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ORIGINAL RESEARCH PAPER

Toxoplasma gondii infection and multiple sclerosis: An age- and a gender-matched case-control seroprevalence study

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ABSTRACT

The link between *Toxoplasma gondii* infection and multiple sclerosis remains controversial. In the present study, we aimed to determine the association between *T. gondii* seropositivity and multiple sclerosis. Using an age- and gender-matched case-control study, we studied 45 patients who had multiple sclerosis attended in two public hospitals and 225 control subjects without this disease and other neurological disorders in Durango City, Mexico. Serum samples of cases and controls were analyzed for detection of anti-*Toxoplasma* IgG using a commercially available enzyme-linked immunoassay. One (2.22%) of the 45 patients with multiple sclerosis, and 15 (6.67%) of the 225 control subjects without this disease were seropositive for anti-*T. gondii* IgG antibodies. No statistically significant difference (OR = 0.31; 95% CI: 0.04–2.47; $P = 0.48$) in seroprevalence of anti-*T. gondii* IgG antibodies between cases and controls was found. The frequency of *T. gondii* seropositivity did not vary among cases and controls about sex or age groups. Results of this study do not support an association between seropositivity to *T. gondii* and multiple sclerosis. However, additional research with larger sample sizes to confirm this lack of association should be conducted.

KEYWORDS

Toxoplasma gondii, infection, seroprevalence, multiple sclerosis, epidemiology, case-control study, Mexico

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INTRODUCTION

Toxoplasma gondii (*T. gondii*) is an intracellular coccidian of the phylum Apicomplexa [1]. Chronic infections with this parasite occur in approximately 30% of the human population worldwide [2]. Humans usually acquire *T. gondii* infection from animals: ingestion of oocysts

shed by cats, and cysts in tissues of animals [2, 3]. Transmission of *T. gondii* may also occur by blood transfusion [4, 5], and organ transplantation [6]. Primary infection with *T. gondii* during pregnancy may lead to infection of the fetus [7]. Most infections with *T. gondii* are asymptomatic [3]. However, infections in immunocompromised patients may cause devastating effects, including neurological and ocular manifestations [7]. Dissemination of *T. gondii* occurs widely within the host's body and can infect the brain [8]. Infection with *T. gondii* in the brain may cause psychiatric diseases, i.e., depression [9], mixed anxiety and depression disorder [9, 10], and schizophrenia [11]. During infection, *T. gondii* induces numerous changes to host neurons and globally alters host neurological signaling pathways [12]. Chronic infections with *T. gondii* may induce changes in neuronal connectivity and synaptic plasticity [13]. Infections with *T. gondii* have also been linked to epilepsy [14]. Whether infection with *T. gondii* is associated with multiple sclerosis is controversial. A negative association between this infection and multiple sclerosis was found in two studies in Turkey [15] and Germany [16]. However, a meta-analysis that assessed this association showed no significant association [17]. In another study, 50 patients with multiple sclerosis and 50 family members in Iran were tested for *T. gondii* serology, and both groups had similar frequencies of anti-*T. gondii* IgG antibodies [18]. In the present study, we aimed to determine the association of *T. gondii* infection and multiple sclerosis in Durango City, Mexico.

MATERIALS AND METHODS

Study design and subjects studied

An age- and gender-matched case-control survey of 45 patients who had multiple sclerosis attended in two public hospitals in Durango City, Mexico and 225 subjects without multiple sclerosis from the general population of the same city was performed.

Inclusion criteria for enrollment of patients were: 1) patients suffering from multiple sclerosis attended in two public hospitals: The General Hospital "450" of the Secretary of Health and the Hospital "Dr. Santiago Ramón y Cajal" of the Institute of Security and Social Services for the State Workers in Durango City; 2) aged 15 years and older; and 3) who voluntarily accepted to participate in the study. Diagnosis of multiple sclerosis was based on the 2010 McDonald criteria [19]. Of the 45 cases, 30 (66.7%) were females, and 15 (33.3%) were males. Cases were 15–73 years old (mean age: 40.76 ± 13.09. Subjects without multiple sclerosis (controls) were randomly selected from the general population of Durango City and matched with cases for age and gender. Of the 225 controls, 150 (66.7%) were females and 75 (33.3%) were males. Controls were 14–73 years old (mean age: 40.64 ± 13.48). Age in cases was similar to that in controls ($P = 0.95$). Cases were enrolled consecutively from January 2014 to June 2016.

A laboratory test for the detection of anti-*T. gondii* IgG antibodies

Blood samples form cases and controls were obtained and centrifuged. Serum samples were obtained and frozen at −20 °C until analyzed. Serum samples were analyzed for detection of anti-*T. gondii* IgG antibodies using the commercially available enzyme immunoassay kit "*Toxoplasma* IgG" (Diagnostic Automation/Cortez Diagnostics Inc., Woodland Hills, CA, USA). This test was performed following the instruction of the manufacturer.

Statistical analysis

The statistical analysis was performed with the software Microsoft Excel 2016, and Epi Info version 7. For calculation of the sample size, we used a two-sided confidence level of 95%, a power of 80%, a 1:5 proportion of cases and controls, a reference seroprevalence of 6.1% [20] as the expected frequency of exposure in controls, and an odds ratio of 4.0. The result of the sample size calculation was 43 cases and 215 controls. We compared the age among cases and controls with the student's *t*-test. The association between multiple sclerosis and *T. gondii* infection was assessed with the Fisher exact test. Odds ratio (OR) and 95% confidence interval (CI) were calculated. A *P* value less than 0.05 was considered as statistically significant.

Ethical aspects

The Ethics Committees of the General Hospital of the Secretary of Health and the Institute of Security and Social Services for the State Workers approved this project. Aims and procedures of the study were explained to all participants. Also, a written informed consent was obtained from each participant. This study was conducted in compliance with the ethical standards of the responsible institution on human subjects as well as with the Helsinki Declaration.

RESULTS

Of the 45 patients with multiple sclerosis, one (2.22%) was seropositive for anti-*T. gondii* IgG antibodies. Whereas of the 225 subjects without this disease, 15 (6.67%) were seropositive for anti-*T. gondii* IgG antibodies. No statistically significant difference (OR = 0.31; 95% CI: 0.04–2.47; $P = 0.48$) in seroprevalence of anti-*T. gondii* IgG antibodies between cases and controls was found. The frequency of *T. gondii* infection was correlated with a stratification by sex and age of cases and controls (Table 1). The frequency of *T. gondii* seropositivity in male cases was equal to that in male controls ($P = 1.0$), and in female cases to that in female controls ($P = 0.21$). The frequency of *T. gondii* seropositivity did not vary among cases and controls about age groups.

Table 1. Stratification by sex and age in cases and controls for seropositivity to *T. gondii*

Characteristics	Cases			Controls			*P* value
	Seroprevalence of *T. gondii* infection			Seroprevalence of *T. gondii* infection			
	No. tested	No.	%	No. tested	No.	%	
Sex							
Male	15	1	6.7	75	5	6.7	1.00
Female	30	0	0.0	150	10	6.7	0.21
Age (years old)							
30 or less	12	1	8.3	70	4	5.7	0.55
31–50	24	0	0.0	92	3	3.3	1.00
>50	9	0	0.0	63	8	13.0	0.6

DISCUSSION

Very little is known about the association between multiple sclerosis and infection with *T. gondii*. Only a few studies on this topic have been reported, and the results are conflicting. Therefore, in this age- and gender-matched case-control seroprevalence study we sought to determine the association between multiple sclerosis and seropositivity to *T. gondii* in a sample of people in Durango City, Mexico. In the present study we found a lower (but not statistically significant) seroprevalence of *T. gondii* infection in patients who had multiple sclerosis than their age- and gender-matched controls. Intriguingly, previous studies have also found a lower seroprevalence of *T. gondii* infection in patients with multiple sclerosis than in their controls. However, some of such studies have found statistically significant differences in seroprevalences among groups whereas other did not. In a German study, sera of 163 patients with multiple sclerosis and 178 age- and gender-matched healthy subjects were analyzed for anti-*T. gondii* IgG antibody and patients had a significantly lower seropositivity rate than controls [16]. In another study, 115 patients with multiple sclerosis and 60 age- and gender-matched healthy subjects in Turkey were compared as to the presence of specific IgG antibodies against *T. gondii*, and researchers found a significantly lower seroprevalence of *T. gondii* infection in patients than in their controls [15]. It is not clear why we did not see a negative association between multiple sclerosis and *T. gondii* infection as previously reported in the German and Turkish studies. However, we studied a small sample of patients with multiple sclerosis, and this factor might have contributed to the lack of negative association found in our study. In an Iranian work, researchers studied 50 patients with multiple sclerosis and 50 healthy family members and found a lower (but not statistically significant) seroprevalence of anti-*Toxoplasma* IgG antibodies in patients than in family members [18]. In a recent meta-analysis including 669 patients who had multiple sclerosis and 770 controls, the seroprevalence of *T. gondii* infection was lower in patients than in controls, but the difference was not statistically significant [17].

The present study has the limitations of a small sample size of patients with multiple sclerosis and the small number of participating health institutions. Further studies with larger samples sizes and performed in more than two health institutions should be conducted. We were able to recruit 45 patients with multiple sclerosis attending two public health institutions in a period of two and a half years. The number of new cases of multiple sclerosis attending health institutions in Durango City is low. The biggest health institution in this city reported only 5 new cases in a period of about 6 months (https://www.elsiglodedurango.com.mx/noticia/967637.reporta-imss-cinco-casos-de-esclerosis-multiple-en-2018.html). Therefore, to obtain a substantial increase in the sample size of patients with multiple sclerosis in the city would require several years. Nevertheless, we increased the proportion of controls (5 controls for every case) to compensate the low number of cases. The present study was performed in a low *T. gondii* seroprevalence population and this condition may help to identify only clear associations when recruiting small sample sizes of cases, as we found in previous studies of 65 patients with mixed anxiety and depressive disorder [10], and 50 schizophrenic patients [21]. Previous studies [15, 16, 18] on the association between *T. gondii* infection and multiple sclerosis have also recruited small number (50–163) of patients. However, results of the present study may help to increase the number of cases in a meta-analysis for identification of an association between *T. gondii* infection and multiple sclerosis.

CONCLUSIONS

Results of this study do not support an association between seropositivity to *T. gondii* and multiple sclerosis. However, additional research with larger sample sizes to confirm this lack of association should be conducted.

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Author's contributions: EMMH, JHT, JMSP, LFSA, AASC, and FXCJ obtained the clinical data and blood samples of participants. OAC reviewed the data analysis and the manuscript. LARC obtained the clinical data and performed the clinical diagnosis in the participants. CAE performed the laboratory tests, the data analysis, the statistical analysis and wrote the manuscript.

Conflict of interest: The authors declare no conflict of interest.

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