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Original Article

Seroepidemiology and Risk Factors of *Toxoplasma gondii* in Iranian COVID-19 Patients: A Case-Control Study

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Abstract

Background: Theoretically, there is a possible association between emerging SARS-CoV-2 infection and parasites such as *Toxoplasma gondii*. We aimed to evaluate the seroepidemiology of *T. gondii* in COVID-19 patients and the control group as well as its correlation with risk factors.

Methods: Totally, 450 sera samples were taken from COVID-19 positive patients and controls from the Tehran, Karaj, and Shiraz cities, Iran. Anti-*T. gondii* IgG and IgM were evaluated using the ELISA technique. After two months, the participants were followed for recovery or non-recovery and even death. The association between seroprevalence and severity of viral infection as well as other risk factors was statistically estimated.

Results: IgG prevalence in patients and healthy individuals was 59.11% and 61.77%, respectively; these values were estimated at 2.22% and 0% for IgM, respectively. There was no significant association between the prevalence of IgG with COVID-19 infection, while this association was statistically significant for IgM prevalence. The Karaj had the highest prevalence, and a significant association was observed between the seroprevalence and some variables.

Conclusion: Despite the non-significant association between the chronic phase of *T. gondii* (sero) prevalence and COVID-19 symptomatic forms, the parasite prevalence was estimated remarkable and the viral infection and parasite-related acute phase antibodies relationship was estimated to be statistically significant. Due to immunosuppressive therapies for this viral inflammatory infection, it makes it more difficult to interpret the results, and because of the vulnerability of the immune system of these individuals, toxoplasmosis is likely to be hazardous in them; therefore, screening for this parasitic complication seems necessary.

Introduction

The emerging viral disease human coronavirus, designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified and reported in Wuhan, China, in December 2019 from infected patients with respiratory symptoms (1). Then the rapidly spreading virus in different countries, causing the WHO to declare a pandemic situation (2). According to the latest statistics, the morbidity and mortality of this deadly viral disease have been more than 504 and 7 million people worldwide, respectively (3).

Infection can be asymptomatic or manifest a wide range of clinical signs such as fever, cough, tiredness, loss of taste or smell, and in severe cases, even thereby the death of the host by Acute Respiratory Distress Syndrome (ARDS) depending on viral load, host immune system, virus variant factors (4). The exact pathogenesis of COVID-19 infection is not yet clear; hence the critical factor is inflammation, which can be involved in the progression of the disease; severe inflammatory responses in the lungs can lead to lung damage and worsen the patient's condition (5).

Many demographic criteria and comorbidities were claimed to be risk factors for disease severity and even death of COVID-19-positive patients (6). The SARS-CoV-2 and other infectious agents such as parasites co-infection can complicate the patient's situation that has been neglected (7). In the latter, surprisingly, parasites can act in dual function, as evidence suggests that some parasites modulate immune mechanisms by regulating immunity as well as "concomitant immunity" properties (8, 9). Conversely, certain parasites provoke hypersensitivity and extreme reactions in the immune system, and *Toxoplasma gondii* falls into this category (10).

Toxoplasma is a ubiquitous opportunistic protozoan parasite, a feline-specific single-cell parasite that can accidentally infect humans (11). Human toxoplasmosis is often asymptomatic, but depending on the age (infants and pediatrics), the state of the immune system, and the extent of parasite exposure, it can be dangerous (12). It can cause miscarriage in pregnant women or congenital disorders (13). To evade the host's immune system, *T. gondii* targets the host's macrophages, leading to an intracellular infection (14).

In the present study, we aimed to evaluate the seroepidemiology of *T. gondii* in COVID-19 patients and the control group and its correlation with risk factors.

Methods and Materials

Ethical consideration

Informed consent was obtained from all participants. The study design, including its ethical aspects, was reviewed and approved by the Ethics Committee of Tarbiat Madares University (IR.MODARES.REC.1401.024).

Study area and serum samples

The present case-control study was conducted between October 2021 and March 2022. A total of 450 peripheral venous blood samples (about 5 ml) were collected under sterile conditions from randomly examined individuals (233 females and 217 males) attending Tehran, Karaj (Alborz), and Shiraz (Fars) metropolises health centers those whose COVID-19 infection were confirmed by Real-time PCR method and clinical symptoms. Among the subjects, 160 belonged to Tehran, 140 and 150 from Karaj and Shiraz, respectively. Half of the samples from each geographical area were healthy people samples and considered the control group. In the patients' group, 42 cases had an acute form of infection, 132 patients had moderate and mild symptoms, and the rest (51 patients) were asymptomatic and admitted on an outpatient basis.

Blood routine biochemical factors (such as eosinophilia, etc.) were measured for patients, then sera of blood samples were separated by centrifuge at 2500 rpm, aliquot, and stored at -20 °C until analyses were carried out. The subjects' demographic characteristics, including age, sex, urbanization status, cat contact, etc. were obtained with a questionnaire. Subjects were homogenized in terms of age and sex as much as possible. Participants were followed again after two months regarding *T. gondii* seropositivity status and severity of symptoms. Details of patients' general characteristics are provided in Table 1.

ELISA for T. gondii IgG and IgM Antibodies

Anti-*T. gondii* IgG and IgM antibodies were detected by a commercial indirect ELISA kit (PT-Toxo-IgG-96 and PT-*Toxoplasma*-IgM-96, Pishtazteb, Iran) following the manufacture's protocol in medical parasitology laboratory of Tarbiat Modares University. The sample OD (optical density) was evaluated at 450 nm using a 630 nm reference filter in an ELISA reader (DANA, DA3200- Iran). In the IgG antibodies quantitative assessment, higher values than 11IU/ml were considered positive; both the sensitivity and specificity of the IgG test were 100%. In the IgM evaluation, after calculation according to the kit formula (Cut-off Index (COI) = Sample OD/Cut-off value), values higher than 1.1 were considered positive for each sample; the sensitivity and specificity of this test are reported to be 100% and 99%, respectively.

Statistical analysis

Statistical analyses were performed in terms of correlation (Odds Ratio: OR) between the seroprevalence of *T. gondii* and the symptoms in the participants. Moreover, the association with demographic variables was done using chi-square tests with 95% confidence interval (CI), and 0.05 ≤ values were considered significant. All data analyzed was conducted by using SPSS 21.0 (IBM Corp., Armonk, NY, USA) and CMA-2 statistical software.

Results

The mean age of patients was 53.6 years, and healthy individuals were 56.1 years old. Of all samples, 54% were female, and 46% were male. According to the ELISA results, the seroprevalence of *T. gondii* was estimated at 59.11% in patients and 61.77% in healthy individuals according to IgG assay. While these values, according to IgM status in patients and the control group, were estimated at 2.22% and 0%, respectively. Based on OR analyses, no significant association was observed between seroprevalence (IgG) in patients and healthy groups (OR: 0.89, 95% CI: 0.61-1.30). *T. gondii* IgG Seroprevalence was higher in men than in women (62.80% *vs.* 58.43%). However, no significant relationship was observed between prevalence and gender. In COVID-19 patients, the seroprevalence of the parasite was higher in asymptomatic people (70.59%, P-*value*= 0.001). According to the geographically based analysis (Fig. 1), protozoa seroprevalence was significantly high in the Karaj region. Among the demographic characteristics, the animal (cat), soil contact, and income level had a significant relationship with the seroprevalence.

Five IgM-positive cases were detected. All of them were in the patients' group, and no one was in healthy individuals (2.22% *vs.* 0.0%, P-*value* <0.05); the statistically significant association between IgM seroprevalence and COVID-19 infection was found (OR=11.25, 95% CI: 0.62-204.65). The highest prevalence was in urban people and people who came in contact with animals (0.1%). Similar to IgG, the highest seroprevalence of IgM was observed in the Karaj region (2.14%, P-*value*=0.00), howbeit two (6.25%) IgM positive cases were in patients with severe symptoms. Further details of the findings are provided in Table 2.

After two months of follow-up, we were able to access 288 precipitants (including 170 healthy people and 118 patients). Four cases were expired, all of whom were patients with severe symptoms; 29 patients had post-infection side effects (respiratory, cardiovascular, etc.), of which ten were patients with severe symptoms, and 19 cases belonged to mild/moderate symptoms patients as well as the rest (255) were recovered.

Table 1: General characteristics of subjects based on demographic variables and the condition of the disease of COVID-19

Characteristics	Total (%)	Patients	Control group
Age (yr) (mean±SD)	54.8	53.6	56.1
>30	26	17	9
30-60	374	190	184
<60	50	18	32
Gender			
Female	243 (54%)	118	125
Male	207 (46%)	100	107
Geographical area			
Tehran	160	80	80
Karaj	140	70	70
Shiraz	150	75	75
CoV infection status			
Sever	42	42	-
Moderate	142	142	-
Asymptomatic	51	51	-
Residence status			
Urban	377	184	193
Rural	73	41	32
Animal (cat) contact			
Yes	172	98	74
No	278	127	151
Soil contact			
Yes	191	102	89
No	259	123	136
Income level			
Low	101	46	55
Medium	315	160	155
High	34	13	21

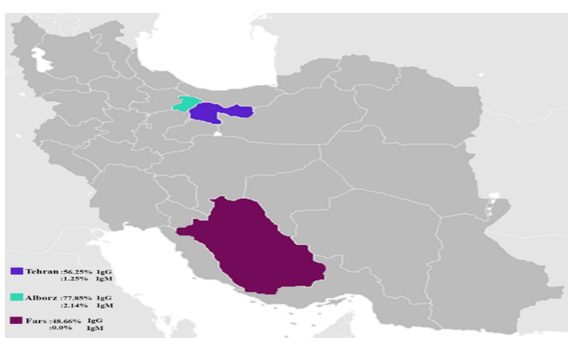

Fig. 1: Geographical distribution and prevalence of anti-*T. gondii* antibodies in the studied areas in Iran

Table 2: Results of *T. gondii* seroprevalence in terms of variables in COVID-19 patients and control group

Characteristics	Total	IgG Positive (%)	IgG Negative (%)	IgM positive (%)	IgM Negative (%)	P-*value**
Total	450	272/450 (60.44)	178/450 (39.56)	5/450 (1.11)	445/450 (98.89)	
Patients	225	133/225(59.11)	92/225 (40.89)	5/225(2.22)	220/225 (97.78)	>0.05
Control group	225	139/225(61.77)	86/225 (38.23)	0/225(0)	225/225 (100)	
OR		0.89 (0.61-1.30)	-	11.25 (0.62-204.6)	-	
Age category						
<30	26	13(50%)	13(50%)	1(3.85)	25(96.15)	>0.05
30-60	374	232(60.03)	142(39.97)	3(0.80)	371(99.20)	
>60	50	27(54)	23(46)	1(2)	49(98)	
Gender						
Female	243	142(58.43)	101(41.57)	2(0.82)	241(99.18)	>0.34
Male	207	130 (62.80)	77 (37.20)	3(1.45)	204(98.55)	
Geographical area						
Tehran	160	90 (56.25)	70(43.75)	2 (1.25)	158 (98.75)	0.11
Karaj	140	109(77.85)	31(22.15)	3 (2.14)	137 (97.86)	0.00†
Shiraz	150	73 (48.66)	77(41.34)	0 (0)	150 (100)	0.74
CoV infection status						
Sever	32	17(53.12)	15(46.88)	2(6.25)	30(93.75)	0.74
Moderate	142	80(56.33)	62(43.67)	3(2.11)	139 (97.89)	0.13
Asymptomatic	51	36(70.59)	15(29.41)	0(0.2)	51 (100)	0.00†
Residence status						
Urban	377	233(61.80)	144 (38.20)	4 (0.1)	373(99.9)	0.18
Rural	73	39(53.42)	34(46.58)	1 (0.13)	72 (99.87)	
Animal (cat) contact						
Yes	172	91(52.91)	81 (47.09)	4(2.33)	168(97.67)	0.01†
No	278	181(65.11)	97(34.89)	1(0.36)	277(99.64)	
Soil contact						
Yes	191	82 (42.93)	109(57.7)	3(1.57)	188(98.43)	0.00†
No	259	190(73.36)	69(26.64)	2(0.77)	257(99.23)	
Income level						
Low	101	76(75.25)	25(24.75)	2(1.98)	99(98.02)	0.00†
Medium	315	178(56.51)	137(43.49)	3(0.95)	312(99.05)	0.02†
High	34	18(52.94)	16(47.06)	0(0)	34(100)	0.73

*: *P*-value was assessed for IgG, **†**: significant association

Discussion

Parasitic infections can regulate the host's immune system, and if they co-occur with another inflammatory infection, the parasites can change the situation to the benefit or detriment of the host (15, 16). The main pathogenicity and the death leading cause of the deadly COVID-19 infection is inflammation (17). Limited studies have been performed on the association between toxoplasmosis – a prevalent parasitic infection- and COVID-19, and the positive or negative impact of parasitic infection is still in a haze of ambiguity (18, 19). Our findings showed no significant association between the seroprevalence of *T. gondii* and the COVID-19 infection. However, the association of toxoplasmosis with some demographic variables was shown. Interleukin (IL) -12 and interferon (IFN-γ) -as innate immune system products- are secreted by immune cells (e.g. neutrophils, monocytes, and dendritic cells) against parasite entry and invasion and toxoplasmosis occurrence (20, 21). Conversely, the parasite dominates the host's immune system with NO (nitric oxide) and reactive oxygen secretions, and can induce the production of pro-inflammatory mediators as well as cause the infected host cells death (22, 23). Parasites can manipulate mitochondrial metabolism, dynamics, and functions to reprogram host immunity and promote their survival (24, 25). What is clear is that in COVID-19, especially in the symptomatic and severe form of the disease, viral load, host immune status, and major risk factors such as age, obesity, and the presence of underlying diseases such as diabetes are considered as key factors in pathogenesis and infection progression (26).

Toxoplasmosis can be considered a risk factor for COVID-19 severity, in this regard, evidence proposes that active toxoplasmosis causes exhaustion of host natural killer (NK) cells and T cells, whereas these cells are involved in COVID-19 infection, and exhaustion of these cells can be in favor of viral infection (18). According to our results, those who were positive for the acute phase of *T. gondii* antibodies (IgM) were all in the symptomatic group of patients, which is in line with other previous studies (27, 28). During COVID-19 infection, cytokine storm syndrome leads to inflammatory reactions of the host and the disease progresses to worsen which is called ARDS (29). The presence of active toxoplasmosis can act as a synergistic co-factor in stimulating inflammation (30). The presence of IgM-positive cases in symptomatic patients can confirm this theory.

In terms of geographical area, Karaj had a high prevalence, which can be related to the abundance of stray and domestic animals (cats and dogs) in human environments and close human contact with these animals. In this regard, previous studies have emphasized the frequency of zoonotic (potential) parasites in animals and the environment (soil, vegetables, etc.) in Karaj (31). Income level and toxoplasmosis possible association statistically evaluation displayed that the prevalence is inversely related to income level, so that in low-income individuals and communities, the prevalence is higher, this phenomenon can be interpreted as the hygiene and health education level are lower in low-income people, and the presence of infected animals and environmental contamination is much more pronounced in poor regions. Latent toxoplasmosis is prevalent in human societies (32).

In immunocompetent people, latent toxoplasmosis is not an earnest problem, but in people with an inadequate immune system, such as HIV (AIDS) people and those with acute COVID-19 treated with corticosteroids can reactivate and cause severe infections and even death, consequently screening immunocompromised patients who treated with immunosuppressants is very imperative in terms of latent and active toxoplasmosis (33, 34).

Although according to the "hygiene hypothesis", some parasites can reduce the severity of other infections by modulating and regulating the host's immunity, this could not be the case with Toxoplasmosis, despite the higher seroprevalence of anti-parasite antibodies (IgG) in the control group; since being positive for chronic phase antibody (IgG) positive does not necessarily represent the current and/or previous infection and may even indicate asymptomatic exposure (35). On the other hand, the seroprevalence of anti-*T. gondii* IgG in the subjects, whether patients or healthy individuals, does not mean the true prevalence, and only shows the apparent prevalence.

The present study has faced limitations such as the lack of access to all samples and the impossibility of tracing the parasite genome by molecular methods and the failure to check for other co-infections were the mains.

Conclusion

There was no significant association between chronic infections of *T. gondii* and COVID-19, but it showed a probably association with acute infections of the parasite. Seroprevalence was associated with some environmental risk factors and the host antimicrobial tools status; because people with viral inflammatory disease (COVID-19) are treated with immunosuppressants, screening of these individuals for active and latent toxoplasmosis is recommended.

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Competing interests

None declared.

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