

Toxoplasma gondii Infection and Headache: A Matched Case-Control Study in a Public Hospital in Durango City, Mexico

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Abstract

Background: *Toxoplasma gondii* (*T. gondii*) can disseminate to brain in infected hosts. Little is known about the magnitude of the association between this infection and headache. Therefore, we sought to determine the association of *T. gondii* seropositivity and headache in patients attending neurological consultations in a public hospital in Durango City, Mexico.

Methods: Through an age- and gender-matched case-control study, 105 patients suffering from headache and 105 subjects without headache were examined for anti-*T. gondii* IgG and IgM antibodies using commercially available enzyme-linked immunoassays. Seropositive cases were analyzed for detection of *T. gondii* DNA by polymerase chain reaction.

Results: Anti-*T. gondii* IgG antibodies were found in five (4.8%) of the 105 cases and in seven (6.7%) of the 105 controls (odds ratio (OR) = 0.70; 95% confidence interval (CI): 0.21 - 2.28; P = 0.76). The frequency of high (> 150 IU/mL) levels of anti-*T. gondii* IgG antibodies among anti-*T. gondii* IgG positive individuals was significantly (P = 0.01) higher in cases (5/5) than in controls (1/7). Anti-*T. gondii* IgM antibodies were found in one (20.0%) of the five IgG seropositive cases, and in three (42.9%) of the seven IgG seropositive controls (P = 0.60). *T. gondii* DNA was not detected in any of the five anti-*T. gondii* IgG positive cases. No association between *T. gondii* infection and specific headache types was found.

Conclusions: This is the first matched case-control study on the association between *T. gondii* infection and headache. Results suggest that high anti-*T. gondii* IgG antibody levels, but not *T. gondii* seropositivity, were associated with headache in the population studied.

Keywords: *Toxoplasma gondii*; Infection; Seroprevalence; Headache; Case-control study; Epidemiology; Mexico

Introduction

Toxoplasma gondii (*T. gondii*) is one of the most successful parasites in the world due to its ability to infect and persist in most warm-blooded animals [1]. About 30% of the human population worldwide is chronically infected with *T. gondii* [2]. Transmission of *T. gondii* may occur horizontally by ingestion of oocysts from the environment or ingestion of tissue cysts contained in meat from many different animals, vertically during pregnancy, by blood transfusion, and organ transplantation [3]. Infections in humans are commonly asymptomatic [2, 4]. When symptoms are present, they typically resemble a mononucleosis or flulike illness [5]. Infection with *T. gondii* may also lead to chorioretinitis [6]. Reactivation of a latent infection in immunodeficiency conditions can cause fatal toxoplasmic encephalitis [7]. When primary infection of the mother occurs during pregnancy, there is likelihood of a transplacental infection that can lead to congenital toxoplasmosis with affection of brain and retina [8].

T. gondii can persist in the central nervous system of a variety of hosts including humans [1]. The parasites persist as intraneuronal cysts that are controlled, but not eliminated by the immune system of the host [9]. Infection with *T. gondii* in brain in immunocompetent individuals may cause headache [10-12], confusion [10], and difficulty concentrating [12]. Headache of the migraine type has been linked to *T. gondii* infection [13]. In a cohort of 261 postnatal cases of acute toxoplasmosis in Brazil, headache was present in 10.7% of patients [14]. Very little is known about the frequency of *T. gondii* infection in patients suffering from headache. In a study of 108 cases of new daily persistent headaches in Dominican Republic, *T. gondii* infection was found in 3.7% of patients [15]. It is unclear how

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Table 1. Diagnoses of Headache and Frequency of *T. gondii* Infection in the Study Population

ICD-10 code	Diagnosis	No. of patients	Seropositivity to <i>T. gondii</i>		P value
			No.	%	
G43.0	Migraine without aura	16	2	12.5	0.74
G43.1	Migraine with aura	17	1	5.88	
G43.3	Complicated migraine	3	0	0	
G43.9	Migraine, unspecified	3	0	0	
G44.0	Cluster headache syndromes	4	0	0	
G44.1	Vascular headache, not elsewhere classified	1	0	0	
G44.2	Tension-type headache	20	1	5	
G44.3	Chronic post-traumatic headache	3	0	0	
G44.8	Other specified headache syndromes	38	1	2.63	

higher the frequency of *T. gondii* infection is in patients with headache as compared to subjects without headache. Therefore, we sought to determine the association between *T. gondii* infection and headache in patients attending at the Department of Neurology in a public hospital in the northern Mexican city of Durango.

Materials and Methods

Study design and study populations

We performed a case-control seroprevalence study of 105 patients suffering from headache (cases) and 105 people without headache from the general population (controls) in Durango City, Mexico. This study was performed from April 2016 to March 2017. Inclusion criteria for enrollment of the cases were: 1) patients suffering from headache attending in the Department of Neurology at the public Hospital "Dr. Santiago Ramon y Cajal" in Durango City; 2) aged 13 years and older; and 3) who voluntarily participate in the study. Only patients with diagnosis of headache made by two neurologists (GQC and JTG) were selected for inclusion in the study. Exclusion criterion was subjects suffering from a severe disease who were unable to decide themselves about their enrollment in the study. Gender, socioeconomic status, and occupation of patients were not restrictive criteria for inclusion in the study. In total, 82 females and 23 males suffering from headache were enrolled in the study. Cases were 13 - 86 years old (mean = 42.71 ± 15.79). Diagnoses of headache in patients were based on the International Statistical Classification of Diseases and related Health Problems version 2016 (ICD-10) (<http://apps.who.int/classifications/icd10/browse/2016/en>). Table 1 shows the types of headache diagnosed in the studied patients. Concerning the control group, 105 subjects without headache were randomly selected from the general population in Durango City. All control subjects were matched with patients by age (± 4 years) and gender. Controls were 15 - 86 years old (mean = 42.65 ± 15.40), and included 82 females and 23 males. There was no difference in age between cases

and controls (P = 0.97).

Detection of *T. gondii* IgG and IgM antibodies

Sera of cases and controls were analyzed for anti-*T. gondii* IgG antibodies with the commercially available enzyme immunoassay kit "Toxoplasma IgG" (Diagnostic Automation Inc., Woodland Hills, CA, USA). This test allows qualitative and quantitative determinations of anti-*T. gondii* IgG antibodies. A cut-off of ≥ 8 IU/mL of specific anti-*T. gondii* IgG antibody was considered for seropositivity. IgG seropositive serum samples of cases and controls were further analyzed for anti-*T. gondii* IgM antibodies by the commercially available enzyme immunoassay "Toxoplasma IgM" kit (Diagnostic Automation Inc.). All IgG and IgM tests were performed following the instructions of the manufacturer. Positive and negative controls included in the kits were analyzed in each run. Criterion for diagnosis of *T. gondii* infection was based on results of the IgG test.

DNA extraction and PCR of *T. gondii*

Cases with anti-*T. gondii* IgG antibodies were further examined for detection of *T. gondii* DNA by nested-polymerase chain reaction (PCR). DNA extraction from whole blood was performed according to the instructions of a commercially available kit (QIAamp DNA Blood Mini kit; Qiagen, Germany). DNA amplification was performed with primers directed against the B1 gene of *T. gondii* and following the protocol described by Burg et al [16]. The PCR products were analyzed by using a 2% agarose gel electrophoresis stained with ethidium bromide. Visualization of the PCR products was performed with an ultraviolet transilluminator.

Statistical analysis

Statistical analyses were carried out using the Microsoft Excel 2010, SPSS version 15.0 (SPSS Inc. Chicago, IL, USA), and

Epi Info version 7 (Centers for Disease Control and Prevention: <http://wwwn.cdc.gov/epiinfo/>) software. For calculation of the sample size, we used a 95% two-sided confidence level, a power of 80%, a 1:1 ratio of cases and controls, and a reference seroprevalence of 7.4% [17] as the expected frequency of exposure in controls. The result of the sample size calculation was 101 cases and 101 controls. The paired Student's *t*-test was used to compare age values among the groups. The association between seroprevalence and characteristics of cases was assessed by bivariate analysis. We calculated the odds ratios (ORs) and 95% confidence intervals (CIs), and statistical significance was set at a P value less than 0.05.

Ethics aspects

The Ethical Committee of the Institute of Security and Social Services for State Workers in Durango City, Mexico approved this project. The purpose and procedures of this case-control study were explained to all participants. A written informed consent was obtained from all subjects and from the next of kin of minor participants.

Results

Anti-*T. gondii* IgG antibodies were found in five (4.8%) of the 105 cases and in seven (6.7%) of the 105 controls. No difference in the seroprevalence of *T. gondii* infection between cases and controls was found (OR = 0.70; 95% CI: 0.21 - 2.28; P = 0.76). All five anti-*T. gondii* IgG positive cases had anti-*T. gondii* IgG antibody levels higher than 150 IU/mL. In contrast, of the seven anti-*T. gondii* IgG positive controls, only one (14.3%) had anti-*T. gondii* IgG antibody levels higher than 150 IU/mL (Table 2). The frequency of high (> 150 IU/mL) levels of anti-*T. gondii* IgG antibodies among anti-*T. gondii* IgG positive individuals was significantly (P = 0.01) higher in cases (5/5) than in controls (1/7). Anti-*T. gondii* IgM antibodies were found in one (20.0%) of the five IgG seropositive cases, and in three (42.9%) of the seven IgG seropositive controls (P = 0.57 by two-tailed Fisher exact test). *T. gondii* DNA was not detected in any of the five anti-*T. gondii* IgG positive cases.

Bivariate analysis of IgG seropositivity to *T. gondii* and ICD-10 codes did not show an association between *T. gondii* infection and specific types of headache (Table 1).

Discussion

The epidemiological impact of *T. gondii* infection on headache has been poorly studied. Some people infected with *T. gondii* suffer from headache [10-12]; however, it is unclear how many people suffering from headache has an infection with *T. gondii*. We are not aware of any age- and gender-matched case-control study on the association between *T. gondii* infection and headaches. Therefore, we decided to carry out this age- and gender-matched case-control study to determine the

Table 2. Anti-*Toxoplasma* IgG Levels in Cases and Controls

Anti- <i>Toxoplasma</i> IgG levels	Cases		Controls		P value
	No.	%	No.	%	
< 100 IU/mL	0	0	4	57.1	0.01
100 - 150 IU/mL	0	0	2	28.6	
> 150 IU/mL	5	100	1	14.3	

association of seropositivity to *T. gondii* and headache in patients attending at the Neurology Department in a public hospital in Durango City, Mexico. The seroprevalence of *T. gondii* infection in patients suffering from headache was low (4.8%), and the seropositivity rate of anti-*T. gondii* IgG and IgM antibodies in patients suffering from headache and in control subjects was similar. The seroprevalence of *T. gondii* infection found in patients with headache is comparable to seroprevalences of *T. gondii* infection reported in the general population (6.1%) [18], and healthy blood donors (7.4%) [16] in the same Durango City. In contrast, the seroprevalence observed in patients suffering from headache is lower than seroprevalences of *T. gondii* infection reported in high risk groups in the same Durango City including waste pickers (21.1%) [19], inmates (21.1%) [20], and psychiatric patients (18.2%) [21]. The low prevalence of *T. gondii* infection found in patients suffering from headache which is similar to or lower than those reported in controls and other population groups of the same city suggests that *T. gondii* infection did not contribute substantially for a higher risk of headache in our population studied. The low frequency of infection with *T. gondii* in patients with headache was unexpected since infection with *T. gondii* is considered a cause of headache in immunocompetent [10-12], and immunocompromised patients [22]. Seroprevalence results suggest that factors other than *T. gondii* infection might contribute for headache in our population studied. No association between *T. gondii* infection and types of headache was found. However, the number of specific types of headache was small. Intriguingly, all five patients with headache and anti-*T. gondii* IgG antibodies had high (> 150 IU/mL) levels of anti-*T. gondii* IgG antibodies. This frequency was significantly higher than that found in control subjects. This finding might suggest a recent infection or a constant high antigenic stimulation. These results indicate an association between high anti-*T. gondii* IgG antibody concentration and headache. Very few studies have reported anti-*T. gondii* IgG concentrations in patients with headache. In a study in Poland, a high anti-*T. gondii* IgG concentration (mean: 189 ± 85 IU/mL) was found in patients with recurrent headache as the main symptom of acquired cerebral toxoplasmosis [11]. In a case report of a patient with systemic erythematosus lupus presenting with fever, headache, and mental change, high anti-*T. gondii* IgG titers in cerebrospinal fluid and in serum were found [23]. In the present study, no testing of anti-*T. gondii* IgM antibodies in subjects seronegative to anti-*T. gondii* IgG antibodies was performed. This was due to the low diagnostic value of an IgM positive test in subjects seronegative to IgG. A high number of false-positive IgM results have been reported using commercially available enzyme immunoassays for detection of anti-*T. gondii* IgM an-

tibodies [24, 25].

The present study has some limitations. A small number of specific types of headache were studied. In addition, patients were enrolled in a single hospital, and most patients had a medium socioeconomic status. Further studies with larger number of patients with specific types of headache, enrolled in several hospitals or health care centers, and of several socioeconomic statuses to determine the association between *T. gondii* infection and headache should be conducted. Additional studies to determine the role of anti-*T. gondii* IgG concentration in headache are needed.

Conclusions

This is the first age- and gender-matched case-control study on the association between *T. gondii* infection and headache. Results suggest that high anti-*T. gondii* IgG antibody levels, but not *T. gondii* seropositivity, were associated with headache in the population studied. Because a small number of specific types of headache were studied, further studies to determine the association between *T. gondii* infection and types of headache are needed.

Competing Interests

The authors declare that they have no competing interests.

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