



Original article

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## Detection of acute and chronic toxoplasmosis amongst multi-transfused thalassemia patients in southwest of Iran

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### ABSTRACT

**Objective:** Since pre-transfusion screening for *Toxoplasma gondii* is not performed on blood packs, thalassemia patients are susceptible to acquiring toxoplasmosis; thus, the aim of this study was to evaluate the seroprevalence status of *T. gondii* in individuals who suffer from thalassemia in comparison to healthy persons in the southwest of Iran.

**Methods:** In this case-control study, 117 thalassemia patients and 205 healthy persons participated. All samples were tested for the presence of specific IgG and IgM antibodies against *T. gondii* using ELISA technique. Data were analyzed using Chi-square test.

**Results:** Seroprevalence of anti-*T. gondii* IgG was detected in 30.76% (36/117) of patients and 20% (41/205) of healthy individuals ( $P=0.04$ ), also anti-*T. gondii* IgM in these groups was detected 1.70% (2/117) and 0.48% (1/205), respectively ( $P=0.3$ ). In present study, nine related risk factors with toxoplasmosis were evaluated and data analysis showed that only contact with cat was significantly correlated with IgG seroprevalence ( $P=0.02$ ).

**Conclusions:** Current research suggests thalassemia patients are more prone than normal persons to acquiring *T. gondii* infection ( $P=0.04$ , OR:1.77). Due to limited studies in this high risk group, further studies are recommended.

## 1. Introduction

Toxoplasmosis is a widespread zoonosis disease, caused by an obligate intracellular protozoan parasite known as *Toxoplasma gondii* which can infect a wide range of warm-blooded vertebrates such as man, livestock, birds and marine mammals. For the first time

it was characterized from liver and spleen smears in a north African rodent known as *Ctenodactylus gondii* by Nicolle and Manceaux in 1908. Some routes of *T. gondii* transmission include: food-borne (consuming raw meat, drinking water contaminated by oocyst, ingestion of undercooked meat contaminated by cyst, etc), zoonotic (ingesting oocysts shed by infected cats), congenital (mother to foetus), organ transplantation as well as blood transfusion[1,2]. *Toxoplasma* is mostly asymptomatic in immunocompetent individuals although it is considered as an opportunistic infectious agent in high risk groups which include: pregnant women, immunocompromised individuals, i.e. cancer patients, HIV+ and organ transplant recipients[3-8]. It is estimated at least one third of the world's population are infected[1, 9]. According to recent studies, it has been reported that seroprevalence of *T. gondii* in Iranian general

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population, Iranian pregnant women and, immunocompromised patients is 39%, 41% and, 50%, respectively[3,5,10]. Based on recent studies, chronic toxoplasmosis may associated with autoimmune and neurodegenerative disorders[11-15].

Thalassemia is one of the most common genetic disorders which happens due to mutation in genes responsible for creation of  $\alpha$  or  $\beta$  globulin chains and leads to non-production or reduced production of globin chains[16]. Due to severe anemia in thalassemia patients, numerous complications occur including: changes in the face, long bones, pathological fractures in spinal column, osteopenia, osteoporosis, short stature, hemolytic anemia, hepatomegaly, hepatocellular carcinoma, splenomegaly, heart failure, zinc deficiency, liver dysfunction, metabolic disorders, endocrine disorders, hypogonadism, spermatogenous cell abnormalities, etc[16, 17].

The national program for thalassemia control was initiated in 1996 in Iran and due to special attention to the disease, the number of afflicted newborns has declined. It is estimated 2-3 million carriers of thalassemia and 14,000 thalassemia patients exist in Iran [16, 18]. Over 25% of total national blood products have been used for thalassemia patients in Iran. Since pre-transfusion screening for *T. gondii* is not performed on blood packs, these patients are susceptible to acquiring toxoplasmosis; thus, the aim of this study was to evaluate the seroprevalence status of *T. gondii* in individuals who suffer from thalassemia in comparison to healthy persons in the southwest of Iran.

## 2. Materials and Methods

### 2.1. Study area

Ahvaz city, capital of Khuzestan province which is located in the southwest of Iran (31°50' N and 49°11' E), is ranked as the 7th largest city throughout the country and based on the latest census, its population is calculated about 1,395,184 in 352,128 families. Weather temperature is highly variable throughout the year so that in summer temperature exceeds 50°C whereas in winter it falls to 5°C. Also, annual average rainfall is approximately 230 mm[19, 20].

### 2.2. Study population

In this case-control study, blood samples were collected from 117 thalassemia patients as case group who referred to the Research Center of Thalassemia and Haemoglobinopathies (RCTH) located at Shafa hospital affiliated to Ahvaz Jundishapur University of Medical Sciences, Iran (the only center working on oncology and

hematology in the southwest region of Iran, Khuzestan province) for routine follow-ups by an oncology specialist. Each thalassemia patient had his/her own folder which included: epidemiological data, laboratory findings, prescribed drugs and the follow-up information. In addition, 205 serum samples were collected from non-thalassemia and apparently healthy persons as control group who were admitted to Golestan hospital, an educational hospital affiliated to Ahvaz Jundishapur University of Medical Sciences, Iran and adjacent to Shafa hospital. Inclusion criteria in our study were as follow: 1) individuals who agreed to participate in the study in both groups (after obtaining a written informed consent); 2) in case group: possessing personal folder as thalassemia patient in Shafa hospital; examination by physician to rule out malignancy or immunodeficiency disorders according to records documented in thalassemia patients folders and using routine laboratory findings; 3) in control group: complete blood count (CBC) to rule out thalassemia and anemia; lack of history of prior blood transfusions, checkup by physician to rule out any serious disease or immunodeficiency disorders.

### 2.3. Serology method

In order to detect specific IgG and IgM antibodies against *T. gondii*, 5 milliliters of venous blood was taken from each subject who met the above mentioned inclusion criteria of the study. Sera were separated by centrifugation at 4000 rpm for 5 minutes and then stored at -20°C until tested using enzyme-linked immunosorbent assay (ELISA) technique according to the manufacturer's guideline as described earlier[6]. Anti-*T. gondii* IgG and IgM antibodies were measured using commercial ELISA kit (IgG and IgM, Trinity Biotech Captia™, Jamestown, NY, USA). For both IgG and IgM antibodies, the levels lower than 1.1 IU/mL were considered as negative samples and levels equal to or higher than 1.1 IU/mL were considered as positive samples.

### 2.4. Questionnaire

A questionnaire was filled out for each participant in both case and control groups containing the demographic information including: age (25 or less, 26-30 and >30), gender (Male or Female), residence (Urban or Rural regions), marital status (Single, Married, Divorced or Widowed), education level (Grade school, 12years/High school or University degree), ethnicity (Fars, Arab, Lor, Kord, Turk or Other), contact with cat (Yes or No), consumption of raw/undercooked meat (Yes or No), exposure to soil (Yes or No) and access to safe drinking water and sanitation.

### 2.5. Statistical Analysis

Data were analyzed using Chi-square test. The probability level of 0.05 was accepted as statistically significant. Statistical analyses were carried using SPSS version 16.

## 3. Results

### 3.1. Participants

In this study, a total of 322 subjects met the inclusion criteria and participated (117 thalassemia patients and 205 apparently healthy individuals). The mean age of participants in case and control groups was 26.01±9.69 and 28.42±10.26 years old, respectively.

### 3.2. Seroprevalence of anti-*T. gondii* antibodies

The seroprevalence of toxoplasmosis amongst thalassemia patients

in terms of IgG and IgM was detected 30.76% (36/117) and 1.70% (2/117), respectively; while frequency of these antibodies in control group was 20% (41/205) and 0.48% (1/205), respectively. IgG seroprevalence between two groups was statistically significant ( $P=0.04$ ), however no significant difference was observed in terms of IgM frequency between thalassemia patients and healthy persons ( $P=0.3$ ) (Table 1).

**Table 1**

Analysis of anti-*T. gondii* IgG and IgM antibodies in thalassemia patients and control group

	Patients' group n (%)	Healthy individuals n (%)	Sig.	OR.
IgG- positive	36/117 (30.76%)	41/205 (20%)	0.04	1.77
IgM-positive	2/117 (1.70%)	1/205 (0.48%)	0.3	3.55

Abbreviations: Sig, Significance; OR, odds ratio.

### 3.3. Risk factors

In current survey, nine risk factors related to toxoplasmosis infection have been recorded. Univariate analysis of demographic

**Table 2**

Demographic characteristics and risk factors related to seroprevalence of *T. gondii*

Characteristic	Thalassemia patients (N=117)				Controls (N=205)				Total (N=322)			
	Number tested	IgG positive	%	P value	Number tested	IgG positive	%	P value	Number tested	IgG positive	%	P value
Gender												
Male	59	19	32.20	0.5	99	21	21.21	0.4	158	40	25.31	0.4
Female	58	17	29.31		106	20	18.86		164	37	22.56	
Age group (years)												
25 or less	51	16	31.37	0.8	84	18	21.43	0.2	135	34	25.18	0.5
26-35	45	13	28.88		77	14	18.18		122	27	22.13	
>35	21	7	33.33		44	9	20.45		65	16	24.61	
Residence												
Urban	83	26	31.32	0.3	166	33	19.87	0.5	249	59	23.69	0.8
Rural	34	10	29.41		39	8	20.51		73	18	24.65	
Education level												
Grade school	90	30	33.33	0.1	18	3	16.66	0.3	108	33	30.55	0.3
12years/High school	27	6	22.22		20	3	15		47	9	19.14	
University degree	0	0	0		167	35	20.95		167	35	20.95	
Ethnicity												
Fars	37	12	32.43	0.7	71	15	21.12	0.1	108	27	25	0.2
Arab	35	12	34.28		56	10	17.85		91	22	24.17	
Lor	37	10	27.02		44	8	18.18		81	18	22.22	
Kord	5	1	20		11	3	27.27		16	4	25	
Turk	2	1	50		10	2	20		12	3	25	
Other	1	0	0		13	3	23.07		14	3	21.42	
Contact with cat												
Yes	83	26	31.32	0.5	80	23	28.75	0.01*	163	49	30.06	0.02*
No	34	10	29.41		125	18	14.4		159	28	17.61	
Consumption of raw/undercooked meat												
Yes	18	5	27.77	0.4	14	3	21.42	0.5	32	8	25	0.2
No	99	31	31.31		191	38	19.89		290	69	23.79	
Exposure to soil												
Yes	47	17	36.17	0.07	127	25	19.68	0.2	174	42	24.13	0.4
No	70	19	27.14		78	16	20.51		148	35	23.64	
Source of drinking water												
Purified water	89	28	31.46	0.5	200	41	20.5	0.4	289	69	23.87	0.3
Unpurified water	28	8	28.57		5	0	0		33	8	24.24	

\*Statistically significant

information was done in order to determine the possible association between seroprevalence of *T. gondii* and these risk factors. Risk factor analysis showed that there is statistically significant association only between seroprevalence of *T. gondii* and contact with cat ( $P=0.01$  for control group and  $P=0.02$  for total population) (Table 2). Based on gender, the frequency of anti-*T. gondii* IgG antibodies was identified as being higher in males than females in both groups but the difference was not significant ( $P=0.4$ ). Other surveyed risk factors, which have been listed in Table 2, show no significant relationship with toxoplasmosis.

#### 4. Discussion

The prevalence rate of toxoplasmosis in the southwest of Iran ranged from 21% to 47% in different groups[4-6,10,15]. Present survey is the first study to evaluate the seroprevalence of toxoplasmosis amongst thalassemic patients in the southwest region of the country. Seroprevalence of anti-*T. gondii* IgG was identified in 30.76% (36/117) of patients and 20% (41/205) of healthy individuals ( $P=0.04$ ), as well as anti-*T. gondii* IgM in these groups which was detected 1.70% (2/117) and 0.48% (1/205), respectively ( $P=0.3$ ). Karakas et al. studied 36 thalassemia patients and 36 healthy individuals in Turkey and the seroprevalence rate of toxoplasmosis in terms of IgG and IgM was reported 19.4% and 5.5% (borderline) among case group, respectively; while IgG was 14% in control group and no significant statistical difference was observed between two groups ( $P=0.752$ )[21], which is lower than our results. The reason for this could be study population, sample size, methodology, cultural habit of the region, etc. In this research, ELISA technique has been employed. Several serological methods are routinely used in order to detect IgG and IgM antibodies against *T. gondii*. Among them, ELISA with high sensitivity and specificity, is able to discern between chronic and acute phase of toxoplasmosis[22].

Approximately 14,000 thalassemic patients have been identified and registered in Iran with the median age of 15 years old[18]. The disease exists throughout the country, but it is more common in the bordering parts of the Oman Sea and Persian Gulf (Hormozgan, Bushehr and Sistan & Baluchestan provinces), adjacent parts of Caspian Sea (Mazandaran, Gilan and Golestan provinces) as well as Fars, Kerman and Khuzestan provinces. In the aforementioned regions, nearly 10% of the total population are  $\beta$ -thalassemia carriers, while in other zones of the country, it is less common and ranges between 3-8%. Also, nearly 1000 thalassemia major individuals are born in Iran every year[16].

Multiple blood transfusion is vital for thalassemia major patients;

hence, they are prone to acquiring the blood-borne pathogens [23]. According to World Health Organization (WHO) strategies, screening of blood packs must be done for some blood-borne pathogens including: hepatitis B virus surface antigen (HBsAg), hepatitis C virus (HCV), human immunodeficiency virus (HIV) and *Treponema pallidum* in all countries and for human T-cell lymphotropic viruses I/II (HTLV I/II), human cytomegalovirus (CMV), Chagas disease and Malaria in some countries and endemic regions[24]; while pre-transfusion screening for *T. gondii* has not yet been performed. Also, there are some reports that documented *T. gondii* could thrive in citrated blood at 5° C for more than 50 days and blood transfusion contributes in the transmission of parasites by blood products[25, 26]. Seroprevalence of toxoplasmosis in healthy blood donors range from 1-75% worldwide[9]. Also, in some studies conducted in different geographical areas of Iran, the prevalence rate of *T. gondii* amongst blood donors from southern, southeastern and northern regions was reported 19.3%, 25%, 56.4%, respectively, which indicates existence of this infectious agent in all major areas of the country[9].

It should be noted that nearly 25% of total national blood products is being used for thalassemia patients in Iran[18]. Numerous reports have documented the prevalence of transfusion-transmissible infections (TTI) in high risk groups, particularly different multi-transfused patients. For instance, seroprevalence of human T- cell leukemia virus Type-1 (HTLV-1) was tested in both thalassemia and hemodialysis patients in the southwest of Iran. The results showed a significant frequency among case group (7.6% - 27/357) in comparison with control group (0.62% - 5/800)[27]that is in agreement with current study. Furthermore, seroprevalence of hepatitis infection in high risk groups such as sickle cell anemia patients, persons with haemophilia, hemodialysis patients and patients with thalassemia is reported by several authors ranging between 1.1-5.1% for HBV and 7.9-54% for HCV in the southwest of Iran[28-31]. In another study, anti-*T. gondii* antibodies were examined in hemodialysis patients. Seroprevalence rate of IgG and IgM was observed in 29.3% and 7.9% of patients, respectively; while in control group seropositivity for these antibodies was 26% and 4%, respectively. The difference between two antibodies was statistically significant ( $P<0.05$ )[6].

In present research, 9 related risk factors with toxoplasmosis were evaluated and data analysis showed that only contact with cat was significantly correlated with IgG seroprevalence ( $P=0.02$ ). Since felines are the definitive hosts for *T. gondii* and they are in close contact to humans particularly in rural regions, accordingly, there is a general agreement that contact with cat with maintaining

the disease transmission chain, is considered as a potential risk factor in the majority of studies [5, 9, 10, 32, 33]. As it was mentioned in study area section, Khuzestan province due to its proximity to the sea, had appropriate humidity that is vital for oocysts sporulation. The oocysts are shed in large amount through feces of infected cats and can live for several months in moist soils [32]. Thus exposure to soil could have a key role in toxoplasmosis morbidity. However, in our results this risk factor was not significant in two groups ( $P=0.07$  and  $P=0.2$  in case and control group, respectively) that corresponds to Zemene et al's study among pregnant women [34], while Cong et al. showed exposure to soil is associated with persistence of *T. gondii* in study population [35]. Consumption of raw/undercooked meat is one of the studied risk factors in our study that was not statistically significant ( $P=0.4$  and  $P=0.5$  in thalassemia patients and control individuals, respectively). In some studies it has been shown that consumption of raw meat is not correlated with toxoplasmosis [5, 35-38] while results of other investigations are in contrast [9, 10]. Risk factors like gender, residence, age group, educational level, ethnicity and source of drinking water which have been detailed in Table 2, were not statistically significant in current study.

There is evidence which suggests that, despite the constant presence of anti-*T. gondii* IgM antibodies in serum, no positive IgM-ELISA can be indisputably considered as an acute infection [6]; thus, PCR-based techniques to explore DNA of *Toxoplasma* are required in future studies.

## 5. Conclusion

Our study suggests thalassemia patients are more susceptible than control persons to acquiring toxoplasmosis ( $P=0.04$ ); thus, due to rare studies in this high risk group, further studies are recommended.

## Conflict of interest statement

The authors declare no conflict of interests.

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