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Sero-prevalence of *Toxoplasma gondii* before and during the COVID-19 pandemic in Northwestern Iran

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Abstract

Background *Toxoplasma gondii (T. gondii)* is a ubiquitous protozoan parasite on our planet that causes toxoplasmosis. This study evaluated the seroprevalence and related risk factors for *T. gondii* infection in a population referred to healthcare centers in Meshkin-Shahr, Northwest Iran.

Methods A total of 400 blood samples were randomly collected from the general population and assessed using the anti-*Toxoplasma* antibodies, Immunoglobulin G and M (IgG and IgM) Enzyme-linked immunosorbent assay (ELISA) Kits in two steps before and during the coronavirus disease 2019 (COVID-19) pandemic, 2019–2020. The results were analyzed through logistic regression via SPSS 26 software.

Results Before the COVID-19 pandemic, anti-*toxoplasma* antibodies were detected in 39% of individuals (IgG: 38%, IgM: 0.5%, and IgG-IgM: 0.5%). Among the eleven risk factors evaluated, contact with soil and people awareness were significantly associated with *T. gondii* infection ($p < 0.05$). However, factors such as females, 20–39 age groups, junior high schools, housewives, rural areas, raw meat or vegetable consumption, vegetable or fruits washed by water, not detergent, and cat owners did not show a significant relationship with seropositivity ($p > 0.05$). After the outbreak of the COVID-19 pandemic, the overall seroprevalence for anti-*T. gondii* antibody increased to 49.7% (IgG: 47.7%, IgM: 0.5%, and IgG and IgM: 1.5%). Among these patients, 26% were positive for COVID-19. Additionally, before the COVID-19 pandemic, 40 samples were negative for anti-*T. gondii* antibodies but later became positive. The crude and adjusted models suggested that toxoplasmosis may be a possible risk factor for increased susceptibility to COVID-19, with an odds ratio (OR) of 1.28 (95% confidence interval (CI), 0.82–1.99; $P < 0.05$). Conversely, a non-significant protective effect against latent toxoplasmosis was observed in COVID-19-positive individuals (OR = 0.99; 95% CI, 0.51–1.92; $P > 0.05$), and COVID-19 positivity did not increase the levels of anti-*T. gondii* IgG antibodies.

Conclusions The general population in this region had a moderate seroprevalence of *T. gondii*. The increased number of COVID-19-positive patients with latent toxoplasmosis highlights the need to pay attention to the early diagnosis and proper treatment of toxoplasmosis in these patients and implement preventive programs in these areas for future possible viral infections.

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Keywords *Toxoplasma gondii*, Seroprevalence, General population, COVID-19, Iran

Introduction

Toxoplasmosis is one of the neglected parasitic illnesses. Its etiologic agent, *T. gondii*, is the most successful intracellular protozoon on our planet [1]. Human infection occurs through the consumption of mature oocysts in contaminated food, vegetables, and water, eating undercooked meat containing tissue cysts of *T. gondii*, congenital, organ transplantation, and blood transfusion. Toxoplasmosis is asymptomatic in immunocompetent people but can be regarded as a life-threatening or life-limiting infectious illness in pregnant women, immunedeficient individuals, and organ transplants [2, 3]. The prevalence of Toxoplasma infection varies from 15 to 85% in different countries, depending on geographic factors, social and cultural habits, and transmission routes [4]. Approximately 39.5% of Iranian residents are infected with the *T. gondii* parasite [5], and the highest rates of anti-*Toxoplasma* IgG are found in the North and Northwest (48.6%) [6]. The overall prevalence of *T. gondii* infection in Meshkin-Shahr was reported to be 18.3% in 2003 [7]. COVID-19 is a severe acute respiratory illness that may lead to pneumonia and other major public health problems [8]. The disease, accompanied by other respiratory infections, reduces T cell immunity, which changes the microbiota in the gut–lung system and makes patients susceptible to secondary infections such as viral, bacterial, parasitic, and fungal infections [9, 10]. Several studies have shown a link between the occurrence of COVID-19 and parasitic illnesses. Of these, toxoplasmosis has been widely mentioned among COVID-19 patients [1, 11–13]. SARS-CoV-2 and *T. gondii* can activate innate immunity through the common mechanism. Indeed, these pathogens activate the toll-like receptors 2, 4, and 7 through the canonical signaling pathway. Hence, the induction of some cytokines may increase COVID-19 severity in patients with toxoplasmosis [14, 15]. Given the lack of information on the anti-*Toxoplasma* antibodies over the past two decades in the Meshkin-Shahr district and the reports of miscarriages of unknown origin in pregnant women, a cross-sectional study was carried out on both the general population and pregnant women (not published). In the present study, the seroprevalence rate of toxoplasmosis and related risk factors were investigated in the general population before and during the COVID-19 pandemic (due to exposure to the COVID-19 outbreak) in the Meshkin-Shahr district, Northwest Iran, from 2019 to 2020.

Materials and methods

Study area

Meshkin-Shahr is one of the mountainous cities of Ardabil province, northwest of Iran (38°44'N, 47°40'E) (Fig. 1) and is located at an altitude of 4811 m above sea level [16].

Sample collection

This cross-sectional study was conducted on the general population referred to health centers in Meshkin-Shahr city in two stages. In the first step, from April to September 2019, before the incidence of COVID-19, blood samples of 400 participants (101 males and 296 females) were collected. The serum was separated and transported in an icebox to the Ardabil University of Medical Sciences and was kept at -20 °C until testing. A questionnaire was completed for each patient, including demographic characteristics and the infection's risk factors, including sex, age, presence or ownership of animals, contact with soil, eating habits, etc. In the second step, following the declaration by the World Health Organization that COVID-19 became a pandemic, all 400 participants were rescreened for *T. gondii* antibodies from April to September 2020. Information was obtained from each patient for coronavirus positivity according to their Reverse transcription polymerase chain reaction (RT-PCR) results. The sample size formula was used to calculate the number of participants according to the seroprevalence of toxoplasmosis in Meshkin-Shahr [7].

Serological analysis

The sera were analyzed for IgG and IgM antibodies using an ELISA kit (PishtazTeb, Tehran, Iran) following the manufacturer's instructions. The optical density was measured at 450 nm with an ELISA reader. Titers of both types of antibodies equal to or above 1.1 were considered positive. High IgG and IgM titers indicate latent and acute or active toxoplasmosis, respectively. The test's sensitivity was 99.9%, and the specificity was 100%.

Statistical analysis

SPSS v. 26.0 software (SPSS Inc., Chicago, IL, USA) with a p-value < 0.05 as statistically significant was used to analyze the results. The chi-square or Fisher's exact test was utilized to investigate the relationships between qualitative variables. A logistic regression analysis (Model 1 and Crude Model) was used to identify the associations between explanatory variables.

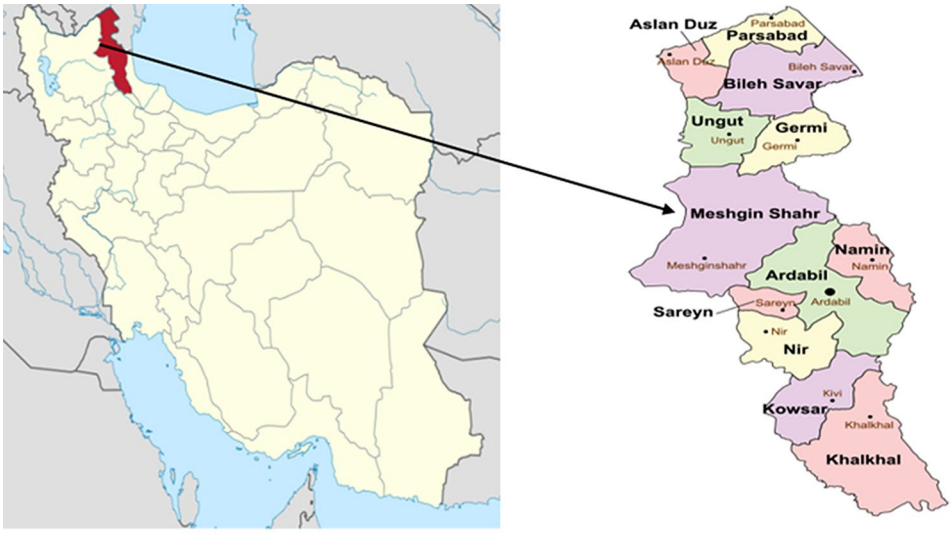

Fig. 1 Map of Iran and Ardabil Province (Left), map of districts in Ardabil Province, Meshkin-Shahr (serum samples were collected, Right)

Results

Seroprevalence and risk factors for *T. gondii* antibodies before the COVID-19 pandemic

In our study, there were 299 (74.8%) females and 101 (25.3%) males with a mean age of 29.3 years (SD=12.1; range=5–82 years) (Table 1). In the first step, the seroprevalence of anti-toxoplasma IgG and IgM was 38% (152 cases) and 0.5% (2 cases), respectively. In addition, 2 samples (0.5%) were positive for both IgG and IgM (Table 2). The highest prevalence of IgG antibodies was observed in individuals aged 20–39 years, with statistically significant differences ($P=0.000$). The mean age of anti-*T. gondii* IgM seropositive patients (2 patients) was significantly lower (19.5 years) than that of negative patients (29.2 years) ($p<0.001$) (Table 1). The results also showed a higher rate of seroprevalence among females, 28.2% (113 cases). The prevalence rate was twice as high for individuals with a history of contact with soil ($P<0.001$) and no awareness ($P=0.006$). There was no significant association between IgG seropositivity and educational level, occupation, residency, raw or undercooked meat or liver, raw or unwashed vegetable consumption, kind of vegetable or fruit washing, blood transfusion or organ transplantation, or animal owners. The seroprevalence of IgG antibodies in the cat ownership and interaction history groups was greater than that in the other groups. However, there was no statistically significant.

Differences in the seroprevalence of *T. gondii* antibodies before and during COVID-19 pandemic

In the 2nd step, all participants were reexamined to determine the differences in *T. gondii* antibodies before and during the COVID-19 pandemic. After the outbreak of the COVID-19 pandemic, 208/400 participants (52%) were positive for COVID-19 from April to September 2020. On the other hand, overall 199 (49.7%) patients were positive for anti-*T. gondii* antibodies ($P<0.001$). Additionally, 40 samples that were seronegative for anti-*T. gondii* antibodies before COVID-19, became seropositive later. Only 19 patients were positive for COVID-19.

Table 2 shows that the results are similar across the different sociodemographic groups except for people's awareness. The group where we observed the significant differences among the independent variables and anti-*T. gondii* IgG antibodies was the soil contact variable ($P<0.001$). During this time, anti-*T. gondii* antibodies were detected in 199/400 (49.7%) of the participants. Among these positive samples, IgG, IgM, and IgG-IgM antibodies were detected in 191 (47.7%), 2 (0.5%), and 6 (1.5%), respectively.

The crude and adjusted models revealed that anti-*T. gondii* IgG is more prevalent in COVID-19-positive individuals and latent toxoplasmosis is considered a possible risk factor that could increase susceptibility to COVID-19 (OR=1.28; 95% CI, 0.82–1.99; $P<0.05$). In contrast, positivity for COVID-19 did not increase the level of IgG antibodies. In other words, in the event of positive PCR results devoid of any associated symptoms, a non-significant protective effect against toxoplasmosis was observed in COVID-19-positive individuals (OR=0.99; 95% CI, 0.51–1.92; $P>0.05$) (Table 3).

Discussion

In the present study, the overall prevalence of anti-*T. gondii* antibodies in the population referred to healthcare centers was 38.5%, which is in accordance with other studies in Iran (30.4-48.6%) [5, 6]. However, there was a wide variation in the seroprevalence results among the different areas of the country. Our findings were less than those of the Caspian Sea Basin, which is an endemic area,

Table 1 Demographic characteristics of the enrolled subjects and IgG anti-*T. gondii* antibodies before and during the COVID-19 pandemic in Northwest Iran ($n=400$)

Variables	Subjects *N* (%)	Pre- COVID-19			During- COVID-19				
		Positive *N* (%)	Negative *N* (%)	*P*-value	Positive *N* (%)		Negative *N* (%)		*P*-value
					Get COVID-19	Not Get COVID-19	Get COVID-19	Not Get COVID-19	
Sex									
Female	299 (74.8)	113 (28.2)	186 (46.5)	0.35	72 (34.6)	73 (38)	81 (38.9)	73 (38)	0.41
Male	101 (25.3)	41 (10.3)	60 (15)		32 (15.4)	20 (10.4)	23 (11.1)	26 (13.5)	
Age groups									
< 10	11 (2.8)	1 (0.3)	10 (2.5)	0.19	2 (1)	0 (0)	5 (2.4)	4 (2.1)	0.4
19-Oct	74 (18.5)	25 (6.3)	49 (12.3)		17 (8.2)	18 (9.4)	20 (9.6)	19 (9.9)	
20–29	130 (32.5)	50 (12.5)	80 (20)		36 (17.3)	29 (15.1)	42 (20.2)	23 (12)	
30–39	117 (29.3)	46 (11.5)	71 (17.8)		28 (13.5)	30 (15.6)	27 (13)	32 (16.7)	
40–49	44 (11)	22 (5.5)	22 (5.5)		15 (7.2)	9 (4.7)	5 (2.4)	15 (7.8)	
≥ 50	24 (6)	10 (2.5)	14 (3.5)		6 (2.9)	7 (3.6)	5 (2.4)	6 (3.1)	
Educational levels									
Illiterate	52 (13)	23 (5.8)	29 (7.2)	0.39	14 (6.7)	14 (7.3)	9 (4.3)	15 (7.8)	0.44
Junior High school	152 (38)	57 (14.2)	95 (23.8)		36 (17.3)	38 (19.8)	35(16.8)	43 (22.4)	
Diploma	93 (23)	34 (8.5)	59 (14.8)		28 (13.5)	18 (9.4)	30 (14.4)	17 (8.9)	
Associate degree	35 (8.8)	16 (4)	19 (4.8)		11 (5.3)	7 (3.6)	10 (4.8)	7 (3.6)	
Bachelor degree	58 (14.5)	23 (5.8)	35 (8.8)		14 (6.7)	14 (5.7)	19 (9.1)	11 (5.7)	
Master's degree	10 (2.5)	1 (0.3)	9 (2.3)		1 (0.5)	2 (3.1)	1 (0.5)	6 (3.1)	
Occupation									
Unemployed	39 (9.8)	19 (4.8)	20 (5)	0.52	13 (6.3)	13 (6.8)	5 (2.4)	8 (4.2)	0.21
Employee	49 (12.3)	17 (4.3)	32 (8)		13 (6.3)	7 (3.6)	17 (8.2)	12 (6.3)	
Self-employment	23 (5.8)	11 (2.8)	12 (3)		5 (2.4)	6 (3.1)	6 (2.9)	6 (3.1)	
Housewife	201 (50.2)	78 (19.5)	123 (30.8)		51 (24.5)	50 (26)	52 (25)	48 (25)	
Student	66 (16.5)	20 (5)	46 (11.5)		14 (6.7)	13 (6.8)	21 (10.1)	18 (9.4)	
Farmer	9 (2.3)	5 (1.3)	4 (1)		4 (1.9)	1 (0.5)	0 (0)	4 (2.1)	
Worker	13 (3.3)	4 (1)	9 (2.3)		4 (1.9)	3 (1.6)	3 (1.6)	3 (1.6)	
Residency				0. 51					0. 20
Urban	176 (44)	68 (17)	108 (27)		43 (20.7)	39 (20.3)	50 (24)	44 (22.9)	
Rural	224 (56)	86 (21.5)	138 (34.5)		61 (29.3)	54 (28.1)	54 (26)	55 (28.6)	
Contact with soil									
Yes	195 (48.8)	100 (25)	95 (23.8)	< 0.001	62 (29.8)	61 (31.8)	32 (15.4)	40 (20.8)	< 0.001
No	205 (51.2)	54 (13.5)	151 (37.8)		42 (20.2)	32 (16.7)	72 (34.6)	59 (30.7)	
Raw or undercooked meat or liver consumption									
Yes	160 (40)	63 (15.8)	97 (24.3)	0.42	40 (19.2)	34 (17.7)	43(20.7)	43 (22.4)	0.19
No	240 (60)	91 (22.8)	149 (37.3)		64 (30.8)	59 (30.7)	61 (29.3)	56 (29.2)	
Raw or unwashed vegetable consumption									
Yes	368 (92)	144 (36)	224 (56)	0.24	97 (46.6)	87 (45.3)	90 (43.3)	94 (49)	0.2
No	32 (8)	10 (2.5)	22 (5.5)		7 (3.4)	6 (3.1)	14 (6.7)	5 (2.6)	
Washing vegetable/fruit									
Water	365 (91.3)	142 (35.5)	223 (55.8)	0.36	93 (44.7)	84 (43.8)	97 (46.6)	91 (47.4)	0.21
Detergent	35 (8.8)	12 (3)	23 (5.8)		11 (5.3)	9 (4.7)	7 (3.4)	8 (4.2)	
Blood transfusion or Organ transplantation									
Yes	18 (4.5)	6 (1.5)	12 (3)	0.37	2 (1)	3 (1.6)	5 (2.4)	6 (3.1)	0.11
No	382 (95.5)	145 (36.2)	237 (59.25)		102 (49)	90 (46.9)	99 (47.6)	93 (48.4)	
Owner									

Table 1 (continued)

Variables	Subjects *N* (%)	Pre- COVID-19			During- COVID-19				
		Positive *N* (%)	Negative *N* (%)	*P*-value	Positive *N* (%)		Negative *N* (%)		*P*-value
					Get COVID-19	Not Get COVID-19	Get COVID-19	Not Get COVID-19	
Cat Owner	140 (35)	55 (13.8)	85 (21.3)	0.35	37 (17.8)	31 (16.1)	31 (14.9)	41 (21.4)	0.73
Dog Owner	24 (6)	6 (1.5)	18 (4.5)		3 (1.4)	7 (3.6)	8 (3.8)	6 (3.1)	
Poultry Owner	31 (7.8)	9 (2.3)	22 (5.5)		10 (4.8)	4 (2.1)	11 (5.3)	6 (3.1)	
Other Pet Owner	9 (2.3)	5 (1.3)	4 (1)		3 (1.4)	3 (1.6)	0 (0)	3 (1.6)	
No	196 (49)	79 (19.8)	117 (29.3)		51 (24.5)	48 (25)	54 (26)	43 (22.4)	
People Awareness									
Yes	154 (38.5)	35 (8.8)	119 (29.8)	0.015	25 (12)	26 (13.5)	35 (16.8)	31 (16.1)	0.08
No	246 (61.5)	82 (20.5)	154 (41)		79 (38)	67 (34.9)	69 (33.2)	68 (35.4)	

Table 2 Comparison of the seroprevalence of *T. gondii* before and during the COVID-19 pandemic in Northwestern Iran ($n=400$)

Anti-T. gondii antibody	Before COVID-19 *N* (%)	During COVID-19 *N* (%)			*P*-value
		Total	COVID-19 Positive	COVID-19 Negative	
Only IgG-Positive	152 (38)	191 (47.7)	101 (25)	90 (22.5)	< 0.001
Only IgM-Positive	2 (0.5)	2 (0.5)	1 (0.25)	1 (0.25)	0.999
Only IgG-IgM-Positive	2 (0.5)	6 (1.5)	3 (0.75)	3 (0.75)	0.610
Negative	244 (61)	201 (50.2)	103 (25.7)	98 (24.5)	-
Total	400 (100)	400 (100)	400 (100)		-

Table 3 IgG anti-*T. gondii* antibody effects on COVID-19 and vice versa

Status	Result	Crude Model OR (95% CI)	Model 1* OR (95% CI)
Effect of IgG anti-*T. gondii* antibody on the COVID-19	Negative 1		
	Positive	1.08 (0.72–1.62)	1.28 (0.82–1.99)
Effect of COVID-19 on the IgG anti-*T. gondii* antibody	Negative 1		
	Positive	0.99 (0.51–1.92)	1.14 (0.55–2.37)

* Adjusted for sex, age, educational level, occupation, residency, soil contact, raw or undercooked meat or liver consumption, raw or unwashed vegetable consumption, washing vegetable/fruit, blood transfusion or organ transplantation, owner, and people awareness

the Northwest, and the capital of Iran, Tehran, whereas they were more than those of the south and southwest [5, 6, 17]. This value was also higher than that reported in a previous study performed by Soltan-Mohammad Zadeh et al. (18.3%) in this area [7]. The Meshkin-Shahr district, mainly an agricultural area, has warm and cold air during the summers and winters. The average daily temperature is less than 0 °C for more than one-third of the year. This weather condition is a convincing reason for the moderate seroprevalence of *T. gondii* infection compared with that in northern areas. The increase in seropositivity, compared with that reported in the previous study [7], may be related to climate fluctuations, and temperature increases in recent years [18], sample size, serology test type, and study design [19]. The seroprevalence results of our study are also in agreement with those of other studies carried out by Jafari et al., Tabriz (35.1%) [20], Keshavarz et al., Tehran (39%), Mostafavi et al., Isfahan (41.1%) and Sarkari et al., Kazerun (36.9%) [5]. The lowest seroprevalence rates were reported in Hormozgan, Kohgiluyeh & Boyer-Ahmad, Kermanshah, Semnan, and Markazi Provinces, Iran [6]. The results also were not comparable with those of our neighboring countries, which is higher than that reported from Iraq (20.87%) [21], Turkey (31.9%) [22], and Qatar (29.8%) [23] as well as Europe (32.1%) [24].

Several sociodemographic and cultural factors including geographical situation, the type of foodstuff, sex, age group, and occupation can affect the seroprevalence estimates of toxoplasmosis [5, 25]. In our study, eleven critical risk factors related to toxoplasmosis were evaluated. Among these individuals, those with the greatest degree of seropositivity were females, in the 20–39 years age group, in junior high schools, housewives, in rural areas, in contact with soil, raw meat or vegetable consumption, vegetable or fruits washed by water, not detergents, cat owners, and people who were not aware of toxoplasmosis. However, the seropositivity for *T. gondii* infection was significantly associated with contact with soil and people's awareness ($p<0.05$), and other risk factors were not

significantly related to *T. gondii* infection. Some studies have likely assessed the risk factors for seroprevalence of *T. gondii* infection and our results are in agreement with their findings [5, 19, 22, 26].

Similar to our results, some studies have shown a non-significant association between the seroprevalence rate of *T. gondii* and sex [5, 6, 19, 27–29].

The 20–39 age group had the highest seroprevalence percentage, and the rate of seropositivity decreased with age increasing. This aligns with findings from studies in Iran and other countries [5, 26, 28, 30, 31]. However, large-scale studies of 26 provinces in Iran reported the highest rate among individuals over a certain age [6]. Another research among the general population in southwestern Iran reported a higher seroprevalence of toxoplasmosis in older subjects [26].

As mentioned above, due to the main occupation of agriculture in this area as a soil-related job, its reason might be clarified the exposure to *T. gondii* through contact with soil, raw/undercooked meat, and barbecue as a cultural habit of people in this area, or vegetable consumption, contact with cats as definitive hosts in cat owners, and lack of awareness or a low level of education about *T. gondii* infection might be clarified. In this respect, the results of our study are consistent with those of other studies [2, 19, 20, 26, 32].

In the current study due to the high number of housewives participants, the highest seroprevalence was observed in them; however, many studies reported similar results [5, 19].

Certain parasites, particularly those causing chronic infections, may influence the symptoms and outcomes of COVID-19. These parasites can impact the immune response and own survival. For example, *T. gondii* is a common parasite that can cause long-term infections and may have a dual effect on the immune system in humans with COVID-19. While it may activate and regulate the immune response, it has also been suggested to provide little protection against COVID-19. However, bifunctional effects related to the association between *T. gondii* and COVID-19 outcome variations have been documented [33–35]. In the second step, we assessed all participants for acute and chronic toxoplasmosis by testing for anti-*T. gondii* antibodies (IgM and IgG) during the initial phase of the COVID-19 pandemic. Among all the participants, 52% tested positive for COVID-19, and 47.7% showed a significant increase in anti-*T. gondii* IgG antibody levels (compared with 38% before COVID-19). Additionally, 1% of the participants displayed evidence of acute *T. gondii* infection on the basis of anti-*T. gondii* IgM testing. However, we did not find a significant association between anti-*T. gondii* IgM levels before and during the COVID-19 pandemic. We also observed that 40 participants who tested negative for *T. gondii* before contracting COVID-19 later tested positive. Among them, 19 patients were also positive for COVID-19.

There is little information about the relationship between *T. gondii* infection and the consequences of COVID-19. Our findings indicate a significantly greater prevalence of IgG antibodies against *T. gondii* in COVID-19 patients. Various factors, such as hygienic and dietary habits, climate and socioeconomic conditions, and contact with different felid hosts, can affect the seroprevalence of toxoplasmosis. Improved sanitizing methods have made it possible to reduce the burden of disease in humans, but despite the sanitation compliance schedules during the COVID-19 pandemic, an increasing trend in seroprevalence was found during the COVID-19 pandemic. Recent studies have shown that in a mouse model of chronic toxoplasmosis, the infection causes dysfunction in T-cells. Both CD4+and CD8+T cells show exhaustion, which is indicated by increased expression of coinhibitory molecules such as the programmed cell death protein 1(PD-1). This leads to the reactivation of latent infection. Among the T cell subset, the CD4 population plays a crucial role in controlling the early stage of acute infection due to their ability to such as IFNγ, whereas the CD8 T cell subset is essential for long-term protection and maintenance of infection in a chronic state [36, 37]. Our increase in seropositivity may be related to T-cell dysfunction.

The crude and adjusted models were used to show the effect of latent toxoplasmosis on the risk of susceptibility to COVID-19 and vice versa. Results showed that toxoplasmosis, with an OR of 1.28 (95% CI, 0.82–1.99, $P<0.05$) appeared markedly effective. In other words, *T. gondii* is a possible risk factor that may increase susceptibility to COVID-19. This result is consistent with the studies by Jaroslav Flegr in Czech and Slovak [38], Sharaf-El-Deen [39], and Ahmed Abed [33] but is not in line with some Iranian studies reporting that toxoplasmosis is not a risk factor for COVID-19 [1, 11, 34, 35]. In a non-matched case-control survey, no marked difference in anti-*T. gondii* IgG antibodies was reported between the cases and controls [12]. Conversely, COVID-19 positivity could dose not increase anti-*T. gondii* IgG antibodies. Alternatively, a non-significant protective effect against latent toxoplasmosis was observed in COVID-19-positive individuals (OR=0.99; 95% CI, 0.51–1.92; $P>0.05$). COVID-19 patients who receive immunosuppressive therapy are at risk for opportunistic infections [40, 41]. However, those with COVID-19 did not require hospitalization and experienced moderate disease severity. Additionally, they had not gotten COVID-19 treatments or corticosteroids that could weaken the immune system and potentially trigger a recurrence of toxoplasma. Some studies have shown a reverse correlation between toxoplasmosis and COVID-19 [42, 43], which does not

support our results. These controversies may be related to the study design, study region, and endemicity of toxoplasmosis [12].

Conclusion

This fundamental study investigated the seroprevalence of *T. gondii* infection before and during the COVID-19 pandemic. The general population in this region had a moderate seroprevalence of *T. gondii*. Contact with soil and lack of awareness about infective sources and transmission routes increase the chance of *T. gondii* infection. Therefore, implementing preventive programs in this area should be considered. Owing to the increased incidence of latent toxoplasmosis in COVID-19 patients and the use of immunosuppressive treatment regimens that may cause toxoplasmosis reactivation, early diagnosis and proper treatment of toxoplasmosis in COVID-19 patients are necessary. In addition, a large-scale multicenter retrospective study should be designed to elucidate the association between COVID-19 and *T. gondii*.

Abbreviations

T. gondii	Toxoplasma gondii
COVID-19	Coronavirus disease 2019
ELISA	Enzyme-linked immunosorbent assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
OR	Odds ratios
CI	Confidence intervals
RT-PCR	Reverse transcription polymerase chain reaction

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Author contributions

SHA and SM conceptualized and wrote the manuscript. SI and SM conducted data analysis. SHA, SM, and SA provided critical revisions. HA, and FF collected samples and completed questionnaires. All authors equally contributed to writing from their perspectives, editing the manuscript, and approving the final manuscript.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Human ethics approval and consent to participate

This study was approved by the Ethics Committee of the Ardabil University of Medical Sciences (No IR.ARUMS.REC.1397.169 and IR.ARUMS.REC.1399.065) and was conducted in accordance with relevant guidelines and regulations or Declaration of Helsinki. Informed consent was obtained from all the participants before sample collection. Participation was completely voluntary, and the participants could choose to quit at any time for any reason during the process of answering the questionnaire.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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