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Iranian Society of Parasitology
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Original Article

Associations between *Toxoplasma gondii* Infection and Multiple Sclerosis: A Case-Control Seroprevalence Study

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Received 14 Jan 2023
Accepted 22 Apr 2023

Keywords:
Toxoplasma gondii;
Multiple sclerosis;
Seroprevalence;
Case-control study;
Iran

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Abstract

Background: Currently, there are conflicting reports on the associations between *Toxoplasma gondii* infection and multiple sclerosis (MS) in humans. In the present study, a case–control study was carried out to assess associations between seropositivity to *T. gondii* infection and MS.

Methods: This case-control study was carried out on 200 MS patients (cases) attended in Sina Hospital affiliated to Tehran University of Medical Sciences, Tehran, Iran, and 200 healthy subjects from the general population of the same city, March to July 2017. Blood samples were collected from individuals and were examined using Enzyme-linked immunosorbent assay (ELISA) for the presence of *T. gondii* IgG antibodies and the IgG-positive samples were further analyzed for specific anti-*T. gondii* IgM.

Results: The overall seroprevalence of anti-*T. gondii* IgG was 44.2% (177/400) in 121 (60.5%) sera of the 200 MS patients (cases) and 56 (28.0%) sera of the 200 controls (OR = 3.94; 95% CI: 2.59–5.99; $P < 0.001$). Seroprevalence of *T. gondii* infection in MS patients increased significantly with increasing of age ($P < 0.001$). In the control group, no statistically significant differences were seen between the seroprevalence of *T. gondii* infection in various age groups ($P = 0.858$). Moreover, no statistically significant relationships were reported between the seropositivity to *T. gondii* and the sex for the cases and controls ($P>0.05$). Anti-*T. gondii* IgM antibodies were not detected in anti-*T. gondii* IgG positive patients.

Conclusion: *T. gondii* infection might be a probability risk factor for MS. However, further studies are necessary to describe clearly the roles of *T. gondii* infection in MS.

Introduction

"Multiple sclerosis (MS) is a chronic inflammatory and neurological autoimmune disease affecting the central nervous system (CNS)" (1). According to the Global Burden of Disease (GBD) report, MS was ranked tenth for prevalence in neurological conditions with 2,012,000 cases estimated globally in 2015 (2). Although the etiology of MS is not clearly understood similar to other autoimmune diseases, combination of genetic proneness and environmental and lifestyle factors can lead to development of the disease (3). Within the environmental factors, infectious agents such as human herpesvirus 6 (HHV-6), Epstein-Barr virus (EBV) and *Chlamydia pneumoniae* increase the risk of future development of MS (4). Furthermore, neurotropic parasites such as *Toxoplasma gondii* are suggested as overlooked risk factors that can contribute to the pathophysiology of the disease (4, 5).

T. gondii, the causative agent of toxoplasmosis, is an obligate intracellular coccidian parasite of all warm-blooded animals and humans worldwide. Toxoplasmosis is a life- threatening disease for organ transplant recipients, cancer patients and patients with human immunodeficiency virus (AIDS) (6–10). Although acute toxoplasmosis is generally asymptomatic in immunocompetent people, behavioral and neurological disorders have shown to associate to latent infections in various hosts (11–14). Neurological disorders of toxoplasmosis could occur due to the brain damages directly induced by the parasite or host immune responses to the parasite and localization of tissue cysts in the CNS (14, 15). *T. gondii* infection can be associated to primary neurologic diseases such as Parkinson disease, Alzheimer's disease, epilepsy, schizophrenia and MS (16–18).

There are conflicting reports on the associations of *T. gondii* infection with MS in humans. Negative associations between the *T. gondii* infection and MS have been reported in studies in Turkey (19) and Germany (20). In contrast, *T. gondii* seropositivity was significantly associated to MS (21–23). In Iran, 50 MS patients and 50 of their family members were assessed for *T. gondii* antibodies and the two groups had similar anti-*T. gondii* IgG titers (24). A recent meta-analysis has shown lower prevalences of *T. gondii* in MS patients, compared to control groups; however, no significant associations were reported between toxoplasmosis and MS (25). Therefore, this case–control study was carried out to assess the associations between seropositivity to *T. gondii* infection and MS.

Materials and Methods

Study design and sample collection

This case-control study was carried out on 200 MS patients (cases) referred to Sina Hospital affiliated to Tehran University of Medical Sciences, Tehran, Iran, as well as 200 healthy individuals from the general population of the city, during Mar–Jul 2017. Diagnosis of MS was carried out by two experienced neurologist based on 2010 McDonald criteria (26). Inclusion criteria for the participants included being MS patient, aged 16 years and older and having full willingness to participate in the study. Two hundred healthy volunteers were set up and evaluated as the control group in the same socioeconomic status with the patient group in terms of the consensus definition of control groups in cerebrospinal fluid biomarker studies in MS from 2013 (27). Venous blood samples (up to 3 ml) were collected from the cases and controls and immediately centrifuged at 1000× g for 5 min. Sera were aliquoted and frozen at -20 °C until use.

Ethics approval and consent to participate

This study was carried out based on the Declaration of Helsinki and approved by the Research Ethics Committee of Tehran Uni-

versity of Medical Sciences, Tehran, Iran. All participants were voluntarily enrolled in the study and informed that the study methodology included no potential risks to their health and all their information were strictly assumed confidential. Informed written consents were collected from the participants or their parent or legal guardian in the case of children under 16 before commencement of the study.

Serological assays

ELISA based on soluble antigens of *T. gondii* was used to assess anti-*T. gondii* IgG in blood sera. Cut-off values of the optical density (OD) were reported as the mean OD values of *T. gondii* negative sample reactivity with two standard deviation (SD) (28). The OD of each sample was compared to that of the cut off and recorded as positive or negative result. The protocol was completely described in a previous study by the current authors (29). Moreover, sera with anti-*T. gondii* IgG were further analyzed for specific anti-*T. gondii* IgM using commercial ELISA kits (Vircell, Granada, Spain) according to the manufacturer's instructions.

Statistical analysis

For the statistical analysis, SPSS Software v.24 was used (IBM Corp., Armonk, NY, USA) (30). Descriptive statistics were used to characterize the samples. Moreover, independent *t*-test was used to compare the mean ages within the two groups. Seroprevalence of anti-*T. gondii* IgG for MS patients and the controls generally and for age and sex subgroups separately were compared using chi-square test. Odds ratio (OR) and 95% confidence interval (CI) were calculated for the associations between multiple sclerosis and *T. gondii* infection. In general, *P*-values less than 0.05 were recorded statistically significant.

Results

The mean age of the patients at the onset of MS was 28.36 ±8.70 yr. The mean ages of the MS patients and controls were respectively 36.72±10.26 and 33.23±8.15 yr, which were significantly different based on the *t*-test ($P < 0.001$). Participants were divided into three age groups of ≤ 30, 31–50 and ≥ 51 years old. A majority of the participants were between 31 and 50 years old. Of the 200 patients in MS group, the mean ages of seropositive and seronegative patients were respectively 38.78±8.96 and 33.57 ±11.34 yr, which were statistically significant ($P = 0.001$). Furthermore, the mean ages of the seropositive and seronegative participants in the MS group at the onset of the MS were respectively 29.73±8.19 and 26.27±9.10 yr with significant differences ($P = 0.006$). The patients were selected among relapsing remitting MS under treatment of different preparations of interferon beta. Patients with a history of recent relapse or steroid use during the past three months were excluded.

In Table 1, the seropositivity to *T. gondii* for cases and controls by sex and age is assessed. Seroprevalence of *T. gondii* infection in general and MS patients increased significantly with increasing of age ($P < 0.001$). However, no statistically significant differences were seen between the seroprevalence of *T. gondii* infection in different age categories of the control group ($P = 0.858$) (Table 1). Moreover, no statistically significant relationships were demonstrated between seropositivity to *T. gondii* and sex for cases and controls. Anti-*T. gondii* IgG antibodies were detected in 121 (60.5%) of the 200 MS patients and 56 (28.0%) of the 200 controls (OR = 3.94; 95% CI: 2.59–5.99; $P < 0.001$). In a multiple logistic regression analysis and after adjustments for covariates, associations were still significant (adjusted OR = 3.57; 95% CI: 2.33–5.47; $P < 0.001$). Moreover, anti-*T. gondii* IgM was

not detected in anti-*T. gondii* IgG positive samples.

Table 1: Stratifications by sex and age in cases and controls for seropositivity to *Toxoplasma gondii*

Characteristics	*Case*			*Control*			*Total*		
	Seroprevalence of *T. gondii*			Seroprevalence of *T. gondii*			Seroprevalence of *T. gondii*		
	n (total)	%	OR (95% CI)	*n (total)*	%	OR (95% CI)	*n (total)*	%	OR (95% CI)
Age (yr)									
≤ 30	19(58)	32.8	1	21(77)	27.3	1	40(135)	29.6	1
31–50	89(122)	73.0	5.54(2.81-10.91)	33(118)	28.0	1.04(0.54-1.97)	122(240)	50.8	2.46(1.57-3.84)
> 50	13(20)	65.0	3.81(1.31-11.11)	2(5)	40.0	1.78(0.28-11.40)	15(25)	60.0	3.56(1.48-8.60)
P-value		< 0.001			0.858			< 0.001	
Sex									
Male	26(44)	59.1	0.93(0.47-1.83)	15(44)	34.1	1.45(0.71-2.98)	41(88)	46.6	1.13(0.70-1.82)
Female	95(156)	60.9	1	41(156)	26.3	1	136(312)	43.6	1
P-value		0.829			0.308			0.617	

Discussion

The relationships of *T. gondii* infection with development of MS are still debated and results of studies on this topic are conflicting. Therefore, the current case-control study was carried out to investigate associations between seropositivity to *T. gondii* and MS in people of Tehran City, Iran.

Results of the current case-control study showed that the seroprevalence of *T. gondii* infection was significantly higher in MS patients than controls. The associations of *T. gondii* infection with MS was positively significant even by adjustment for covariates. Similarly, studies in Turkey (21) and Iran (22, 23) showed significant positive associations between latent toxoplasmosis and MS, however some others in Mexico (31), Italy (32), Germany (20), Iran (24) and Latin America (33) indicated a negative association. More recently (2021), in a meta-analysis the seroprevalence of *T. gondii* infection was lower in MS patients than in controls; however, differences were not statistically significant (OR = 0.68, 95% CI = 0.50–0.93) (18). In general, it was not clear why dissimilarities and controversies were reported in the associations between seroprevalence of *T. gondii* infection and MS in the highlighted studies. Various designs of the studies (population based cross-sectionals and case controls), types of the populations (children, adults and general ages), types of the MS patients (clinically isolated syndrome, relapsing-remitting, primary and progressive) and serological methods with various sensitivities and specificities might be possible reasons for inconsistencies in the results of individual studies. However, pathophysiological mechanisms underlying the roles of *T. gondii* infection in development of MS are not discovered clearly and additional studies are needed in human and animal models (18).

In the present study, positive associations between *T. gondii* seropositivity and MS might be due to the increased risks of infection in MS patients. Generally, individuals with mental disorders are at increased risks of exposure to *T. gondii* infection, which might be linked to their living facilities and behaviors. Furthermore, these people are generally at increased risks of medical comorbidities due to their decreased general resistance to infections (34–36). The current results have revealed needs of studies with a wide range of sociodemographic, clinical and behavioral variables to investigate associations of infection with various types and courses of MS. In general, *T. gondii* might be responsible for a small proportion of MS cases and other infectious agents could be linked to MS; as already reported (37). However, the present study was unable to assess the time between the *T. gondii* infection and MS onset.

Another finding of the present study included those MS patients with positive anti-*T. gondii* IgG had a higher mean age than those with negative anti-*T. gondii* IgG, which was statistically significant ($P = 0.006$). This might be due to *T. gondii* infection that could delay the onset of MS development. However, it is noteworthy that IgG assessment in a single serum sample cannot estimate the exact acquire time of *T. gondii* infection. Similarly, Fleming et al. (38) showed that persistent parasitic infections could be a possible explanation for the suppression of MS development in regions with low prevalence rates of MS. In support of the hygiene hypothesis, immunomodulatory molecules produced by infectious agents can be beneficial (39). In the current study, anti-*T. gondii* IgG was assessed on the collected sera using ELISA and anti-*T. gondii* IgM was further analyzed on positive samples. Assessment of anti-*T. gondii* IgM in all the collected samples could be appropriate but was not possible due to the limited financial resources. These limitations possibly affected the overall seroprevalence rates of *T. gondii* IgM reported in the current study.

Conclusion

The seroprevalence of *T. gondii* in MS patients was higher than that in the healthy people. It seem that *T. gondii* infection might be a probability risk factor for MS. However, further studies are needed to demonstrate roles of *T. gondii* infection in MS development.

Acknowledgements

This study was financially supported by the Vice-Chancellor for Research and Technology Affairs of Tehran University of Medical Sciences, Tehran, Iran.

We would like to acknowledge all staff from the toxoplasmosis laboratory (Department of Medical Parasitology and Mycology, Tehran University of Medical Sciences, Tehran, Iran) for their useful collaboration.

Conflict of interest

The authors declare that they have no competing interest.

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