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The role of *Toxoplasma gondii* in multiple sclerosis: A matched case-control study

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Abstract

Background & Objectives: Toxoplasma (T.) gondii is an intracellular parasite that has recently been reported in association with multiple sclerosis (MS) and other autoimmune diseases, with an unidentified function. The aim of this project was to investigate the seroprevalence of *T. gondii* in MS patients in comparison with healthy people. *Method:* This case-control prospective study was conducted on 90 patients with MS and 90 age and gender-matched healthy participants. All patients and healthy individuals filled a sociodemographic questionnaire and MS patients were evaluated for clinical status. The presence of specific IgG and IgM antibodies against *T. gondii* was explored by using an enzyme immunoassay test in the sera of the participants. *Results:* The mean age of MS patients was 34.47±8.74. Out of 90 MS patients, 70 (77.8%) were female. No significant difference was observed between both groups with respect to age and gender. Anti-*T. gondii* IgG antibodies were found in 47 (52.2%) of the 90 cases and in 79 (87.8%) of the 90 controls (P = 0.0001). Mean age and disease duration of the seropositive MS patients were 36.76±7.78 and 5.12±3.64 years, respectively. There was significant association between *T. gondii* seropositivity and age and also disease duration (P=0.009 and 0.033, respectively).
Conclusion: The results of this study showed that the seroprevalence of *T. gondii* in MS patients is lower than the healthy group. These results suggest that there is a negative association between infection with *T. gondii* and MS and toxoplasmosis can be considered as a possible protective factor for the development of MS.

Keywords: Toxoplasmosis, multiple sclerosis, prevalence, IgG antibody, IgM antibody

INTRODUCTION

Multiple Sclerosis (MS) is a chronic, inflammatory, and demyelinating multifocal disease that affects the central nervous system (CNS).[1-2] The etiology of MS, similar to other autoimmune diseases, is still unclear; but the combination of genetic proneness and environmental influences can lead to creating of the development of this disease.[3-4] One of the most prominent environmental factors in MS pathogenesis is infectious agents such as human herpesvirus 6 (HHV-6), Epstein-Barr virus (EBV), and *Chlamydia pneumoniae*.[5] Recently, there has been growing interest in the correlation between parasitic infections and autoimmune disorders such as MS. This issue has been studied widely since 1989.[6] Previous research has established that parasitic infections may be accompanied with a lower possibility of MS.[7-8] Also, some earlier studies evaluated the association between toxoplasmosis and MS and found contradictory findings.[9-12] *Toxoplasma Gondii* (*T. gondii*) is an intracellular parasite that can cause a lifelong chronic infection in the host.[13] The prevalence of *T. gondii* infection in humans worldwide, based on IgG measured against *T. gondii*, has been reported to be around 6 billion.[13] Daryani *et al*. showed that the general prevalence of *T. gondii* infection in the Iranian population is approximately 40%.[14] Moreover, data from several studies suggest that chronic *T. gondii* infection may have a role in some disorders, such as cognitive impairment, cryptogenic epilepsy,

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headache, and neurodegenerative disorders, which may be due to direct parasite attack or immunological damage affected by the parasite or both.[15-18] There is evidence that interferon-gamma (IFNγ), produced by microglial cells, plays a crucial role in immunological protection against toxoplasmosis.[19] However, IFNγ may result in the production of nitric oxide (NO) that is a key element of tissue damage.[19-20] On the other hand, Rozenfeld *et al*. reported that activated microglia produce interleukin-10 (IL-10) and indirectly induce astrocyte activity and also prostaglandin E2 (PGE2) production.[21] It has been conclusively shown that PGE2 may play a neuroprotective role, as a result of the extinguishing Th1 pro-inflammatory cytokine, the inducing Th2 cytokines like the interleukin- 10, and the decreasing NO production via activated microglia.[22] This mechanism is indicated as an indirect protective effect of neurons by toxoplasmosis.[21] The main challenge faced by many researchers is disagreement on the role of *T. gondii* in MS. While several researchers identified *T. gondii* as a protective factor, others reported it as a risk factor.[10-12,23] A meta-analysis study demonstrated a lower prevalence of *T. gondii* in MS patients compared to control group, but no significant relationship was found between toxoplasmosis and MS.[24] Thus, the actual relationship between toxoplasmosis and MS remained uncertain. Therefore, the aim of this study was to evaluate the role of *T. gondii* in MS.

METHODS

This case-control prospective study was conducted on 90 patients with clinically definite diagnosis of MS and clinically isolated syndrome (CIS) according to McDonald's criteria for diagnosing MS.[25] All patients were randomly selected, then assessed and followed up in the outpatient clinic of MS at Bou-Ali Sina Hospital, Mazandaran province, Iran over one year from December 2017 to January 2019. Meanwhile, the control group included a total of 90 healthy volunteers matched with cases for age and gender; all individuals were randomly selected from the population referring to the clinic.

The inclusion criteria were definite diagnosis of MS and CIS based on McDonald's criteria, aged 18 years and older, and the patients in the remission period who accepted to participate in the study.

The exclusion criteria were receiving intravenous corticosteroids in the past 3 months, consuming anti-parasitic drugs, having immune deficiencies (leukemia, lymphoma, malignancy, and AIDS) and other neurological disorders such as Parkinson's disease, Alzheimer's disease, epilepsy, and diabetes mellitus.

This study was approved by the Institutional Research Ethics Review Board of Mazandaran University of Medical Sciences (IR. MAZUMS. REC.1398.3570). The study has been extracted from a medical student thesis with the project number, 3570.

All patients and healthy individuals filled a sociodemographic questionnaire, which included such information as age, gender, place of residence, educational level, occupation, marriage status, and cat keeping or touching. In addition, other information was obtained from the patients regarding disease duration, annualized relapse rates, extended disability status scale (EDSS), pattern of MS, and type of medication consuming.

Venous blood samples (5 ml) were achieved from all participants for serological tests. Sampling was done in MS clinic and testing was carried out at the university Clinic Laboratory (Bagheban). All blood samples were stored at –20°C and analyzed for anti-toxoplasma IgG and IgM antibodies using enzyme-linked immunosorbent assay (ELISA) (ELISA Kit, Toxo IgG & IgM, Pishtazteb, Iran). The sensitivity of both IgG and IgM kits was 100%, and the specificity of IgG and IgM kits was 100 and 99%, respectively. Repeated freezing and defrosting were avoided for all samples. We used automatic ELISA reader with capacity of optical density at 450 nm, and the results were interpreted according to the related guidelines. The IgM values at 0.90 IU/mL were interpreted as negative, those at 0.91 to 1.09 IU/mL as borderline, and those at 1.10 and above as positive. The IgG values at 8 IU/mL were considered as negative, 11 IU/mL and above as positive, and between 8.1-11 IU/mL as suspicious.

Sample size and statistical analysis

For the sample size calculation, we used the following values: a 95% confidence level, a power of 80%, a 1:1 proportion of cases and controls, and a reference seroprevalence of 55%[25] as the expected frequency of exposure in controls and 33.9% in intervention group. Thus, a total of 90 cases and 90 controls were randomly selected. Statistical analysis was performed using SPSS software version 24 (SPSS Inc., Chicago, Illinois, USA). Continuous data were stated as

mean±standard deviation (SD), whereas frequency data were presented as percentages (%). The Chi-square and Student's t-test were used to test statistically significant differences for parametric data. P-values less than 0.05 were considered statistically significant.

RESULTS

A total of 180 subjects (90 MS patients and 90 healthy controls) participated in this study. The mean age of MS patients and healthy controls was 34.47±8.74 and 34.25±8.11, respectively. Out of 90 MS patients, 70 (77.8%) were female. No significant difference was observed between the two groups with respect to age and gender (P=0.86 and P=0.71, respectively). Regarding the place of residence, 68 patients (75.6%) and 73 healthy people (81.1%) lived in urban areas, and no significant difference between the two groups was reported (P=0.47). In terms of being in contact with cats, none of the participants kept a cat in the house but 36 patients (40%) and 42 controls (46.7%) stated that they had touched cats. No significant difference was found between the two groups (P=0.45). The results of sociodemographic characteristics in two groups are illustrated in Table 1. The mean of EDSS score and mean disease duration was 2.62 ±1.91 (range: 1-7.5) and 6.05±4.29 (range: 0.50-20) years, respectively. Also, the annualized relapse rate (ARR) was 0.62±0.91 (range: 0-4). Regarding the patterns of disease progression, 66 (73.33%), 16 (17.77%), 10 (11.11%), and 4 (4.44%) of patients were relapsing-remitting (R-R), CIS, secondary progressive (S-P), and primary progressive (P-P), respectively. All MS patients received disease-modifying therapies (DMTs) who, 34 (37.8%) patients administrated interferon-beta and 27 (30%) received rituximab. The remaining patients 29 (32.2%) obtained oral DMTs.

Seroprevalence and titer of anti-T. gondii IgM and IgG

The results obtained for anti-*T. gondii* IgG titer were among 0 to 276 IU/mL with the mean value of 67.51±86.21 IU/mL in the case group, and 0 to 260 IU/mL with the mean of 97.74±72.59 in the control group. There was a significant differences

Table 1: Demographic data and serologic characteristics of participants

Variables	MS patients	Healthy group	* *p*-value
Age; Mean± SD	34.47±8.74	34.25± 8.11	0.86
Gender; n (%)			
Male	20(22.2)	17(19.9)	
Female	70(77.8)	73(81.1)	0.71
Educational level; n (%)			
Illiterate	4(4.4)	6(6.7)	
Primary school	6 (6.7)	1(1.1)	
Under diploma	30 (33.3)	32(35.6)	
Diploma	11(12.2)	10(11.1)	
University education	39(43.3)	41(45.5)	0.51
Place of residency; n (%)			
Urban	68(75.6)	73(81.1)	
Rural	22 (24.4)	17(18.9)	0.47
Occupation; n (%)			
Housewife	51(56.7)	58(64.4)	
Employed	14(15.6)	17(18.9)	
Unemployed	25(27.8)	15(16.7)	0.19
Touched cat; n (%)			
Yes	36(40)	42(46.7)	
No	54(60)	48(53.3)	0.45
[a]Seropositivity IgM; n (%)	1(1.1)	1(1.1)	0.75
[a]Seropositivity for IgG; n (%)	47(52.2)	79(87.8)	0.0001
IgG titer; Mean ±SD	67.51±86.21	97.74±72.59	0.012
IgM titer; Mean ±SD	0.15±0.63	0.18±0.74	0.76

* p-value <0.05 is significant. [a]Anti- *T. gondii* antibodies; Anti- *T. gondii* Ig G seropositivity

between the two groups (t (178)= -2.54, P=0.012). All participants were negative for anti-*T. gondii* IgM with the exception of one participant in both case and control groups, who had anti-*T. gondii* IgM titer above 1.1 IU/mL (6.1 and 7.1 IU/mL, respectively). T-tests found no significant differences in mean scores of anti-*T. gondii* IgM titer between the two groups (P=0.76). Overall, 47 patients (52.2%) and 79 controls (87.8%) had anti-*T. gondii* IgG titer of 11 IU/mL and above. Also, there was a significant difference between the two groups (OR: 0.15; 95% CI: 0.72–0.32; P=0.0001, Chi-square test). Table 1 presents the obtained results of the serological data of the two groups. In terms of positive and negative *T. gondii* IgG with sociodemographic data in both groups, overall, no significant association was observed.

The relationship of and anti-T. gondii IgG with demographic data among MS patients

The mean score for age was 36.76± 7.78 and 31.97± 9.13 in anti-*T. gondii* IgG seropositive and seronegative MS patients, respectively. There was a significant differences between the two groups (t(88)=2.68, P=0.009). Out of 47 seropositive MS patients, 32 (68.1%) were female and 15 were male (31.9%). Also, there was a significant difference between seropositive and seronegative MS patients with gender (OR:3.56; 95%CI:1.16-10.87; P=0.021). The relative risk of seropositivity in men was 2.7 times higher than that of women. In addition, out of 47 seropositive MS patients, 12 (25.5%) lived in the rural areas and 22 (46.8%) had a history of touching cats. None of these differences, with regard to either residence place or contact with cats, in the two groups of seropositive and seronegative MS patients were statistically significant (P=0.80 and P=0.20, respectively). The mean of EDSS in seropositive and seronegative MS patients was 2.61±1.95 and 2.62±1.89, respectively. Also, there was no significant difference between EDSS and anti-*T. gondii* IgG (P=0.99). But there was a significant difference between anti-*T. gondii* IgG seropositivity and mean disease duration, so that seropositive MS patients had a shorter disease duration (t(88)=-2.19; P=0.033). Table 2 demonstrates the correlation between anti-*T. gondii* IgG and characteristics of MS patients. Out of 47 seropositive MS patients, 34 (72.3%) were R-R, 7 (14.9%) CIS, 2 (4.3%) P-P, and 4 (8.5%) S-P. There was no significant difference between seropositivity and type of disease (P=0.67). Regarding the patients receiving DMTs, out of 34 (44.1%) patients receiving interferon-beta, 15 were seropositive; and out of 27 patients receiving Rituximab, 18(67.7%) were seropositive. There was no significant difference between seropositivity and kind of DMTs received (P=0.37). Figure 1 presents the frequency of seropositive and seronegative anti-*T. gondii* IgG based on type of MS and DMTs received.

DISCUSSION

The role of *T. gondii* infection in MS is still not completely clear. Currently, there are few studies about this association. Meanwhile, several studies have described contradictory outcomes. Accordingly, this study set out with the aim of assessing the frequency and serum levels of toxoplasma antibodies (IgG and IgM) in MS patients compared to healthy population. Our findings indicated lower seroprevalence of *T. gondii* infection and lower antibody titers of anti-*T.*

Table 2: The association between characteristics of MS patients with anti- *T. gondii* IgG.

Variables	IgG positive (N=47)	IgG negative (N=43)	* *p*- value
Age; mean±SD	36.76±7.78	31.97±9.13	0.009
Gender; n (M/F)	15/32	5/38	0.021
Touch the cat; n (yes/ no)	22/25	14/29	0.20
Residency; n(urban/rural)	35/12	33/10	0.80
Disease duration; mean±SD	5.12±3.64	7.06 ±4.73	0.031
EDSS score; mean±SD	2.61±1.95	2.62±1.89	0.97
ARR; mean±SD	0.63±0.89	0.60±0.96	0.86

* p-value <0.05 is significant. SD: standard deviation, M/F: male/female, n:number;, EDSS: Expanded Disability Status Scale, ARR: Annualized Relapse Rate.

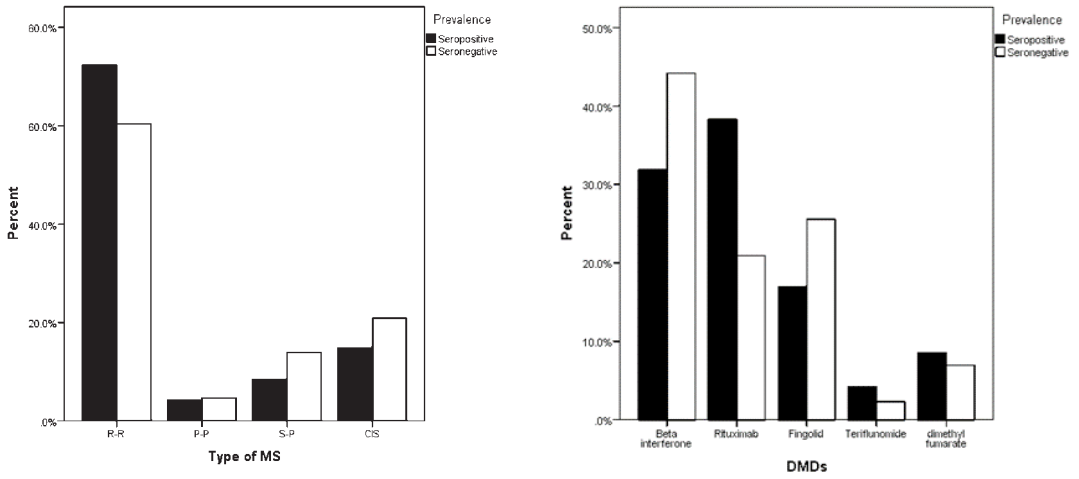

Figure 1: frequency seropositive and seronegative anti-*T. gondii* IgG in according to type of MS and type of DMTs. R-R=relapse-remitting; S-P=secondary-progressive; P-P=primary-progressive; CIS=clinical isolated syndrome.

gondii IgG in MS patients than age- and gender-matched healthy controls. Therefore, these results may support hygiene hypothesis. According to the 'hygiene hypothesis', the diminishing incidence of infections in early childhood can play an important role in the development of both autoimmune and allergic diseases due to inappropriate response of the immune system and the dysregulation of Th1 and Th2 cell activity.[26] In line with the present study, some previous studies demonstrated a negative association between infection with *T. gondii* and MS.[9,11] Koskderelioglua *et al*. conducted a study in Izmir, Turkey and reported a low *T. gondii* seropositivity (33.9%) in MS patients in contrast to the control group (55%); also, Stascheit *et al*. found low levels of antibodies against *T. gondii* in MS patients in Berlin, Germany.[9,11] In a recent study, Nicoletti *et al*. reported a low level of anti-*T. gondii* antibodies in MS patients (29.5%) compared to healthy participants (45.4%).[27] Accordingly, the low prevalence of toxoplasmosis in MS patients may represent a protective effect of this infection on MS. As mentioned in the literature review, the neuroprotective property of toxoplasmosis can be related to the indirect effect of infection on the microglia and astrocytes and the secretion of transforming growth factor beta (TGF-β), PGE2, and IL10. Beside, other protective factors may include the reducing of IL-2 due to the activation of the forkhead box P3 (FoxP3) T cells as an immune-suppressive element, and suppressing of the inflammatory molecules including inducible actin filament (F-actin) depolymerization, nitric oxide synthase (iNOS) and nuclear factor-kappa B (NF-jB) through inducing of TGF-β functions.[20-21,28] Contrary to this study, other studies demonstrated a higher seroprevalence of *T. gondii* antibody in MS patients than in healthy controls; and therefore these studies suggested that *T. gondii* is a possible risk factor for MS development.[10,12] But a study conducted in Kurdistan, Iran rejected any association between *T. gondii* infection and MS.[29] Furthermore, a meta-analysis of five studies about the role of *T. gondii* in MS revealed a lower seroprevalence of *T. gondii* in the MS patients compared to the healthy group; but no significant correlation was found between toxoplasmosis and MS.[24] It is unclear why there were dissimilarities and controversy in the relationship between MS and seroprevalence of toxoplasmosis in the mentioned studies. This is presumably due to the discrepancy in study population and prevalence of toxoplasmosis in the general papulation in certain regions. In the current study, there was no significant relationship between the residence place and contact with cats with the presence of anti-*T. gondii*. The findings of this study are similar to those of the study by Koskderelioglua *et al*. and contrary to the results of Stascheit *et al*.[9,11] In our study, similar to some previous studies, almost two thirds of the participants lived in urban areas. Hence, it could be hypothesized that the prevalence of toxoplasmosis depends on individuals' lifestyle and hygiene habits rather than place of residence. In this study, we investigated the relationship between seroprevalence of toxoplasmosis and MS. Our findings showed that there was a significant difference in age and gender between seropositive

and seronegative MS patients. Older age and male gender had higher seropositivity rates among MS patients. However, previous studies found no significant difference between age and gender with serological status of MS patients.[9,11] One study in Germany reported that older age and male gender are independent risk factors for seropositivity of toxoplasmosis.[30] In terms of disease duration, we demonstrated that seropositive MS patients have a lower disease duration than seronegative MS patients; this is contrary to the results of other studies which reported no significant difference in MS duration with serum anti *T. gondii* antibody.[9,11] Similar to the study by Stascheit *et al*., we found no evidence for the effect of *T. gondii* in ARR and EDSS scores with seroprevalence status of MS patients.[9] But Koskderelioglu *et al*. reported that ARR and EDSS scores were significantly lower in seropositive MS patients than seronegative patients.[11] Regarding the relationship between the seroprevalence of *T. gondii* and the kind of disease-modifying therapies (DMTs) received, similar to the study by Stascheit *et al*., no correlation was observed among MS patients.[9] In general, considering the limited and debated studies on the effect of toxoplasmosis in the pathogenesis of MS, more extensive studies with serological and molecular assay are needed.

In conclusion, the findings of this research indicate that the seroprevalence of *T. gondii* in MS patients is lower than the healthy group. These results demonstrate a negative association between infection with *T. gondii* and MS and toxoplasmosis can be considered as a possible protective factor for the development of MS. On the other hand, this study found no evidence to correlate EDSS, kind of DMTs received, and different types of disease with anti- *T. gondii* antibody among MS patients. In the future, further research needs to explore more closely the correlation between toxoplasmosis and MS patients' characteristics.

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DISCLOSURE

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