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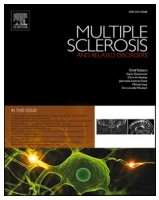

Original article

Toxoplasma gondii and multiple sclerosis: a population-based case-control seroprevalence study, Central Anatolia, Turkey

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ABSTRACT

Background: Toxoplasma gondii, an obligate intracellular parasite, is prevalent in various mammalian species, as well as certain avian, reptilian, and cold-blooded organisms. While immunocompetent individuals generally remain asymptomatic, immunocompromised individuals may experience severe and life-threatening conditions. Multiple sclerosis (MS), a chronic autoimmune disease affecting the central nervous system (CNS), is characterized by inflammation, demyelination, and axonal damage. Despite extensive research, the etiology and pathogenesis of MS remain incompletely understood. Given the strong affinity of *T. gondii* for the CNS, researchers have explored the potential association between *T. gondii* and autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes, and MS. This study aimed to investigate the possible relationship between MS and *T. gondii*.

Methods: A population-based incident cohort of MS patients in Sivas, Turkey, was used to randomly select MS patients. Age- and sex-matched controls were also randomly selected from the general population. A total of 182 MS patients and 182 controls were included in the study. Clinical and socio-demographic variables were recorded using a structured questionnaire. Blood samples were collected from MS patients, and *Toxoplasma* IgG and IgM antibodies were measured using the enzyme-linked immunosorbent assay technique.

Results: Anti-*Toxoplasma* IgG antibodies were detected in 78 cases (42.9%) and 73 controls (40.1%) ($p>0.05$). Age, female sex, and consumption of raw meat were identified as risk factors for toxoplasmosis in both MS patients and controls.

Conclusion: In contrast to previous studies, this study did not find a significant difference in *T. gondii* seropositivity between the control group and MS patients. Further investigations are recommended to elucidate the precise relationship between MS patients and *T. gondii*.

1. Introduction

Toxoplasma gondii (*T. gondii*) is a medically significant parasite due to its wide range of host species it can infect. In humans, infection with *T. gondii* is referred to as toxoplasmosis. The clinical manifestations of toxoplasmosis vary depending on the timing of infection transmission and the immune status of the individual. These manifestations include acute infection, congenital infection, ocular toxoplasmosis, latent infection, and reactivation (Lourido, 2019; Hajj et al., 2021). In the life cycle of *T. gondii*, all mammals and poultry, including humans, serve as intermediate hosts, while cats act as both intermediate and definitive hosts. The parasite exists in three infective forms: tachyzoite, bradyzoite, and sporozoite, which undergo transformation at different stages of their life cycle (Al-Malki, 2021; Attias, 2020; Tong et al., 2021). *T. gondii* has been identified on all continents except Antarctica, and it is estimated that approximately 30% of the global population is infected with the parasite (Aguirre et al., 2019; Skariah et al., 2010). A review study conducted by Molan et al. (2019) reported a worldwide anti-*T. gondii* seropositivity rate of 25.7%.

Human infection can occur through the consumption of raw or undercooked meat containing *T. gondii* tissue cysts, ingestion of water and food contaminated with oocyst-contaminated cat and feline feces, and vertical transmission from an infected mother to her fetus. Transmission can also occur through organ transplantation and blood transfusion from an infected donor to a recipient (Al-Malki, 2021; Lourido, 2019; Tong et al., 2021).

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Multiple sclerosis (MS) is a chronic autoimmune disease affecting the central nervous system (CNS) and is characterized by inflammation, demyelination, and axonal damage. The etiology and pathogenesis of MS are currently unknown, although it is considered a multifactorial disease influenced by complex interactions between genetic and environmental factors (infections such as the Epstein-Barr virus, vitamin D deficiency, smoking, cultural factors, and dietary habits) (Ascherio and Munger, 2016; Correale and Gaitán, 2015; Olsson et al., 2017). The global prevalence of MS ranges from 0.67 to 288 per 100,000 individuals, with an annual incidence ranging from 1.4 to 12.2 per 100,000 people. Over 50% of individuals with MS reside in Europe, while the lowest prevalence is observed in black South Africans (Ascherio and Munger, 2016; Gokce et al., 2019; Howard et al., 2016).

Recent years have witnessed the demonstration of the involvement of certain viruses and bacteria in the pathogenesis of autoimmune diseases (Kivity et al., 2009; Smatti et al., 2019). Numerous studies have reported an increased risk of autoimmune diseases in developed countries, which may be associated with the "hygiene hypothesis" (Fleming and Cook, 2006; Zandman-Goddard and Shoenfeld, 2009). The role of parasites, particularly helminths, in autoimmune diseases has been established through research (Versini et al., 2015). Although some studies have investigated the potential relationship between *T. gondii*, a parasite, and autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (Fleming and Cook, 2006; Krause et al., 2009; Fischer et al., 2013), limited research has examined the possible association between *T. gondii* seropositivity and MS, and the findings from these studies are contradictory (Saberi et al., 2018; Shapira et al., 2012; Nicoletti et al., 2020). Most studies have reported a negative correlation between *T. gondii* and MS (Cicero et al., 2021; Köşkderelioğlu et al., 2017; Méndez-Hernández et al., 2020; Oruç et al., 2016; Pestehchian et al., 2014; Shapira et al., 2012; Stascheit et al., 2015).

The aim of this study is to investigate the potential relationship between *T. gondii* seropositivity and MS in patients from Sivas, a region in Central Anatolia where MS is prevalent.

2. Materials and methods

2.1. Study population and ethical considerations

The study population consisted of 700 individuals diagnosed with MS who were being followed up at the Neurology Outpatient Clinic of Sivas Cumhuriyet University Health Services Application and Research Hospital. Inclusion criteria for patient selection were as follows: first diagnosis of MS, no treatment after diagnosis or treatment with interferon beta, glatiramer acetate, teriflunamide or dimethyl fumarate. No patient was received immunosuppressive therapy at any time. The sample size was determined to be 182 individuals randomly selected from the study population. The control group consisted of 182 individuals matched for age and gender to the MS group (Naing et. al., 2006). Exclusion criteria included speech and language impairments, psychiatric diagnoses and ongoing treatment. Individuals with no history of migration were also excluded from the study.

Ethical approval for this study was obtained from the Clinical Research Ethics Committee of Sivas Cumhuriyet University (approval number: 2017-01/02).

2.2. Measurement of anti-T. gondii IgG and IgM antibodies

II-A. Blood sample collection: A nurse collected 5 mL of whole blood from each participant, which was then centrifuged to obtain serum. Serum samples were stored at -20°C until further analysis.

II-B. Laboratory methods: Detection of anti-*T. gondii* IgG and IgM antibodies in serum samples was performed using a commercially available *Toxoplasma* IgG enzyme-linked immunosorbent assay kit (GenWay Biotech, NovaLisa, San Diego, USA). The assay was performed according to the manufacturer's instructions.

2.3. Questionnaire administration

A standardised, face-to-face, semi-structured questionnaire was administered to both MS patients and control participants who agreed to participate in the study. The questionnaire was designed to assess their socio-demographic characteristics and to identify potential risk factors.

2.4. Statistical analysis

The data collected were entered into SPSS (Statistical Package for the Social Sciences, version 22.0 for Windows) software. Descriptive statistics, including percentiles and means, were used to evaluate the data. Chi-square tests were also used, with a significance level of 0.05.

3. Results

The initial sample size for the case-control study was 182 MS cases and 182 controls.The clinical and serolological features of MS patients are presented in Table 1. Seropositivity for anti-*T. gondii* IgG antibodies was observed in 78 MS patients, giving a seroprevalence rate of 42.9%, and in 73 (40.1%) controls (p-value = 0.59). No individuals in either group tested positive for anti-*T. gondii* IgM antibodies (Table 2).

The mean age of the MS patients was 37.14±9.96 years, and 139 (76.4%) were female. The mean age of the control group was 36.07 ±9.60 years, with 145 (79.7%) being female. The female-to-male ratio of *T. gondii* IgG seropositivity was 4.57 in the MS patient group and 3.29 in the control group. However, there was no statistically significant difference in *T. gondii* IgG seropositivity between the sexes ($p>0.05$). Although a higher prevalence of *T. gondii* IgG seropositivity was observed in women compared to men, the difference was not statistically significant ($p>0.05$). In addition, although an increase in *T. gondii* IgG seropositivity with age was observed in both the MS and control groups, no significant difference was found ($p>0.05$) (Table 2).

No statistically significant correlation was observed between educational status, place of residence, consumption of raw meat and *Toxoplasma* IgG seropositivity in MS patients ($p>0.05$). However, *T. gondii* IgG seropositivity was significantly associated with MS patients

Table 1
The clinical characteristics of Multiple Sclerosis patients. n, number

	n (%)	Anti- *T. gondii* IgG seropositivity- n (%)	X^2/ p
Age, y (mean ± SD) 44.7 ± 11.0	37.14 ±9.96	25 (41.0)	-
Sex (women), n (%) 86 (66.7)	**139 (76.4)**	64 (46.0)	-
Disease duration (year)			
≥1	43 (23.6)	13 (16.7)	0.08
1-5	75 (41.2)	31 (39.7)	
6-10	38 (20.9)	21 (26.9)	
11-15	14 (7.7)	5 (6.4)	
16≤	12 (6.6)	8 (10.3)	
Disease type			
Relapsing-Remitting	128 (70.3)	57 (73.1)	0.19
Secondary Progressive	33 (18.1)	15 (19.2)	
Primary Progressive	10 (5.5)	1 (1.3)	
Progressive Relapsing	11 (6.0)	5 (6.4)	
Early signs of MS			
Vision problems	86 (47.3)	35 (40.7)	0.11
Balance problems or dizziness and headache	36 (20.3)	12 (75.0)	
Tingling and numbness in arms and legs.	48 (26.3)	28 (58.3)	
Tingling and numbness in face.	12 (6.6)	3 (25.0)	

Table 2
Sociodemographic and serologic data in multiple sclerosis (MS) patients and controls and results of potential risk factors for seropositivity.

	Multiple Sclerosis patients		Controls		x^2/p
	n (%)	Anti- *T. gondii* Ig-G seropositivity- n (%)	n (%)	Anti- *T. gondii* Ig-G seropositivity- n (%)	
	182	78 (42.9)	182	73 (40.1)	0.59
Sex					
Female	139 (76.4)	64 (46.0)	145 (79.7)	56 (38.6)	0.41
Male	43 (23.6)	14 (32.6)	37 (20.3)	17 (45.9)	
Age					
19-30	60 (33,0)	19 (31,7)	65 (35,7)	23 (35,4)	0.17
31-40	61 (33,5)	25 (41.0)	73 (40,1)	30 (41.1)	
41-50	43 (23,6)	21 (48.8)	28 (15,4)	10 (35.7)	
51-68	15 (8,2)	13 (72,2)	11 (6,0)	10 (62.5)	
mean ± SD	37.14 ±9.96		36.07 ±9.60		
Marital status					
Married	133 (73.1)	76 (50.4)	150 (82.4)	61 (40.7)	0.04
Single	49 (26.9)	11 (22.4)	32 (17.6)	12 (37.5)	
Profession					
Professional/ Office job	40 (22.0)	17 (42.5)	52 (28.6)	7 (2.7)	0.01
Industry	16 (8.8)	9 (56.3)	31 (17.0)	3 (1.9)	
Housewife	91 (50.0)	46 (50.5)	78 (44.5)	32 (41.0)	
Retired	8 (4.4)	1 (12.5)	8 (4.4)	4 (50.0)	
Student	11 (6.0)	1 (9.1)	11 (6.0)	5 (45.4)1	
Other	16 (8.8)	4 (25.0)	5 (2.7)	2 (40.0)	
Education					
Primary school	63 (34.6)	35 (55.6)	38 (20.9)	13 (34.2)	0.06
Secondary school	27 (14. ()	13 (48.1)	30 (16.5)	15 (50.0)	
High school	41 (22.5)	10 (24.4)	52 (28.6)	18 (34.6)	
University	43 (23.6)	18 (41.9)	54 (29.7)	24 4(4.4)	
Master/ Doctorate	8 (4.4)	2 (25.0)	8 (4.4)	3 (37.5)	
Living place					
City	145 (79.7)	65 (44.8)	155 (85.2)	61 (39.4)	0.09
Town	26 (14.3)	8 (30.8)	16 (8.8)	9 (56.3)	
Rural	11 (6.0)	5 (45.5)	11 (6.0)	3 (27.3)	
Socio-economic status					
Low	19 (10.4)	9 (11.5)	35 (18.2)	22 (30.13)	0.01
Middle	157 (86.3)	69 (88.4)	134 (73.6)	46 (63.01)	
Hight	6 (3.3)	0 (0)	13 (7.1)	5 (6.84)	
Eating raw meat					
Yes	161 (88.5)	72 (44.7)	152 (83.5)	60 (39.5)	0.17
No	17 (11.5)	6 (28.6)	30 (16.5)	13 (43.3)	
Frequency of eating raw meat					
Once a week	24 (14.9)	6 (25.0)	24 (15.8)	9 (37.5)	0.06
Once a month	73 (45.3)	38 (52.1)	49 (32.2)	17 (34.7)	
Once every six months	64 (39.8)	28 (43.8)	79 (52.0)	34 (43.0)	
Pet ownership					
Yes	71 (39.0)	39 (54.9)	63 (34.6)	27 (42.9)	0.38
No	111 (61.0)	39 (35.1)	119 (65.4)	46 (38.7)	
Cat ownership	31 (43.7)	17 (54.8)	36 (57.1)	12 (33.3)	0.20
Dog ownership	8 (11.3)	3 (37.5)	8 (12.7)	6 (75.0)	
Bird ownership	32 (45.1)	19 (59.4)	19 (30.2)	9 (47.4)	
Chronic disease					
Yes	28 (15.4)	17 (60.7)	21 (11.5)	12 (57.1)	0.28
No	154 (84.6)	61 (39.6)	161 (88.5)	61 (37.9)	
Chronic diseases					
Diabetes mellutus	6 (21.4)	3 (50)	6 (27.3)	5 (83.3)	0.28
Hypertension	6 (21.4)	5 (83.3)	3 (13.6)	2 (66.7)	
Heart disease	5 (17.9)	4 (80)	1 (4.5)	1 (100)	
Thyroid disease	6 (21.4)	3 (50)	7 (31.8)	4 (57.1)	
Vertigo	5 (17.9)	2 (40)	5 (22.7)	0 (0)	

* $p<0.05$ is statistically significant.

who were housewives, workers, had low income, were aged 51-60 years, lived in rural areas, owned cats and were married ($P<0.05$) (Table 2).

In addition, no statistically significant association was found between MS type, initial symptoms and anti-*Toxoplasma* IgG seropositivity in MS patients ($p>0.05$). Among MS patients with chronic disease, 60.7% (n=17) were positive for *Toxoplasma* IgG antibodies, while in the control group 57.1% (n=12) of patients with chronic disease tested positive. Anti-*Toxoplasma* IgG antibodies were significantly higher in MS patients with coexisting chronic diseases than in those with MS alone ($p<0.05$). When evaluating the disease duration of MS patients, a higher rate of anti-*T. gondii* IgG antibodies was found in patients aged 1-5 years since MS diagnosis ($p<0.05$) (Table 1).

4. Discussion

MS is a chronic autoimmune disease that affects the immune system. The "hygiene hypothesis", proposed by Strachan in 1989, suggests that microorganisms play a crucial role in the regulation of the immune system (Strachan, 1989). Lack of exposure to infectious agents in populations has been associated with an increased autoimmune and allergic background, and forms the basis of the hygiene hypothesis. In 1966, Leibowitz et al. first suggested an association between the increasing prevalence of MS and high levels of childhood hygiene (Leibowitz et al., 1966). According to the hygiene hypothesis, parasitic infections also play an important role in the development of autoimmune diseases. Helminths induce a Th2 immune response, while protozoa induce the production of pro-inflammatory mediators such as IL-12, IFN-γ and nitric oxide, leading to a Th1 response (Munoz et al., 2011). Studies have shown the impact of helminth infections on autoimmune diseases (Ben-Ami Shor et al., 2013; Versini et al., 2015), as helminths can

modulate the course of MS by promoting the development of regulatory cells that produce IL-10 and TGF-β, inhibiting T-cell proliferation and suppressing IFN-γ production (Fleming and Cook, 2006; Jackson et al., 2009).

In recent years, there has been growing interest in investigating the relationship between helminths and the obligate intracellular parasite *T. gondii* and autoimmune disease. Interestingly, *T. gondii*, a protozoan parasite, has shown anti-inflammatory properties similar to those of helminths (Stumhofer et al, 2006), which may be relevant in the context of MS. A study investigating anti-*Toxoplasma* antibodies in the sera of 1514 patients with various autoimmune diseases and 437 geographically matched controls in Europe and Latin America reported a seropositivity rate of 42% in individuals with autoimmune diseases compared with 29% in the control group (Shapira et al., 2012). The researchers suggested that *T. gondii* may indirectly influence the pathogenesis of several autoimmune diseases. However, they found anti--*Toxoplasma* seropositivity in 30% of MS patients, which was not statistically significant (Shapira et al., 2012). In a cross-sectional study by Hezarjaribi et al. evaluating 272 patients with rheumatoid arthritis and 33 with systemic lupus erythematosus, anti-*Toxoplasma* IgG antibodies were detected in 38.6% of patients with rheumatoid arthritis and 33% of patients with systemic lupus erythematosus (Herzerjaribi et al., 2021).

The relationship between MS and *T. gondii* infection remains poorly understood, and existing studies have yielded conflicting results. Therefore, in this case-control seroprevalence study, we aimed to investigate the association between MS and *T. gondii* seropositivity in a sample of age- and sex-matched individuals from Sivas, Turkey. Our results showed a higher seroprevalence of *T. gondii* infection in patients with multiple sclerosis compared with their age- and sex-matched controls, although the difference was not statistically significant.

A limited number of studies have been conducted to evaluate the potential association between *T. gondii* seropositivity and MS (Cicero et al., 2021; Köşkderelioğlu et al., 2017; Méndez-Hernández et al., 2020; Oruç et al., 2016; Pestehchian et al., 2014; Shapira et al., 2012; Stascheit et al., 2015). Some studies investigating the relationship between *T. gondii* and MS have suggested a potential protective role of *T. gondii* infection based on the hygiene hypothesis. For instance, Stascheit et al. found *T. gondii* seropositivity in 33.3% of MS patients and 47.9% of individuals in the control group, suggesting a negative association between *T. gondii* infection and MS development, thereby proposing *T. gondii* infection as a potential protective factor against MS (Stascheit et al., 2015). Similarly, Köşkderelioğlu et al. reported *T. gondii* seropositivity in 33.9% of MS patients and 55% of the control group, supporting a negative relationship between *T. gondii* infection and the presence of MS (Köskderelioglu et al., 2017). In a meta-analysis by Cicero et al., *T. gondii* infection was suggested to exhibit protective properties against the development of MS (Cicero et al., 2021). Additionally, Nicoletti et al. found evidence of a negative association between *T. gondii* and MS, further supporting the hygiene hypothesis (Nicoletti et al., 2020). In contrast to these findings, Oruç et al. observed anti-*T. gondii* IgG positivity in 44.2% of MS patients and 24.4% of the control group, proposing *T. gondii* infection as one of the environmental risk factors for MS (Oruç et al., 2016).

Saberi et al. investigated the potential role of *T. gondii* in MS and found no significant relationship between toxoplasmosis and MS disease (Saberi et al., 2018). Consistent with our study results, although the seropositivity was higher in MS patients (42.9%) compared to the control group (40.1%), no statistically significant difference was found between MS disease and *T. gondii*. While most studies reported a negative correlation between *T. gondii* and MS, suggesting a protective effect of *T. gondii* against MS, our evaluation did not find any correlation. The findings obtained in Sivas, where the prevalence of MS is high, may present controversial implications in relation to existing hypotheses.

Considering the data from various studies on this subject, larger sample groups should be investigated to determine the detailed relationship between MS patients and *T. gondii* infection. Furthermore, experimental studies utilizing animal models of MS are warranted to elucidate the underlying pathophysiological mechanisms of *T. gondii* infection.

CRediT authorship contribution statement

Gülgün Sevimligul: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Zubeyda Akın Polat:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Seyda Figul Gokce:** Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare that there is no conflict of interest and competing interests regarding the publication of this article.

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