Original Article

Seroprevalence of toxoplasma-specific antibodies in patients suspected to have active toxoplasmosis: A cross-sectional survey

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Abstract

Background: The aim of this study was to investigate the presence and distribution of anti-toxoplasma-specific IgM and IgG tantibodies in patients suspected to have toxoplasmosis and investigate for any association between IgM and IgG antibodies and some toxoplasmosis risk factors as well.

Materials and Methods: In a comparative cross-sectional study, 70 patients suspected to had active toxoplasmosis and 30 control volunteers, who gave informed consent, entered the study. In each group, patient age, sex, signs of appearance, education level, residency status (urban / rural), occupation, frequency of toxoplasma-specific IgG and IgM antibodies, abortion history, and some risk factors (Direct cat exposure, Occupational exposure to raw meat, and Raw vegetable consumption) were recorded. The enzyme-linked immunosorbent assay (ELISA) kits (EUROIMMUN®, United Kingdom) were used for the evaluation of anti-toxoplasma IgG and IgM antibodies according to the manufacturer›s instructions. All analyses were done using SPSS-20.

Results: The frequency of toxoplasma-specific IgG and IgM antibodies like: Direct cat exposures, Occupational exposure to raw meat, and Raw vegetable consumption were not statistically significant between the two groups ($P > 0.05$). The history of previous abortions in women in the toxoplasmosis-suspected group was significantly higher than that in the controls (31.4% versus 6.7%; $P = 0.009$).

Conclusion: The frequency of specific IgM and IgG antibodies in toxoplasmosis suspected in the toxoplasmosis and control groups was not statistically significant.

Key Words: IgG antibody, IgM antibody, Isfahan, *Toxoplasma gondii*, toxoplasmaspecific antibodies

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INTRODUCTION

Toxoplasma gondii is one of the most common intracellular protozoan parasites, with up to 30% of the world's human population affected by this parasite and the third main cause of food-related deaths in the United States of America[1-3]. In Iran, the rate of toxoplasmosis, in 2008, was 40.7% for Isfahan, 44.2% for Lorestan, and 34.2% for Bandar-e-Abbas.[4] Albeit cats are the

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certain hosts, the parasite can infect approximately all mammals and birds.[5-7] Humans are infected mainly by ingestion of *T. gondii* oocysts from contaminated soil, undercooked meat containing the parasite cysts, direct contact with cat feces or by vertical transmission from mother to fetus, via the placenta.[1,2,8] Although human toxoplasmosis is generally asymptomatic in a wide range of cases, it is of clinical importance when early infection occurs or when there is a reactivation of infection in immunosuppressed patients.[9-11] Toxoplasmosis commonly causes mild symptoms in immunocompetent individuals; whereas, in immunocompromised patients it is often fatal. Acute toxoplasmosis in the pregnancy period can lead to abortion, neonatal death, and poor growth or delivery before time.[12,13] Histopathological assessment and serological procedures, including the dye test (DT), indirect fluorescent antibodies (IFA), modified agglutination test (MAT), and enzyme-linked immunosorbent assay (ELISA) are generally used for the diagnosis of *T. gondii* infection.[14-17] Nowadays, a number of new methods are widely used for the diagnosis of *T. gondii* infection in Europe. These methods are: The serum IgG avidity test, polymerase chain reaction (PCR) with body tissues, and Western blots of serum.[18] Also the Real-time PCR (RT-PCR) is another method that has newly been introduced for the diagnosis of toxoplasmosis infection.[19,20] The majority of serological assays rely on anti-*T. gondii* total immunoglobulin antibodies in the serum specimens. IgM antibodies are the first class of antibodies that appear during a primary infection with *T. gondii*.[21,22] Also, the IgG-avidity test has been used to help the diagnosis of acute infection, which has produced at little amount after early infection and has increased over time.[23] The most generally applied methods for the measurement of IgM and IgG antibodies are enzyme-linked immunosorbent assay kits.[21,24] The ELISA test for diagnosis of antibodies to *T. gondii* has the potential to generate stable results in different laboratories, while using different kits.[25]

All in all the presence and titration of IgM and IgG antibodies in patients suspected with toxoplasmosis are not clearly understood. The present investigation seeks to determine the presence and titration of IgM and IgG antibodies in patients suspected with toxoplasmosis, using ELISA. Furthermore, it also tries to find an association between IgM and IgG antibodies with some risk factors, such as, direct exposure to cats, occupational exposure to raw meat, and consumption of raw fruits and vegetables.

MATERIALS AND METHODS

The present comparative cross-sectional study was conducted during 2012, in Isfahan, Iran. Before initiation, the study protocol was approved by the Ethics Committee of the Isfahan University of Medical Sciences. Written informed consent was obtained from all participants after full explanation of the nature of the study. They received a description of the study procedures and potential risks and benefits.

Seventy patients with suspected toxoplasmosis and 30 healthy volunteers, who passed informed consent, entered the study. In this study, the sample size was determined using the Krejcie and Morgan table. The non-probability consecutive sampling method was used.

In each group, patient age, sex, signs of appearance, education level, residency status (urban/rural), occupation, frequency of toxoplasma-specific IgG and IgM antibodies, abortion history, and some risk factors (direct cat exposure, occupational exposure to raw meat, and raw vegetable consumption) were recorded.

The ELISA kits (EUROIMMUN®, United Kingdom) were used for the evaluation of anti-toxoplasma IgG and IgM antibodies according to the manufacturer›s instructions.

In this study, toxoplasma-specific IgG antibodies with results greater than 11 IU / ml were considered to be positive. Also sera with titers of IgM >1.1 IU / ml were regarded as positive.

The data were presented as Mean ± SD for continuous variables and Number (Percent) for categorical ones. Statistical differences among the studied groups were assessed by Independent-Samples *T*-test, Pearson chi-square, and Fisher›s Exact procedures. The Cohen›s kappa statistic was used as a standardized measure of agreement.

All analyses were done using Statistical Package for Social Sciences Version 20 (SPSS Inc., Chicago, IL, USA). Statistical significance was accepted at P less than 0.05.

RESULTS

A total of 94 females and six males entered in the study. The mean age of participants was 27.7 ± 7.9 years (range, 13 to 62 years), and there were no significant statistical differences in age and sex distributions between the two groups (P = 0.25 and P = 0.78, respectively). The demographic and clinical characteristics of the study population were categorized by groups and are summarized in Table 1.

As shown in this table, the frequency of toxoplasma-specific IgG and IgM antibodies like; Direct cat

exposures; Occupational exposure to raw meat, and Raw vegetable consumption were not statistically significant between the two groups ($P > 0.05$).

A history of previous abortions in the women of the toxoplasmosis-suspected group was significantly higher than that in the controls (31.4% versus 6.7%; $P = 0.009$).

Also, there were no statistically significant differences between the seropositivity of the toxoplasma antibodies and the mentioned risk factors [Table 2].

Table 1: Comparison of demographic and clinical features and the risk factors of the studied groups

Characteristics	Groups		*P*-value*
	Suspected to toxoplasmosis (*n* = 70)	Control (*n* = 30)	
Age (year)	27.46±8.2	29.40±6.1	0.25
Gender (male/female)	4/66	2/28	0.78
Signs of appearance	13(18.6)	–	–
Education			0.86
Uneducated and under diploma	32 (45.7)	13 (43.3)	
Diploma	25 (35.7)	10 (33.3)	
Academic	13 (18.6)	7 (23.4)	
Residence			0.39
Urban	46 (65.7)	23 (76.7)	
Rural	24 (34.3)	7 (23.3)	
Occupation (housewife/other)	54/16	19/11	0.24
Toxoplasma IgG-positive	23 (32.8)	9 (30)	0.96
Toxoplasma IgM-positive	3 (4.3)	0	0.55
Direct cat exposures or cat ownership	8 (11.4)	0	0.1
Occupational exposure to raw meat	2 (2.8)	0	1
Raw vegetable consumption	62 (88.6)	26 (86.7)	0.93
Abortion history (*n* = 94)	22 (31.4)	2 (6.7)	0.009

Data are expressed as Mean ± SD, Number and number (percent), *P-values calculated by independent sample *T*-test, Chi-square and Fisher's exact test

In this study, the consistency between the EUROIMMUN ELISA kits and Toxoplasma Serology Laboratory evaluations was found high and acceptable (kappa = 0.845; and 95% confidence interval = 0.734-0.955)

DISCUSSION

Toxoplasmosis, is a wide-spreading zoonotic disease found all over the world. Infection with *Toxoplasma gondii* during pregnancy could cause drastic sequelae in the fetus. Toxoplasmosis generally causes mild symptoms in immunocompetent individuals; whereas, in immunocompromised patients it is more lethal. IgM and IgG are the two specific antibodies that have their presence in the sera, and reveal the stage and kind of infection to *T. gondii*. It has been shown that IgM antibodies appear earlier and decrease more quickly than IgG antibodies and are frequently the first class of antibodies detected after primary infection.

The aim of this investigation was to determine the presence and titration of anti-toxoplasma IgM and IgG antibodies in patients suspected with toxoplasmosis, using the ELISA technique, and evaluating the association between the seropositivity of toxoplasma antibodies and some risk factors as well.

According to the main findings of the present study the prevalence of positive IgM in the suspected-to-be-toxoplasmosis and the control group was 4.3 and 0%, respectively, and there were no statistically significant differences. Also the prevalence of positive IgG in the suspected-to-be-toxoplasmosis and the control group was 32.8 and 30%, respectively.

There were no statistically significant relationships between the seropositivity of toxoplasma antibodies and risk factors. We observed a significant association between the seropositivity of toxoplasma antibodies and abortion history.

For confirming the results of the present investigation we can mention the study of Liu *et al.*; they have

Table 2: Association between sociodemographic risk factors and toxoplasmosis (toxoplasma-specific IgG and IgM antibodies) in 100 under-studied participants

Characters (variables)	Anti-toxoplasma IgG			Anti-toxoplasma IgM		
	Positive *n* = 32	Negative *n* = 68	*P* -value*	Positive *n* = 3	Negative *n* = 97	*P* -value*
Age (year)	25.9±6.2	28.2±8.7	0.32	31±11.3	27.4±8.1	0.46
Gender (F/M)	32/2	62/4	1	2/1	92/5	0.17
Residence (Urban/Rural)	20/2	49/19	0.46	3/0	66/31	0.55
Abortion history (*n* = 94)	12 (37.5)	12 (19.4)	0.097	1 (50)	23 (25)	0.98
Eating raw vegetables	27 (84.4)	50 (73.5)	0.34	1 (33.3)	76 (78.4)	0.26
Direct cat exposures	4 (12.5)	9 (13.2)	0.82	0	13 (13.4)	0.85
Occupational exposure to raw meat	7 (21.9)	7 (10.3)	0.21	0	14 (14.4)	0.89

Data are expressed as Mean ± SD, Number and number (percent), *P-values calculated by independent sample *T*-test, Chi-square and Fisher's exact test

reported that in a sample of 235 pregnant women, 25 (10.6%) were positive for IgG and Zero (0%) for IgM. Their finding was in contrast with ours. With regard to the correlation between the seropositivity of toxoplasma antibodies and eating undercooked meat, unwashed raw vegetables or fruits, living in rural areas, and contact with cats, we could not find any correlation between such factors and the seropositivity of the toxoplasma antibodies in our study. In contrast to our findings, Fouladvand *et al*., showed a significant correlation between the seropositivity of toxoplasma antibodies and a contact with cats, raw vegetables, and milk and food consumption habits.

In accordance with our findings, Aali *et al*., also showed that chronic infection could lead to abortion. Also, they could not find any significant difference between positive IgG and the level of education, place of residence, contact with cats, or consumption of raw vegetables and half-cooked meat.

The impossibility of controlling other confounding variables in abortion, such as, the chromosomal abnormalities, infectious agents, and toxic agents in the environment should be mentioned as one of the limitations of the present study.

As a general conclusion, it can be stated that the frequency of specific IgM and IgG antibodies in toxoplasmosis, in the suspected-to-have toxoplasmosis and control groups was not statistically significant. Also, we can conclude that abortion is involved in the development of chronic toxoplasmosis.

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