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Original Article

Investigating Risk Factors and Seroprevalence of *Toxoplasma Gondii* Antibodies in Patients Referred to the Central Laboratory of Ilam City, Iran

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Background and Aims: Toxoplasmosis is a protozoan infection caused by a forced intracellular parasite called *Toxoplasma gondii*, a branch of the apicomplexa found in humans and numerous species of animals. This study aimed to determine the titer of anti-Toxoplasma antibodies in patients referred to the Central Laboratory of Ilam City and to define their effective factors in the prevalence of this disease using the enzyme-linked immunosorbent assay (ELISA) method.

Materials and Methods: The current study was a descriptive cross-sectional study with a random sampling of 116 patients' serum referred to the Central Laboratory of Ilam City. Samples were analyzed using ELISA for the presence of Immunoglobulin (Ig)M, IgG antibodies against *Toxoplasma gondii*.

Results: Twenty-three samples (19.8%) out of 116 serum samples were detected using anti-Toxoplasma antibodies. Nineteen samples out of these 23 (16.4%) samples were antibody IgG while the other 4 were IgM antibodies. The results of the Chi-square test showed a significant difference between IgG-positive titer and sex, level of education, place of residence, age, and marital status; however, no significant difference was found between these variables and positive IgM titer.

Conclusion: As a large number of people are susceptible to acute toxoplasmosis infection and lack proper health information, it is necessary to adopt monitoring and control measures by health care officials to prevent the infection through the necessary training and health recommendations.

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Introduction

Toxoplasmosis is a zoonotic disease caused by an intracellular parasite called *Toxoplasma gondii*. It is one of the most common parasitic infections in humans with a worldwide distribution. Although the only definitive host is a cat, the parasite can infect all warm-blooded animals and humans [1-5]. Prevalence statistics in the country vary spatially and according to differences in culture and health information. Toxoplasmosis has been reported in 55% and 29% of Northern and Southern Iran, respectively, and 51.8% in different parts of Iran [2]. In humans, infection with this parasite has been reported in all age groups. Humans become infected through a variety of ways including consuming water and food contaminated with oocysts, eating raw or uncooked meat infected with oocysts, along with the placenta, and organ transplants [6-9]. Risk factor analyses show that 30% to 63% of human infections occur due to the consumption of uncooked meat and meat products [10]. Toxoplasmosis is usually a benign infection in people with a healthy immune system and causes no particular problem. The most prevalent symptom in these people is general swelling of the lymph nodes, associated with symptoms similar to infectious mononucleosis, including fever and chills, headache, severe fatigue, and muscle pain [11]. Although most *Toxoplasma* infections are asymptomatic in healthy individuals, they can be hazardous in patients with defective immune systems or following organ transplants [12-15]. There are several methods for assessing the prevalence of *Toxoplasma* in humans and animals. The most common one is the serological technique immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA). With these approaches, the prevalence of antibodies against Toxoplasma in humans has been reported in different parts of the country [16-19]. ELISA is a simple biochemical laboratory method with high sensitivity, through which, it is possible to analyze a large number of samples simultaneously. Antibodies that are formed in response to certain infections or diseases can also be detected by this test. The high level of Immunoglobulin (Ig)M antibody with clinical symptoms indicates acute infection, and the high level of IgG antibody indicates chronic toxoplasmosis infection [16, 19]. The ELISA test is an economical and accurate test in which a blood sample is taken and centrifuged at high speed. After separating the blood serum from the particles, a reagent (conjugate) is added to the blood serum that stains the solution and detects the presence of antiviral antibodies. It is normally used to detect antigens or antibodies. One of these two substances is fixed in the solid bed and is used to track the latter [17].

Therefore, due to the sensitivity of the ELISA method in determining the titer of antibodies against parasites and lack of comprehensive knowledge and lack of appropriate information in the field of common diseases, the serological study of the prevalence of human toxoplasmosis in patients referred to the Central Laboratory of Ilam City is necessary.

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Materials and Methods

Study population

The present descriptive cross-sectional study was performed in 2019 in one set of random 116 blood samples of patients referred to the Central Laboratory of Ilam City. This laboratory is the largest laboratory in the province and several doctors refer their patients to the mentioned laboratory and individuals are referred to them from various parts of the country. initially, the participant's consent was obtained before sampling. During the completion of a questionnaire, demographic information of individuals such as gender, age, marital status, education level, and place of residence were recorded. This study was approved by the ethical committee of Ilam University of Medical Sciences, Iran. (Code: 978049/1135).

Serum sample preparation

Firstly, 3 ml of venous blood was taken from each patient, poured into the clot tube, and centrifuged at 2500 g for 10 min to separate the serum. The serum was then poured into 2 ml micro-tubes and stored at a temperature of -20 ºC until experimenting. Afterward, the patient code and sampling date were printed on each sample.

ELISA test

Secondly, the relevant ELISA kits and serum samples are taken out of the freezer and placed at room temperature. To examine both antibodies, each patient's serum was first fully vortexed, then using a dilute SD buffer and IgM antibody level, the patients' IgG using a kit made by Trinity Biotech Company and with ELISA reader (Stat fax 4200, USAb) in the wavelength of 450 nm was evaluated according to the protocol of each kit and antibody concentration in terms of Immune Status Ratio (ISR) was reported that according to the instructions in the kit manufacturer, negative samples (0.9 ≥ ISR) were excluded from the study while positive (ISR < 1.1) and suspicious samples (0.9 < ISR <1.1) were maintained for further studies.

Statistical analysis

The results were analyzed using the statistical software SPSS 20 and the data were then compared with the Chi-square test. Value was to be significant at $p < 0.05$.

Results

The results of this study demonstrated that 23 (19.8%) out of 116 serum samples had anti-*Toxoplasma* antibodies, while the remaining 93 (80.2%) had no antibody against *Toxoplasma* (Table 1). 19 samples (16.4%) of these 23 samples were antibody IgG while the other 4 (3.4%) were IgM antibodies (Fig. 1). Antibodies (IgG positive and IgM negative) were found in 18 (15.5%) samples, and only one sample (0.9%) was positive for both antibodies (IgG positive and IgM positive). Among the mentioned individuals, only three cases (2.6%) were observed with IgM positive.

Regarding gender, 104 (89.7%) were male and 12 (10.3%) were female. The percentage of IgG antibodies in men and women was 11.53% and 58.3%, respectively. On the other hand, the amount of IgM antibody was 1.9% in men and 16.7% in women. There was a significant relationship between gender (male and female) and IgG antibody positivity ($p < 0.05$), but there

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was no significant relationship between sex, and IgM antibody positivity ($p > 0.05$, Table 2). The maximum age of the subjects in this study was 70 years old, while the least age was four years old. Among age groups, the highest prevalence of IgG antibodies was observed in the age group 31-40 years (42.8%) and IgM antibody in the age group over 50 years (12.5%), and the antibody process increases with age. The results of the Chi-square test showed a significant difference between the age groups of the subjects and the prevalence of IgG antibodies ($p < 0.05$), but no significant difference was found between the age groups of the subjects and the prevalence of IgM ($p > 0.05$) (Table 2). Concerning the location, the results showed that the level of IgG was 30.3% and the level of IgM was 3.03% in rural areas. In urban areas, IgG and IgM levels were 10.8% and 3.6%, respectively. There was a significant relationship between people with positive IgG in urban and rural areas ($p < 0.05$), but no significant difference was found between people with positive IgM in urban and rural areas ($p > 0.05$, Table 2).

The results revealed that serum levels of IgG and IgM antibodies in illiterate people are higher than in educated people and there was also a significant relationship between positive IgG in illiterate people and educated people ($p < 0.05$, Table 2). In this study, out of a total of 116 serum samples, 47 serum samples (40.5%) related to single individuals, and 69 samples (59.5%) related to married individuals were examined. The serum level of both antibodies in married individuals showed an increasing trend. Results statistical test indicated a significant difference between single and married IgG-positive individuals ($p < 0.05$), but there was no difference in IgM-positive antibodies between single and married individuals ($p > 0.05$, Table 2).

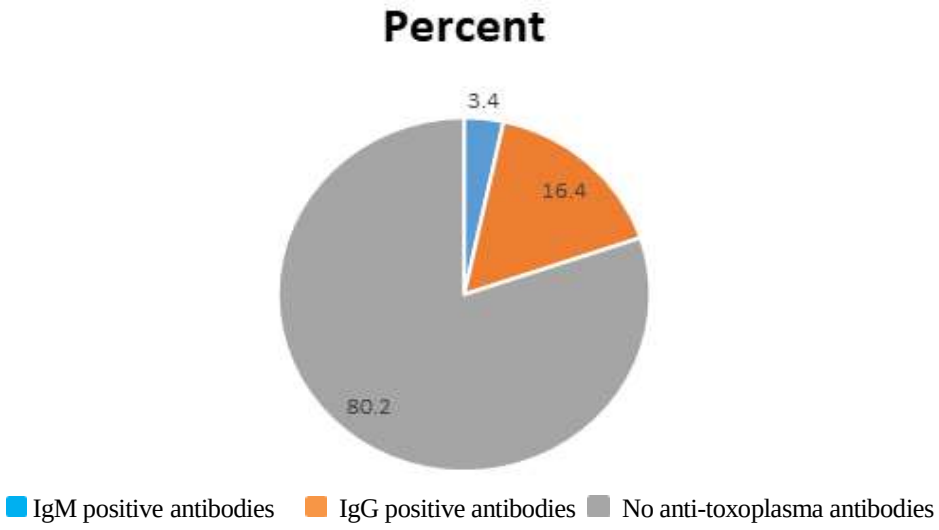

Fig. 1. Frequency of positive and negative antibodies in patients referred to the Central Laboratory of Ilam City

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Table 1. Frequency of serological prevalence of human toxoplasmosis in patients referred to the Central Laboratory of Ilam City

Variables		Frequency	Percent
Range-age (years) No anti-toxoplasma antibodies	<12	9	7.8
	12-20	22	19.0
	21-30	10	8.6
	31-40	4	3.4
	41-50	36	31.1
	> 50	12	10.3
No anti-toxoplasma antibodies		93	80.2
Range-age (years) anti-toxoplasma antibodies	<12	2	1.72
	12-20	5	4.31
	21-30	3	2.58
	31-40	2	1.72
	41-50	8	6.89
	> 50	3	58.2
anti-toxoplasma antibodies		23	19.8
Total		116	100.0

Table 2. Frequency of serological prevalence of human toxoplasmosis based on demographic variables in patients referred to the Central Laboratory of Ilam City

Variables	Risk factors	Frequency N (%)	IgG positive N (%)	IgM positive N (%)	P-Value IgG	P-Value IgM
Sex	Male	104 (89.7)	12 (11.53)	2 (1.9)	0.002	0.113
	Female	12 (10.3)	7 (58.3)	2 (16.7)		
Age range (years)	< 12	10 (8.6)	1 (10)	0 (0.0)	0.042	0.198
	12-20	27 (23.3)	5 (18.5)	0 (0.0)	0.004	0.238
	21-30	16 (13.8)	5 (31.2)	1 (6.2)	0.003	0.108
	31-40	7 (6)	3 (42.8)	0 (0.0)	0.002	0.531
	41-50	40 (34.5)	3 (7.5)	1 (2.5)	0.015	0.101
	> 50	16 (13.8)	2 (12.5)	2 (12.5)	0.010	0.088
Place	City	83 (71.6)	9 (10.8)	3 (3.6)	0.025	0.841
	Village	33 (28.4)	10 (30.3)	1 (3.03)		
Level of education	Illiterate	11 (9.5)	6 (54.5)	2 (18.2)	0.045	0.104
	Literate	105 (90.5)	13 (12.4)	2 (1.9)		
Marital status	Married	69 (59.5)	14 (20.3)	3 (4.3)	0.001	0.493
	Single	47 (40.5)	5 (10.6)	1 (2.1)		

The P-value is considered significant at <0.05

Discussion

The present study displayed that a large percentage of people in Ilam are prone to acute toxoplasmosis infection. There are various methods for detecting *Toxoplasma* parasites

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including serological methods such as ELISA, indirect IFA, Electro chemi luminescence Immunoassay, histology, parasite isolation, as well as molecular methods such as polymerase chain reaction. The most common techniques are IFA and ELISA, of which ELISA is more sensitive [16-18]. Therefore, it was used as an efficient method to evaluate the titers of antibodies against this protozoan. According to this method, 19 samples (16.4%) had IgG antibodies and 4 samples (3.4%) had IgM antibodies which was by the reported results of Aqeely et al. In Jazan [20]. Similar studies conducted in Urmia by Rasouli et al. In 2010 and Davoodi et al. IgG-positive serum titer against *Toxoplasma gondii* was estimated to be 47% and 36.5% and IgM was 3.5% and 6%, respectively [17, 21]. The results of the above studies were higher than the results obtained in the present study. This bias can be due to differences in food culture, climate, personal and environmental health, prevalence, or density. Oocyte-repellent cats in these areas have differences in the type of statistical population tested as well as the sensitivity of different methods in the evaluation of toxoplasmosis. Transmission of the infection depends on the level and frequency of contact with these factors [16-18, 22, 23]. In the present study, the prevalence of IgG positive cases was higher among rural residents (30.3%) than urban residents (10.8%). However, in the case of IgM antibody, this amount was lower in rural areas than in urban areas. These results are similar to studies conducted in Mianeh, Gorgan, Urmia, and Khoy counties [16, 17, 21, 24]. In contrast to studies conducted in Qazvin and Chaharmahal Bakhtiari States, the number of positive serum cases in the city was reported to be higher compared to rural areas [25]. Studies showed that the number of positive serological cases in hot and humid areas is generally high. These findings also indicated that this number increases with age. According to previous research, its prevalence in Iran varies in different parts of the country [17]. In this study, the age group of 31-40 and above among the age groups had the highest prevalence of IgG and IgM antibodies, respectively, which can be directly related to the age of individuals as the possibility of infection with the parasite increases with age. This age group showed a significant difference with IgG antibody titer compared to other age groups, but no significant difference was observed with the prevalence of IgM, with previous studies from other parts of the world and the country match or difference can be detected. The study of Davoodi et al. (2012) claimed that the highest serum level of antibodies is in the age group of 31-40 and women more than men [17].

In another study conducted in the city of Urmia, similar results were obtained [21]. The results of the present study are consistent with the results of the above-mentioned studies. In other studies conducted by Tabatabai et al. and Manouchehri Naeini et al. it is shown that by increasing age, the number of positive serum cases exhibits an increasing trend [25, 26]. In contrast to studies in Venezuela and Croatia, the number of positive serum cases was reported in children under the age of 15 [27,

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28]. In this study, the prevalence of antibodies tended to be higher in females than in males. This finding is consistent with the results of research conducted by Rasouli et al. in Urmia City and Manouchehri Naeini et al. in Chaharmahal Bakhtiari Province [21, 25]. In the present study, positive serum levels of IgM and IgG antibodies were higher in the married group than in the single group showing a significant difference between single and married IgG-positive individuals, but no significant difference was observed in the prevalence of positive IgM between single and married individuals. Similar results were also obtained in the study of Davoodi et al. (2010) and Rasouli et al. (2012) held in the cities of Urmia and Mianeh for serological evaluation of the prevalence of toxoplasmosis in patients referred to the laboratories [17, 21]. In this study, the highest serum level of IgG and IgM antibodies was reported in illiterate people and the lowest belonged to the educated ones. There was a significant relationship between the level of positive antibodies and the level of education of individuals indicating the lack of awareness of these people towards literate people in the community. These results are similar to the research of Davoodi et al. in Mianeh City, Saeedi et al. in Gorgan City, Rasouli et al. in Urmia City, and Manouchehri Naeini et al. in Chaharmahal Bakhtiari Province [13, 14, 17, 19]. Therefore, education can be regarded as a crucial factor in reducing the prevalence of *Toxoplasma*. However, studies in Turkey, Qazvin city, and Hamedan city showed no significant relationship between the level of education and the level of positive serum antibodies [26, 29, 30]. The prevalence of serum levels of *Toxoplasma* antibodies and the high susceptibility of this parasite in this city are related to multiple factors. These factors include: eating poorly cooked meat, reflecting the special eating habits of the people of the region, high traffic of stray cats around houses and parks, and the expulsion of resistant oocysts by infected cats. There is a possibility of widespread contamination of soil and environment. Other factors of transmission such as contamination of food and vegetables along with the lack of healthy drinking water are among other key factors in the transmission of the organism that should be taken into consideration.

Conclusion

The results of this study, considering the centrality of the laboratory to some extent, can indicate the status of immunity and susceptibility to this disease, and recognizing the risk factors affecting this parasite can have a significant impact on reducing the prevalence of this organism. Therefore, it is necessary to educate and increase awareness about the ways of transmission and the importance of this disease and to use it in educational and prevention programs in the region.

Conflict of Interest

The authors have no conflicts of interest to declare.

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