Original Article

Genetic characterization of Toxoplasma gondii in women of reproductive age in the North of Iran

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Abstract

Context: Toxoplasmosis in females plays a prominent role in fetal health owing to serious pathological problems including abortion, hydrocephaly, mental retardation, and chorioretinitis.

Aims: The purpose of the present investigation was to study the seroprevalence and genetic characterization of *Toxoplasma gondii* in female population using ELISA and polymerase chain reaction (PCR) techniques in Mazandaran Province, Iran.

Setting and Design: This was a cross-sectional study on girls and women of childbearing age in Mazandaran Province from 2018 to 2019.

Materials and Methods: A total of 500 serum samples were collected and studied employing ELISA and PCR assays. Prior to sampling, a questionnaire was filled out for each case.

Statistical Analysis Used: Chi-square or Fisher's exact test was used to analyze the obtained data, and associations were considered statistically significant at $P < 0.05$.

Results: The total seroprevalence of the studied sera was 26.6% for IgG antibody, and also, 4.2% of the samples showed anti-*Toxoplasma* IgM antibody. Furthermore, there was a statistically significant relationship between seroprevalence of *Toxoplasma* and residential area and marital status. Genetic characterization of *T. gondii* revealed that 20% of the positive samples were Type II and 0.2% was Types I and II.

Conclusions: The seroprevalence of toxoplasmosis in girls and women of childbearing age in the north of Iran is considerable. Residential area and consumption of vegetables are identified as potentially preventable risk factors for acquiring toxoplasmosis in Mazandaran Province.

Keywords: Antibody, Genotyping, Polymerase chain reaction, Seroprevalence, Toxoplasma gondii

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INTRODUCTION

Toxoplasma gondii, an obligate and opportunistic protozoan with global distribution (one-third of the world population), is able to infect a wide spectrum of animals.[1] *T. gondii* imposes not only a great burden on human but also on animal health.[2] Cat as a final host is responsible for these wide distribution, health, and economical burdens. The wide geographic distribution of the parasite is attributed to some risk factors including population of cat (stray and domestic) and contact to cat and its feces, geographical climate, dietary habits, and residential area.[3] The overall seroprevalence of toxoplasmosis among Iranian general population was reported 39.3%.[1]

Although infection with the parasite in healthy people usually is considered benign, immunocompromised individuals such as cancer and HIV patients and organ transplant recipients may suffer some serious pathological effects.[4] Another at-risk group for toxoplasmosis is women whose first exposure to the parasite is during pregnancy. During this crucial period, tachyzoite can transmit via placental and reach to fetus and cause important clinical manifestations including abortion, hydrocephaly, mental retardation, and chorioretinitis.[5] Thus, considering the mentioned facts, it is significant to determine whether infection occurs in the initial stage of pregnancy or prior since females who have been exposed to toxoplasmosis early to pregnancy are not in danger of having infected fetus, whereas girls and women of reproductive age who are seronegative and nonimmune are at risk of acquiring toxoplasma infection.[6]

In order to manage a proper diagnosis, treatment, and control strategies for toxoplasmosis, it is essential to prepare comprehensive information concerning the seroprevalence rate and genetic determination of *Toxoplasma* in different groups, particularly in girls and women of reproductive age. To diagnose toxoplasmosis, serology assays, particularly ELISA which is considered as the gold standard for detection of anti-*Toxoplasma* IgG and IgM antibodies, are frequently carried out in many medical laboratories.[7] In addition, *Toxoplasma* genetic analysis of humans is significant to comprehend epidemiology, transmission routes, and mechanisms of toxoplasmosis. Considering all the abovementioned facts concerning serious pathological effects during conception period, determination of *Toxoplasma* status in girls and women of childbearing age, and importance of genetic analysis, the current study was conducted to determine the seroprevalence of *T. gondii* in girls and women of childbearing age using ELISA method in Mazandaran Province, North of Iran, 2018–2019, and also identify potentially preventable risk factors of the disease. Another purpose of this work was to identify the genetic characterization of the parasite in order to elucidate their putative role using polymerase chain reaction (PCR) and nested PCR techniques.

MATERIALS AND METHODS

Study area

Mazandaran Province (36°33'56"N 53°03'32"E) is placed at the north of Iran also is located on the south coast of the Caspian Sea. This province is surrounded by five provinces including Tehran, Qazvin, Golestan, Gilan, and Semnan provinces. Mazandaran covers a region of 23,842 km^2 and population of 2,922,432 inhabitants. Mazandaran Province has favorable and appropriate conditions for survival of *Toxoplasma* oocysts and the life cycle of the parasite due to particular geographical condition including moderate and subtropical climate, average temperature of 8 and 25°C in winter and summer seasons, respectively, 70%–100% relative humidity, and annually rainfall of 800–1200 mm.[8]

Sampling

A cross-sectional study was designed on 500 females of Mazandaran Province, North of Iran, 2018–2019. An informed consent form was prepared for every case. A questionnaire was completed for each individual to collect data for different factors which are related to the disease. Then, the blood specimen was prepared from each individual and transferred to laboratory for ELISA assay.

Serological examination

For immunodiagnostic assay, 5 mL of blood specimen were obtained from each woman. Afterward, the serum was separated from blood samples and preserved at −20°C until examination. The titer of IgG and IgM anti-*T. gondii* antibodies was determined applying conventional ELISA method, according to the manufacturer's instructions (PISHTAZ TEB DIAGNOSTICS Co., Tehran, Iran). The optical density of the antibodies was observed at 450 nm after 15 min using an automatic microplate reader (Stat Fax® 2100, Awareness, USA). Moreover, the cutoff value of the test for IgG and IgM anti-*T. gondii* antibodies was determined.[8]

Molecular studies and genotype characterization

DNA extraction and polymerase chain reaction

DNA extraction was performed on IgM-positive samples using Bioneer kit (South Korea) according to the manufacture instruction. The PCR reactions were done in a final volume of 20 μL, using 100 μg/μL of extracted DNA, 1 μM each primer (Fermentas Company, Germany), 250 μM dNTPs, 10 mM Tris-HCl (PH: 9.0), Taq DNA

Pol 1.0 unit, 1.5 mM $MgCl_2$, 30 mM KCl, and master mix (Qiagen, Hilden, Germany). The PCR assay was carried out using primer pairs including TOXO F2 and TOXO R2 premiers [Table 1] based on the RE sequence with duplicates of the fragments with length of 200 and 134 bp. The PCR technique was initiated at 95°C for 10 min for initial denaturation, and at 92°C for 30 s for denaturation, at 55°C for 50 s for annealing, at 72°C for 30 s for extension, and final extension at 72°C for 10 min.

The PCR-positive samples were tested again using nested PCR technique in four phases, based on the SAG2 target, and the first phase products were used as DNA for the second phase. Four pairs of primers were used for nested PCR technique; the prime's sequences are shown in [Table 1]. Products in forward direction (5') was 242 bp and in reverse direction (3') was 221 bp. *T. gondii*-positive samples were genotyped using the *Sau3A I* restriction endonuclease enzyme (Ansan, South Korea) in the forward direction and *Hba I* restriction endonuclease enzyme (Ansan, South Korea) in the reverse direction at the SAG2 locus. For separation of Type III genotype from Type I and Type II, *Sau3A I* restriction endonuclease enzyme (Ansan, South Korea) were used.[9]

Statistical analysis

The collected data were analyzed using Chi-square test (SPSS 11.5 version) (Chicago, IL, USA). Either Chi-square or Fisher's exact test was utilized to analyze the relations between seroprevalence and influence of the following risk factors such as age, married status, and residential area. When $P < 0.05$, the differences were considered statistically significant.

RESULTS

From 500 studied samples, 26.6% (133) and 4.2% (21) were seropositive for IgG and IgM antibodies, respectively. In the age group of 15–29 years, 54.13% (72) of females were positive for *Toxoplasma* antibody, and in the age group of 30–45 years, 45.87% (61) of females were positive. Considering residential area, 80.45% (107) of the cases were seen in a rural area, whereas 19.55% (26) were in an urban area. In addition, a study of marital status of the participants indicated that 63.9% (85) and 36.1% (48) of the positive samples were single and married, respectively. There was a statistically significant relationship between seroprevalence of *Toxoplasma* and age groups, residential area, and marital status ($P < 0.05$). Regarding occupation, employment 33.3% showed the highest rate of *Toxoplasma* antibody that was followed by homemaker 29.4% and student 25.06%. Raw vegetable consumers had more antibodies of the parasite, 27.35%, compared to cooked vegetable consumers, 18.6%. Individuals who consumed undercooked meat showed 21.6% of the antibody while cooked meat consumers had 27.27%. Different variables of the examined participants for detection of *Toxoplasma* antibody in Mazandaran Province are displayed in Table 2. Out of 21 IgM-positive samples, 21 (100%) were positive for PCR method. Genetic characterization of *T. gondii* revealed that 95.2% (20) were Type II and 4.8% (1) were Types I and II.

DISCUSSION

In the current study, the seroprevalence rate of anti-*T. gondii* IgG antibody in the studied girls and women of childbearing age was 26.6%. One may conclude that women who are seronegative (73.4%) are in danger of acquiring infection during pregnancy that can lead to miscarriage and future symptoms and signs for unborn child. A systematic review and meta-analysis which was conducted on girls and childbearing age women showed that the prevalence of *Toxoplasma* using a random-effect model was 33% in Iran.[9] The toxoplasmosis seroprevalence for IgG in the examined participants varied in other regions of the country such as Abadan 12%, Kerman 16.9%, Bojnord 20.4%, and Mashhad 38.01%.[10-12] In all the abovementioned studies, no IgM anti-*T. gondii* antibody was detected, whereas in our study, 4.2% (21) of the specimens showed IgM anti-*T. gondii* antibody. *Toxoplasma* prevalence applying ELISA technique in Mazandaran was reported 55.5%.[13] This considerable

Table 1: The primer sequences in polymerase chain reaction and nested polymerase chain reaction methods

Primer	Primer name	Sequence
Forward primer 1	TOXO F2	5' AGG CGA GGG TGA GGA TGA 3'
Reverse primer 1	TOXO R2	5' TCG TCT CGT CTG GAT CGC AT 3'
Forward primer 1	SAG2.F4	5' GCT-ACC-TCG-AAC-AGG-AAC-AC 3'
Forward primer 2	SAG2.R4	5' GCA-TCA-ACA-GTC-TTC-GTT-GC 3'
Forward internal primer 1	SAG2.F	5' GAA-ATG-TTT-CAG-GTT-GCT-GC 3'
Forward internal primer 2	SAG2.R2	5' GCA-AGA-GCG-AAC-TTG-AAC-AC 3'
Reverse primer 1	SAG2.F3	5' TCT-GTT-CTC-CGA-AGT-GAC-TCC 3'
Reverse primer 2	SAG2.R3	5' TCA-AAG-CGT-GCA-TTA-TCG-C 3'
Reverse internal primer 1	SAG2.F2	5' ATT-CTC-ATG-CCT-CCG-CTT-C 3'
Reverse internal primer 2	SAG2.R	5' AAC-GTT-TCA-CGA-AGG-CAC-AC 3'

Table 2: Demographic and baseline characteristics of participants for detection of *Toxoplasma gondii* IgG antibody

Demographic characteristics	Total number	Toxoplasma-positive number (%)	Chi-square statistics	*P*
Age group (year)				
15-29	150	72 (54.13)	50.26	<0.001
30-45	350	61 (45.87)		
Residential area				
Urban	216	26 (19.55)	41.30	<0.001
Rural	284	107 (80.45)		
Marital status				
Single	356	85 (63.9)	4.69	0.0302
Married	144	48 (36.1)		
Occupation				
Student	375	94 (25.06)	2.00	0.358
Employment	57	19 (33.3)		
Homemaker	68	20 (29.4)		
Undercooked meat consumption				
Yes	60	13 (21.6)	0.849	0.356
No	440	120 (27.27)		
Raw vegetable consumption				
Yes	457	125 (27.35)	0.278*	0.214
No	43	8 (18.6)		
Total	500	133		

*Fisher's exact test statistic

prevalence rate of toxoplasmosis of the studied cases of Mazandaran Province in comparison to other regions of our country may is related to some significant agents such as feeding of raw or undercooked meat and high humidity that prepares a favorable condition for survival of the oocyst of *Toxoplasma*.[14]

Based on the findings, although statistically no significant relationship was seen between seroprevalence of toxoplasmosis and age, a relationship between residential area and toxoplasmosis was statistically significant. This may originate from the fact that in the rural area, the probability of getting infected is higher compared to urban one due to the presence of stray cats and considerable abundance of cats. The seroprevalence of toxoplasmosis was higher in vegetable consumers rather than those who do not consider vegetables in their diet. The fact may be justified by saying that vegetables either washed carefully or not is one of the major sources for transmission of *Toxoplasma* oocysts.[15]

Different factors are attributed to toxoplasmosis severity and clinical manifestations such as parasite strain, immune response, and host genetic variability. Recently, researchers have been paid a great attention to genetic variation of *T. gondii* strains as an important and interesting research area. Till now, three major lineages including Types I, II, and III are known for *T. gondii*.

In this study, although just one case (4.8%) was detected as Type I strain, this type is highly virulent and pathogenic can lead to acquired ocular toxoplasmosis in individuals with disseminated congenital form of *Toxoplasma*. Type I of the parasite has LD100 that one tachyzoite can lead to lethal infection in mouse even at a low dose of inoculations. Genotype II is the most prevalent genotype in human toxoplasmosis. Although Type II strains are causative agents for numerous asymptomatic toxoplasmosis cases in Europe, it can be pathogenic for two important categories including immature fetuses and immunocompromised individuals. In our study, genotype II was the most prominent type[16,17] that was in agreement with the findings of Hosseini *et al*., 2018, in a systematic review which was conducted on genetic diversity of clinical samples. This type of lineage is high cyst-forming and considered generally less virulent with LD100 ≥103. Identification of genotype of *Toxoplasma* in each area is so important and valuable due to probable prediction of symptoms and clinical manifestation of the parasite in that area and patient. In addition, it helps to design new policies for treatment, vaccination, diagnosis, control and prevention of the parasite.

CONCLUSIONS

The authors from the results deduce that the seroprevalence of toxoplasmosis in girls and women of childbearing age in the north of Iran is considerable. Around 73.4% of girls and women of reproductive age were seronegative, and hence, they were susceptible to *Toxoplasma* infection and should be monitored. Residential area and consumption of vegetables are identified as potentially preventable risk factors for acquiring toxoplasmosis in Mazandaran Province. In the current study, genotype II is the most prominent type in the studied participants. In addition, future investigation should conduct to determine the source of contamination chain in the north of Iran.

Conflicts of interest

There are no conflicts of interest.

Authors' contribution

All authors contributed to this study.

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