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ORIGINAL PAPER

Exploring the Association Between Latent *Toxoplasma gondii* Infection and COVID-19 in Hospitalized Patients: First Registry-Based Study

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Abstract

Purpose This study aimed to determine the possible association between *Toxoplasma gondii* infection and COVID-19 outcomes among 133 patients with an RT-PCR-positive test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), hospitalized at Imam Khomeini Hospital, Sari, Mazandaran Province, northern Iran, during August to November 2020.

Methods A questionnaire was used to collect baseline data from the patients who were registered to the Iranian National Registry Center for Toxoplasmosis (INRCT). Also, blood samples were taken from each patient for detecting anti-*T. gondii* antibodies and *T. gondii* DNA using enzyme-linked immunosorbent assay (ELISA) and conventional-PCR methods, respectively. Variables related to the COVID-19 severity and outcomes were indicated based on multiple multinomial logistic regression models.

Results Of 133 patients enrolled in the INRCT with COVID-19 through RT-PCR, 50 (37.59%), 52 (39.1%), and 31 (23%) suffered from mild, moderate, and severe COVID-19, respectively. 57.1% of the patients who died had severe COVID-19, while among those with other outcomes, only 18.60% had severe COVID-19 ($P < 0.05$). Anti-*T. gondii* IgG was detected in 109/133 (81.95%) patients, which was not statistically significant ($P > 0.05$). Among those with negative and positive anti-*T. gondii* IgG, 2 (8.30%) and 29 (26.60%) had severe COVID-19, respectively ($P > 0.05$). *T. gondii* DNA and anti-*T. gondii* IgM were not found in any of the patients. Moreover, all deaths occurred in those with moderate or severe COVID-19 and a positive anti-*T. gondii* IgG.

Conclusion To our knowledge, this is the first registry-based study concerning *T. gondii* infection among patients with COVID-19. Our data show the high rate of latent *T. gondii* infection among COVID-19 with different severity. However, there is no significant relationship between latent *T. gondii* infection and COVID-19 severity and outcomes. Thus, conducting multicenter studies in different geographic regions of the world could offer a better understanding of this relationship.

Keywords *Toxoplasma gondii* · Latent toxoplasmosis · COVID-19 · Serology · PCR · Iran

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Introduction

Toxoplasma gondii (*T. gondii*), the most successful intracellular parasite on the planet, is the etiologic agent of toxoplasmosis [1]. Humans are infected with *T. gondii* parasite through ingestion of raw and undercooked infected meat and consumption of mature oocysts from the environment, congenital, blood transfusion, and organ transplantation [2, 3]. Approximately, 15–85% of individuals in the world, 39.3% of the residents in Iran, and 54% of people in the north of Iran are infected with *T. gondii* parasite [4, 5]. Toxoplasmosis is often asymptomatic in healthy individuals, but can cause severe or life-threatening disease in pregnant women, immunosuppressed individuals, and patients with organ transplants [2, 3]. It has been shown that appropriate immune responses have an important role in parasite control. As *T. gondii* causes an inflammatory infection, the killing of the parasite needs innate and adaptive immune reactions [6].

In December of 2019, the first cases of coronavirus infection with the unknown source were reported in Wuhan, China, and spread to an increasing number of countries [7–11]. World Health Organization (WHO) reports a global public health emergency over the 2019 novel coronavirus disease (COVID-19) outbreak created by SARS-CoV-2 on January 30, 2020. Globally, there have been 200, 840 and 180 proven cases of COVID-19, including 4,265,903 deaths, according to the WHO report until August 6, 2021. In Iran, there have been 4,057,758 confirmed cases of COVID-19 with 92,628 deaths, until August 6, 2021 reported to WHO [7]. COVID-19 appears as a febrile respiratory illness that may progress to respiratory failure and pneumonia [8]. The initial immune response to COVID-19 is produced by stimulation of innate cells and virus-specific T cells and -cells. In severe cases, however, a systemic inflammatory syndrome with uncontrolled production of pro-inflammatory cytokines and chemokines occurs [9, 11].

A recent study has revealed a reverse correlation between the occurrence of COVID-19 and parasitic diseases [12]. It is interesting to note that SARS-CoV-2 and *T. gondii* can activate innate immunity through a similar pathway. In fact, in both pathogens, toll-like receptors, including TLR 2, TLR4, and TLR7, are activated via the canonical pathway. On the other hand, it is also possible that some induced cytokines in patients with toxoplasmosis increase the severity of COVID-19 [13, 14]. Thereby, it is hypothesized that *T. gondii* may be associated with COVID-19 in hospitalized patients. Given that Mazandaran Province, northern Iran, has the highest prevalence of *T. gondii* in Iran [15], as well as the fact that the Iranian National Registry Center for Toxoplasmosis (INRCT) was hosted in the province (settled at Imam Khomeini Hospital), this study was well justified. With these premises, the main goal of this study was to answer the question of whether toxoplasmosis has any effect on the risk of SARS-CoV-2 infection and COVID-19 outcomes. For this purpose, we detected *T. gondii* infection among 133 hospitalized patients with COVID-19 who were registered in the INRCT using serological and molecular tests at Imam Khomeini Hospital, Sari, northern Iran.

Materials and Methods

Study Area and Participants

From August to November 2020, we examined 133 patients enrolled in the INRCT with an RT-PCR-positive test for SARS-CoV-2 hospitalized at Imam Khomeini Hospital, a provincial COVID-19 referral hospital in Sari, Mazandaran Province, northern Iran. This study complied with the joint Ethical Committees of Mazandaran University of Medical Sciences (Ethic No. IR.MAZUMS.REC.1399.8630).

Clinical Evaluation

The information collected included demographic data (age, gender, past medical history, and job), prognostic factors (C-reactive protein (CRP) and lymphopenia), hospitalization ward, use of mechanical ventilation, corticosteroid consumption history, length of hospital stay (LOS), severity of COVID-19, and death. Then, blood samples were collected from patients with an RT-PCR-positive test for SARS-CoV-2 virus, in tubes containing k2-EDTA as anticoagulant and centrifuged at 3000 rpm for 3 min. Then, the plasma and buffy coat were stored at − 20 °C and submitted to the laboratory of INRCT at Imam Khomeini Hospital for further examination.

Serological Assay

The plasma was screened for anti-*T. gondii* IgG, and IgM was determined using a commercially available enzyme-linked immune sorbent assay (ELISA) kit (PishtazTeb, Iran), based on the manufacturer's protocol. The optical density (OD) of the samples was measured at 450 nm using a plate reader. Both IgG and IgM titers > 1.1 were considered positive. Both the sensitivity and specificity of the IgG test were 100%, and for the IgM test, they were 100% and 99%, respectively.

PCR Assay

T. gondii DNA was extracted from 133 buffy coat specimens according to the phenol–chloroform isoamidalcol method. Then, conventional PCR (PCR) was done by forward primer F 5′-CGCTGCAGGGAGGAAGACGAAAGTTG-3′ and reverse primer R 5′-CGCTGCAGACAC AGTGCATCT GGATT-3′, amplifying a 529-bp fragment of gene RE with master mix (12.5 µl; Fermentas) mixed with 4 µl of the extracted DNA to give to an ultimate content of 25 ml comprising 7.3 µl distilled water and 0.6 µl of each primer at a concentration of 1 pmol/µl. Then, 32 cycles were performed in a thermocycler (Corbett Research, Sydney, Australia) at 94 °C, for 3 min by 1 cycle (initiation denaturation), 94 °C for 30 s (denaturation), 55 °C for 30 s (annealing), 72 °C for 20 s (extension), 30 cycles and 72 °C for 7 min 1 cycles (final extension). Subsequently, PCR product was evaluated by electrophoresis on 1% (w/v) agarose gel in Tris–borate EDTA at 85 V for 25 min and observed using UV transillumination after staining with SafeView™ DNA Stains (Applied Biological Materials, Inc.).

Data Analysis

Quantitative and qualitative variables were presented as mean ± SD and frequency (%), respectively. Chi-square, Fisher's exact, and extended Fisher (Fisher–Freeman–-Halton) tests were applied to measure the association between categorical variables. In addition, to compare the mean of quantitative variables in the two and three groups, independent *T* test and ANOVA were used, respectively. Furthermore, a multiple multinomial logistic regression model was utilized to examine the association between the COVID-19 severity and anti-*T. gondii* IgG results, adjusting the confounders. Thus, variables that had $P < 0.30$ in the univariate model were entered into the multiple models. Statistical analyses were calculated using the SPSS version 20.0 and STATA release version 12. $P < 0.05$ was considered statistically significant.

Results

In this study, 70 (52.63%) participants were female with a mean age of 60.90 ± 16.70 and 63 (47.37%) were male with a mean age as 56.59 ± 17.33. The results of the independent *t* test showed that the mean age of males and females did not have a statistically significant difference ($P > 0.05$). Also, the mean age in patients with severe COVID-19 was higher compared to other severity groups; however, the difference in mean age among these three groups is not statistically significant ($P > 0.05$). Of 133 patients submitted to the INRCT with COVID-19, 50 (37.59%), 52 (39.1%) and 31 (23%) had mild, moderate and severe COVID-19. The COVID-19 was severe in 21 (30%) females and 10 (15.90%) males, but this difference was not statistically significant and there was also no significant association between gender and COVID-19 severity ($P > 0.05$).

Anti-*T. gondii* IgG was detected among 109/133 (81.95%) of the patients. *T. gondii* DNA and anti-*T. gondii* IgM were not detected in any of the patients. Also, in patients with negative and positive anti-*T. gondii* IgG, 2 (8.30%) and 29 (26.60%) of them had severe COVID-19, respectively, which was not statistically significant ($P > 0.05$). In addition, there is no significant association between COVID-19 severity with past medical history, job, lymphopenia, corticosteroid consumption history and CRP variables ($P > 0.05$). But, mechanical ventilation, hospitalization ward and lengths of hospital stay (LOS) variables were significantly associated with COVID-19 severity ($P < 0.05$) (Tables 1, 2, 3).

Furthermore, 57.10% of the patients who died had severe COVID-19, while in patients with other outcomes, only 18.60% of patients had severe COVID-19 ($P < 0.05$) (Table 3). Figure 1 shows that all deaths occurred in cases with moderate or severe COVID-19 and a positive anti-*T. gondii* IgG. The mean age of patients who died of COVID-19 was significantly higher than that of patients with other outcomes ($P < 0.05$). Also, among patients ≤60 <60 years and > 60 years, 1.40% and 21% of patients experienced death, respectively, and there is a significant association between age and COVID-19 outcomes ($P < 0.05$). COVID-19 outcomes had a statistically significant association with CRP, mechanical ventilation, hospitalization variables as well ($P < 0.05$). However, other variables had no statistically significant association with COVID-19 outcomes ($P > 0.05$) (Table 4).

The results from the multiple multinomial logistic regression revealed that only the CRP had a significant effect on moderate COVID-19 so that the odds of moderate COVID-19 compared to mild COVID-19 (moderate vs. mild) in patients with CRP < 50 and > 50, respectively, were 5.34 and 9.45 times those of patients whose CRP result was negative ($P < 0.05$). However, the CRP variable had no statistically significant impact on severe COVID-19 compared to mild COVID-19 (severe vs. mild) in these patients ($P > 0.05$). Moreover, the anti-*T. gondii* IgG variable had no statistically significant influence on COVID-19 severity, so that the odds of moderate and severe COVID-19 compared to mild COVID-19 in patients with positive vs. negative anti-*T. gondii* IgG were 0.63 and 2.71 times, respectively. This means that the odds of patients with positive anti-*T. gondii* IgG are more likely to have severe COVID-19 ($P > 0.05$). Other variables were not substantially associated with COVID-19 severity as well ($P > 0.05$) (Table 5).

Table 1 Frequency distribution of the demographic characteristics of patients with COVID-19

Characteristics	Total	COVID-19 severity			*P*
		Mild	Moderate	Severe	
Age					
Mean ± SD	58.95 ± 17.10	54.84 ± 16.47	60.58 ± 15.42	62.84 ± 19.76	0.08
≤ 60	70 (100)	31 (44.30)	24 (34.30)	15 (21.40)	0.23
> 60	63 (100)	19 (30.20)	28 (44.40)	16 (25.40)	
Gender					0.11
Female	70 (100)	26 (37.10)	23 (32.90)	21 (30.00)	
Male	63 (100)	24 (38.10)	29 (46.00)	10 (15.90)	
Past medical history					0.27
No	30 (100)	12 (40.00)	11 (36.70)	7 (23.30)	
DM/HTN/CHD	76 (100)	26 (34.20)	35 (46.10)	15 (19.70)	
Other	27 (100)	12 (44.50)	6 (22.20)	9 (33.30)	
Job					0.055*
Housewife	59 (100)	22 (37.30)	29 (49.20)	8 (13.60)	
Employee/retired employee	18 (100)	6 (33.30)	4 (22.20)	8 (44.50)	
Other	56 (100)	22 (39.30)	19 (33.90)	15 (26.80)	

HTN; hypertension, DM; diabetes mellitus, CHD; coronary heart disease

*Significant at level of 0.05, values are reported as frequency (percent) and mean ± SD

Table 2 Frequency distribution of the clinical characteristics of patients with COVID-19

Characteristics	Total	COVID-19 severity			*P*
		Mild	Moderate	Severe	
Hospitalization					< 0.001*
Ward	110 (100)	49 (44.50)	44 (40.00)	17 (15.50)	
ICU	23 (100)	1 (4.30)	8 (34.80)	14 (60.90)	
Lymphopenia					
No	58 (100)	27 (46.60)	21 (36.20)	10 (17.20)	0.14
Yes	74 (100)	23 (31.10)	30 (40.50)	21 (28.40)	
CRP					0.09
No	17 (100)	11 (64.70)	3 (17.60)	3 (17.60)	
< 50	50 (100)	20 (40.00)	18 (36.00)	12 (24.00)	
> 50	66 (100)	19 (28.80)	31 (47.00)	16 (24.20)	
Mechanical ventilation					< 0.001*
Negative	120 (100)	50 (41.70)	48 (40.00)	22 (18.30)	
Positive	12 (100)	0 (0.00)	4 (33.30)	8 (66.70)	
Corticosteroid consumption history					0.53
No	126 (100)	48 (38.10)	50 (39.70)	28 (22.20)	
Yes	7 (100)	2 (28.60)	2 (28.60)	3 (42.90)	

*Significant at the level of 0.05; the values are reported as frequency (percent)

Discussion

Little is known regarding the relationships between *T. gondii* and COVID-19 outcomes at a global scale. Our findings indicate that 81.95% of the patients with COVID-19 were positive for anti-*T. gondii* IgG, but no evidence of acute *T. gondii* infection by PCR or anti-*T. gondii* IgM was shown among them. Also, all deaths occurred among cases with moderate or severe COVID-19 and a positive anti-*T. gondii* IgG; however, this relationship was not significant. Furthermore, the anti-*T. gondii* IgG variable had no statistically significant influence on the severity of COVID-19. Accordingly, there was no statistically significant association between anti-*T. gondii* IgG and the severity and outcomes of COVID-19.

Table 3 Evaluating the relationship between the lengths of hospital stay (LOS), anti-*T. gondii* IgG results, and disease outcome with COVID-19 severity

Characteristics	Total	COVID-19 severity			*P*
		Mild	Moderate	Severe	
LOS					< 0.001*
Mean ± SD	8.56 ± 4.98	6.84 ± 3.69	8.25 ± 4.20	11.84 ± 6.38	
T. gondii IgG					0.14
Negative	24 (100)	10 (41.70)	12 (50.00)	2 (8.30)	
Positive	109 (100)	40 (36.70)	40 (36.70)	29 (26.60)	
Outcome					< 0.001*
Death	14 (100)	0 (0.00)	6 (42.90)	8 (57.10)	
Other	118 (100)	50 (42.40)	46 (39.00)	22 (18.60)	

*Significant at the level of 0.05; the values are reported as frequency (percent) and mean ± SD

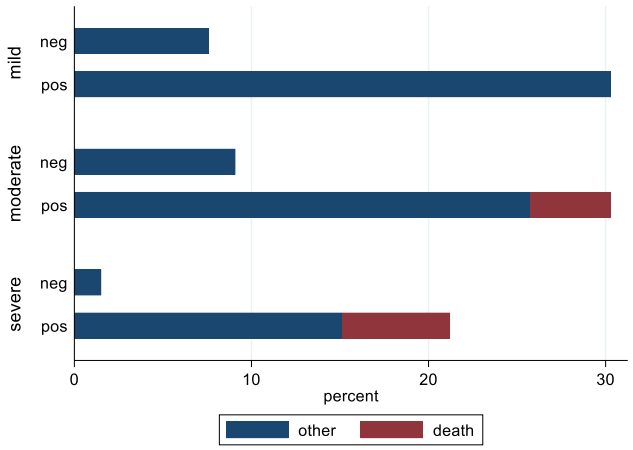

Fig. 1 Frequency distribution of COVID-19 outcome by anti-*T. gondii* IgG results and COVID-19 severity

The *T. gondii* parasite has a complex life cycle with different stages, creates complex interactions with the host, and exerts differential immune responses on humans. The innate immune system is the first response to infection with the production of interleukin (IL)-12 and interferon (IFN-γ) by neutrophils, dendritic cells, and monocytes [16–18]. The parasite persists in cells by overcoming host antimicrobial tools, including reactive oxygen species and nitric oxide production, host cell death induction, and the secretion of pro-inflammatory cytokines and chemokines [19]. After entry and invasion of *T. gondii* tachyzoites into the host cell and activation of the immune system, the parasite converts to the bradyzoite form, which persists in tissue cysts for the lifetime of the host. In this step, the humoral response to toxoplasmosis results in increased levels of specific circulating immunoglobulins. Furthermore, TNF- is involved in the resistance to both acute and chronic toxoplasmosis [20, 21]. According to this scenario, a chronic inflammatory condition in toxoplasmosis might aggravate COVID-19 severity and hyperinflammatory conditions in these patients.

In a recent study, Wolday et al. argue that parasitic co-infection could be an indicator of COVID-19 severity [22]. This viewpoint is supported by findings that show the TH1 immune response, which is accompanied by high levels of IFN- production, causes severe tissue damage, such as antimicrobial peptides and paneth cells, a type of immunopathology that is also seen in people with inflammatory bowel disease [23]. In another study, Bradbury et al. discussed the prospective role of helminth co-infections in the modulation of hyperinflammatory reactions in COVID-19 [13]. Helminth parasites could alter the outcome of COVID-19 in Africa and Latin America, where helminthic contamination is still common. Indeed, the helminth parasites create a modified Th2 response with a controlled inflammatory component that reduces morbidity and or mortality in COVID-19 patients [24]. Given that animal co-infection studies show both negative and positive effects on antiviral immunity, more research on the severity of COVID-19 in helminth-endemic areas is needed [25, 26].

Abdel-Hamed et al. investigated the role of IFN- in reducing the severity of COVID-19 within parasitic infections in patients hospitalized at Zagazig University Hospital and Al-Ahrar Hospital in Sharkia Province, Egypt. They reported that the rate of parasitic infections was 68.8%, with a highly statistically significant increase in mild cases compared with severe cases ($P < 0.001$). Furthermore, high levels of IFN-γ were found in mild cases versus low levels in severe COVID-19 cases with parasitic infections. However, in 92.3% of the patients, the severity of COVID-19 was mild, and a severe situation was found in 7.7%. In this survey, *T. gondii* was the most commonly identified parasite (22.4%) in COVID-19 patients [27]. However, in another study, Sharaf-El-Deen demonstrated that the prevalence of toxoplasmosis was significantly higher in patients with COVID-19 (admitted to the Isolation Department of Shibin El-Kom Hospital, Egypt) than in healthy individuals. Their findings also showed the number of *T. gondii* positive cases was statistically significantly higher in the severe patient group compared with the moderate group [28]. In a study from the north of Iran,

Table 4 Evaluation of the association between COVID-19 outcomes for patients following hospitalization with some important variables

Characteristics	Total	COVID-19 outcomes		*P*
		Death	Other	
Age				
Mean ± SD	58.84 ± 17.08	75.93 ± 10.86	56.69 ± 16.44	< 0.001*
≤ 60	70 (100)	1 (1.40)	69 (98.60)	< 0.001*
> 60	62 (100)	13 (21.00)	49 (79.00)	
Gender				0.70
Female	69 (100)	8 (11.60)	61 (88.40)	
Male	63 (100)	6 (9.50)	57 (90.50)	
T. gondii IgG				
Negative	24 (100)	0 (0.00)	24 (100.00)	0.07
Positive	108 (100)	14 (13.00)	94 (87.00)	
Past medical history				0.68
No	30 (100)	2 (6.70)	28 (93.30)	
DM/HTN/CHD	76 (100)	10 (13.20)	66 (86.80)	
Other	26 (100)	2 (7.70)	24 (92.30)	
CRP				0.04*
No	17 (100)	0 (0.00)	17 (100.00)	
< 50	50 (100)	3 (6.00)	47 (94.00)	
> 50	65 (100)	11 (16.90)	54 (83.10)	
Hospitalization				< 0.001*
Ward	110 (100)	2 (1.80)	108 (98.20)	
ICU	22 (100)	12 (54.50)	10 (45.50)	
Mechanical ventilation				< 0.001*
Negative	120 (100)	2 (1.70)	118 (98.30)	
Positive	12 (100)	12 (100.00)	0 (0.00)	
Corticosteroid consumption history				0.55
No	125 (100)	13 (10.40)	112 (89.60)	
Yes	7 (100)	1 (14.30)	6 (85.70)	

*Significant at the level of 0.05; the values are reported as frequency (percent) and mean ± SD

Ghaffari et al. reported that latent *T. gondii* infection is common (%84) among COVID-19 patients, similar to our results. However, the association between COVID-19 and latent *T. gondii* infection was not significant in COVID-19-positive and COVID-19-negative individuals [29]. The results of the available studies are contradictory in different areas. However, it seems that differences in the predominant genotypes of *T. gondii* and COVID-19 variants in several periods of the pandemic as well as the level of endemicity of *T.* gondii in various geographical regions are possibly the main reasons for these controversial findings.

Based on our findings, mechanical ventilation, hospitalization, LOS, and outcome variables were significantly associated with COVID-19 severity ($P < 0.05$). Also, an increase in CRP levels was detected in 11 of the 14 patients who died in this study. Moreover, CRP had a statistically significant association with moderate COVID-19 compared to mild disease. However, this association is not observed when severe COVID-19 is compared to mild COVID-19. CRP is an important factor in the non-specific immune response, which is increased during infections and inflammation [30]. The major limitation of this study could be the sample size. It is suggested that future studies be conducted as multicenter studies in different geographic regions to make more accurate health decisions.

Conclusion

To our knowledge, this is the first registry-based study concerning *T. gondii* infection among patients with COVID-19. According to our study, latent *T. gondii* infection is common among COVID-19 with different severity. However, there was no significant relationship between the infection and COVID-19 severity and outcomes. The authors have suggested that more prospective multicenter studies with valuable diagnostic methods should be conducted in various geographical regions of the world to address the accurate

Table 5 Determining the associated risk factors with the severity of COVID-19 (moderate and severe vs. mild) based on multiple multinomial logistic regression model

Characteristics	Moderate vs. mild		Severe vs. mild	
	Odds ratio (%95 CI)	*P*	Odds ratio (%95 CI)	*P*
Age	1.02 (0.99, 1.05)	0.14	1.02 (0.99, 1.06)	0.13
Gender (female) Male	2.02 (0.83, 4.89)	0.11	0.83 (0.29, 2.32)	0.72
T. gondii IgG				
Negative Positive	0.63 (0.20, 1.89)	0.41	2.71 (0.49, 14.96)	0.25
Past medical history (no)		0.97		
DM/HTN/CHD	0.98 (0.29, 3.22)		0.53 (0.13, 2.13)	0.37
Other	0.72 (0.16, 3.09)	0.66	1.17 (0.26, 5.20)	0.82
CRP (no)				
< 50	5.34 (0.99, 28.57)	0.049*	2.01 (0.40, 10.12)	0.39
> 50	9.45 (1.73, 51.42)	0.009*	3.03 (0.57, 15.98)	0.19
Lymphopenia (no)				
Yes	1.49 (0.63, 3.52)	0.36	2.54 (0.93, 6.91)	0.06
Corticosteroid consumption history (no)				
Yes	1.33 (0.14, 12.32)	0.79	3.69 (0.48, 28.36)	0.20

*Significant at the level of 0.05

association of toxoplasmosis and COVID-19. It is also necessary that more molecular epidemiology be done on these patients to better understand the distribution status of *T. gondii* genotypes.

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Declarations

Conflict of Interest The authors participated in the study or in the preparation of the report and declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval Ethical approval for this study was obtained from the Ethical Committees of Mazandaran University of Medical Sciences and Iranian National Registry Center for Toxoplasmosis in Imam Khomeini Hospital, Iran (IR.MAZUMS.REC.1399.8630).

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