					
A	399	27	89	22.5	>0.05
B	360	24.3	66	18.3	
AB	92	6.2	19	20.7	
O	618	41.8	110	17.8	
Rh					
Positive	1354	91.5	263	19.5	>0.05
Negative	115	7.8	21	18.3	

While most of donors (44.7%) gave blood on a regular basis, 28.1% of them had no experience of blood donation before. Blood group O was the most frequent group (41.8%) and AB was the least frequent (6.2%) blood group. No correlation was found between *Toxoplasma* seropositivity and ABO or Rh blood group ($p > 0.05$). Moreover, no association was found between the occupations of participants and *Toxoplasma* seropositivity ($p > 0.05$).

All of IgM-positive samples were tested for the presence of *Toxoplasma* DNA. *Toxoplasma* DNA was detected in two of IgM-positive samples (1.9%). These two samples were positive for only IgM but not IgG. Correlation between IgM positivity and PCR results was statistically significant ($p = 0.005$).

Discussion

Presence of organism in blood during the course of infection ensures its transmission through transfusion [9]. Moreover, the ability of organism to survive in the stored blood is another factor which increases the chance of transmission through transfusion. It has been found that tachyzoites of *Toxoplasma* can survive in stored blood for several weeks [19]. During the course of active infection, *Toxoplasma* might be present in blood and this would be a real threat for blood recipients especially patients undergoing multiple transfusion or those who require blood transfusion during the course of transplantation.

The current study is the first seroprevalence study of *Toxoplasma* infection among healthy blood donors in south of Iran. Samples were taken from healthy volunteers donating blood from five blood transfusion centers in different geographical areas of Fars province, south of Iran. The Iranian blood transfusion organization (IBTO) is a nationally qualified organization which performs blood transfusion procedures. IBTO has a main center in each province and different branches in counties of each province. Recent data show that there are twenty-three blood donors per 1,000 population in Iran. More than 90% of blood donations in Iran are collected from voluntary non-remunerated blood donors and the rest is donated as family replacement donation [20].

We found an overall seroprevalence of 19.3% in blood donors. This rate of seroprevalence in healthy blood donors is more or less similar to the rates reported from Malaysia, South India, United Arab Emirates and Turkey [15-16, 21-22], but lower than those reported from Brazil, Egypt and Saudi Arabia [11,13-14].

In a study by Ormazdi *et al*., the rate of *Toxoplasma* infection in blood donors referred to Tehran blood transfusion organization has been evaluated. Among 250 healthy volunteer blood donors, 132 (52.8%) cases have been positive for IgG and nine cases (3.6%) for IgM anti-*Toxoplasma* antibodies [23].

In the current study, differences in the seroprevalence rate of *Toxoplasma* in different donation centers of the province were statistically significant as people living in Kazeroun and Nourabadhad had a higher seroprevalence rate of *Toxoplasma* than others. This might be due to the differences in climate condition of these two areas whose temperature is milder than Firozabad, Jahrom or Darab. This might increase the chance of survival of oocysts in the environment and a higher transmission rate of *Toxoplasma* through contaminated food or soil.

High seroprevalence of *T. gondii* with age detected in this study is consistent with other studies conducted on this subject [11,17-18]. The increase in risk of acquiring *Toxoplasma* for elder people may be due to a longer lifetime exposure of these people to *Toxoplasma*-contaminated environmental sources.

Seroprevalence of *Toxoplasma* was higher in married compared to unmarried subjects in this study. Considering the association of age and *Toxoplasma* seropositivity, this might be more related to the age of the married subject, rather than to the marital status, which is higher for married participants.

The inverse rate of seroprevalence with the level of education detected in our study has been also documented in previous studies [11,14,18].

Gender was not associated with *Toxoplasma* seropositivity and it is difficult to draw any relation between sex and seroprevalance since more than 90% of people donating blood are males. The presence of IgM anti-*Toxoplasma* antibodies reflects the risk of transmission through transfusion. The seroprevalence rate of IgM anti-*Toxoplasma* antibodies in blood donors varies from 2.4 to 5% [12]. In our study, 5.4% of the blood donors were seropositive for IgM and 1.6% was tested positive for both IgG and IgM. More importantly, *Toxoplasma* DNA was detected in blood samples of two of IgM positive cases. The presence of parasitemia revealed by PCR in IgM-positive healthy blood donors ensures the likelihood of transmission of *Toxoplasma* through blood transfusion.

Conclusion

In conclusion, considering the relatively high seroprevalence rate of *Toxoplasma* infection in blood donors reported in this study, and in view of the fact

that IgM-positive individuals might have tachyzoites in their blood, toxoplasmosis should be considered as a significant transfusion risk in this region and also in any region with similar conditions. Appropriate strategies should be adapted to reduce the risk of acquiring toxoplasmosis through blood transfusion. It can be suggested that immunosuppressed recipients and pregnant women receive *T. gondii* antibody-negative blood components for transfusion.

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