Evaluation of *Toxoplasma gondii* in the Etiology of Cryptogenic Epilepsy: A Case-control Study

Selahattin Aydemir[1], Milad Torkamanian Afshar[1], Özlem Sarı Torkamanian Afshar[2], Zeynep Taş Cengiz[1], Mustafa Kiraz[3], Caner Baydar[4], Hasan Yılmaz[1]

[1]Van Yüzüncü Yıl University Faculty of Medicine, Department of Parasitology, Van, Turkey
[2]University of Health Sciences Turkey, Van Training and Research Hospital, Clinic of Medical Biochemistry, Van, Turkey
[3]University of Health Sciences Turkey, Van Training and Research Hospital, Clinic of Neurology, Van, Turkey
[4]Van Yüzüncü Yıl University Faculty of Medicine, Department of Neurology, Van, Turkey



Selahattin Aydemir PhD

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Corresponding Author: Selahattin Aydemir PhD, Van Yüzüncü Yıl University Faculty of Medicine, Department of Parasitology, Van, Turkey, E-mail: saydmr23@gmail.com

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Abstract

Objectives: This study was conducted to evaluate the relationship between toxoplasmosis and cryptogenic epilepsy (CE) by comparing the rate of *Toxoplasma gondii* seropositivity identified in CE patients with those without any neurological disorder.

Methods: The study included 200 cryptogenic patients and 164 individuals without neurological disorders. *T. gondii* seropositivity was studied in individuals included in the study using the *T. gondii*-IgG ELISA kit.

Results: The study found *T. gondii* IgG in 15.50% of CE patients and 28.66% of individuals without any neurological disorder. The difference between the two groups was determined to be statistically significant.

Conclusion: Toxoplasmosis did not increase the risk of epilepsy in the region where this study was conducted. However, for a better understanding of the CE-*T. gondii* relationship, we believe that seroprevalence studies should be conducted in larger populations.

Keywords: Cryptogenic epilepsy, dopamine, GABA, neurology, toxoplasmosis

INTRODUCTION

Epilepsy is one of the most common neurological disorders after stroke and Alzheimer's disease, and it has a global prevalence of approximately 1%. The underlying cause of epilepsy is unknown in two-thirds of patients, and this type of epilepsy is called cryptogenic epilepsy (CE).[1] Infectious diseases, especially those associated with the central nervous system (CNS), are assumed to play an important role in epilepsy.[2]

Toxosoplasma gondii (*T. gondii*) is an intracellular zoonotic parasite. The prevalence of this parasite varies depending on the cultural structures of the societies and especially their eating habits, and it has been reported that approximately one-third of the world's population is infected with this parasite.[3] Using the Trojan horse strategy, *T. gondii* may penetrate into non-immune organs such as dendritic cells and monocytes, as well as the testicles, eyes, and notably the brain. It may infect many cell types in the brain, including neurons, Purkinje cells, and microglial cells in the amygdala, hippocampus, cerebral cortex, and basal ganglia regions.[3,4] It may lead to changes in the cognitive and psychological state of the host by disrupting the electrical activity of the cells in which it settles.[3] In addition, a study on mice reported that *T. gondii* chronic infection increased brain dopamine levels by up to 15%. Therefore, the neurobehavioral and neurological symptoms assumed to be associated with *T. gondii* infection may be associated with potential dopamine modulation in the host brain.[5] Based on these studies, the *T. gondii* positivity rate was reported to be higher in patients with various neuropsychiatric disorders, such as schizophrenia, CE, bipolar disorder, obsessive-compulsive disorder, unipolar depression, substance use disorder, suicides, murders, generalized anxiety disorder and panic disorder, personality disorders, and mood disorders, than in healthy controls.[3,4,6] Current studies consistently support the relationship between *T. gondii* and schizophrenia, although it is necessary to conduct more detailed studies on other disorders.[5]

This study was conducted to evaluate the relationship between toxoplasmosis and CE by comparing the rate of *T. gondii* seropositivity identified in CE patients with patients without any neurological disorder and by performing a meta-analysis of the results of studies that previously discussed the relationship between *T. gondii* and CE.

METHODS

Study Population and Sample Collection

This study was conducted on 200 patients diagnosed with CE and followed up at the University of Health Sciences Turkey, Van Training and Research Hospital between August 2021 and October 2022 in Van province, Turkey. Also included as the control group were 164 healthy individuals without neurological disease. The patient group included CE patients with normal brain magnetic resonance imaging and no history of head trauma, brain surgery, meningitis, encephalitis, or alcohol dependence; the control group included healthy subjects in the same age range as well as the patient group without any neurological complaint. To provide similar populations of the patient and control groups, people not residing in Van were not included in the study. For the routine examinations of the individuals included in the study, the remaining blood samples that they gave to the biochemistry laboratory were used in the study. Serum was separated from the collected blood samples and stored at -20 °C.

Serological Antibody Detection

The presence of anti-*T. gondii* IgG antibody in sera was determined using an ELISA IgG kit (DRG, Germany). The serum samples were allowed to thaw at room temperature before testing. The procedure was performed according to the manufacturer's instructions.

Statistical Analysis

The Minitab 14 package program was used for statistical analysis of the seroprevalence study with the control group. The Z test and Fisher's exact tests were used to determine statistical significance and $p<0.05$ was considered significant.

RESULTS

The ratio of females to males in the patient and control groups was 95/105 and 100/64, respectively. The mean age of the patients was 31.05±12 years (minimum 19-maximum 72), whereas in the control group was 42.27±20.12 years (minimum 19-maximum 80) (Table 1).

MAIN POINTS

- Current studies consistently support the link between *T. gondii* and schizophrenia. However, more detailed studies are needed for other disorders such as cryptogenic epilepsy (CE).
- Studies have reported that neurological disorders of toxoplasmosis depend on the localization of tissue cysts in the central nervous system. *T. gondii* infections may not be associated with all types of epilepsy but only some types of epilepsy and only some strains, not all *T. gondii* strains, may lead to epilepsy.
- Toxoplasmosis did not increase the risk of CE in the region where this study was conducted.

The study found *T. gondii* IgG in 31 (15.50%) of 200 CE patients and 47 (28.66%) of 164 non-epileptic individuals. Based on the statistical analysis, the higher rate in non-epileptic individuals compared with CE patients was determined to be statistically significant (p=0.003).

The distribution of *T. gondii* IgG positivity rates by age and gender is shown in Table 2. When the positivity rates of CE patients and non-epileptic individuals by age were statistically compared, no significant difference was found in individuals under 35 years of age (p=0.47) but a significant difference was found in individuals over 35 years of age (p=0.001).

DISCUSSION

It is estimated that 30% of people worldwide are infected with *T. gondii*. However, *T. gondii* seropositivity may vary among countries, different regions within the same country, and communities of different ethnic origins living in the same region.[5] Studies conducted in Turkey in the last decade have determined *T. gondii* seropositivity to be 26.8-32.4% in the general population.[7] *T. gondii* seropositivity was found to be 29% in the control group of a study investigating the relationship between schizophrenia and toxoplasmosis in Van province,[6] and 28% in the control group of a study investigating *T. gondii* seropositivity in hemodialysis patients.[8] This study also showed 28.66% positivity in individuals without any neurological disease selected as the control group. This study and previous studies have demonstrated that approximately one in three people in Turkey are exposed to *T. gondii*. Therefore, studies on the pathogenicity of this protozoon are important.

Toxoplasma gondii may cause serious illnesses in humans, particularly in congenitally infected children and those with a weakened immune system or immunocompromised.[7] In particular, it has been discussed that individuals may develop behavioral and neurological disorders due to latent toxoplasmosis. Studies have reported that *T. gondii* with an increasing prevalence recently may be associated with some neurological disorders such as Parkinson's disease, Alzheimer's disease, schizophrenia, and epilepsy.[3,4,6] While there is consistency in the results of studies on schizophrenia,[5] there is no clear information about the role of *T. gondii* in the etiology of neurological disorders such as epilepsy. There are studies reporting that there is no statistically significant difference between the *T. gondii* seropositivity rate of epileptic patients and that of the healthy control group,[9,10] as well as studies reporting that the seropositivity rate is higher in epileptic patients.[11,12] Researchers also evaluated the seroprevalence of *T. gondii* in patients with CE, a type of epilepsy with unknown etiology.

Table 1. Comparison of demographic characteristics of the patient with CE and control groups

	CE patient group	Control group	p
Mean age±SD	31.05±12	42.27±20.12	0.001
Gander			
Female	95 (47.50%)	100 (60.98%)	0.01
Male	105 (52.50%)	64 (39.02%)	

CE: Cryptogenic epilepsy, SD: Standard deviation

Table 2. Distribution of *T. gondii* IgG positivity rates by age and gender

Veriable	CE patient group		Control group		p
	Number (%)	Seropositivity to *T. gondii* (%)	Number (%)	Seropositivity to *T. gondii* (%)	
Gender					
Female	95 (47.50)	15 (15,79)	100 (60.98)	28 (28.00)	**0.037**
Male	105 (52.50)	16 (15.24)	64 (39.02)	19 (29.69)	**0.031**
Age					
≤35	144 (72.00)	25 (17.36)	77 (46.95)	17 (22.08)	0.407
>35	56 (28.00)	6 (10.71)	87 (53.05)	30 (34.48)	**0.001**
Total	200 (100.00)	31 (15.50)	164 (100.00)	47 (28.66)	**0.003**

CE: Cryptogenic epilepsy, IgG: Immunoglobulin G

Table 3. Characteristics of studies investigating the relationship between cryptogenic epilepsy and toxoplasmosis

Authors	Country	Age groups	CE patient group		Control group	
			N	n (%)	N	n (%)
El-Tantawy et al.[13]	Egypt	Child age group	132	80 (60.06)	60	26 (43.33)
Abd El-Aal et al.[14]	Egypt	2-46 age	72	25 (34.72)	60	7 (11.67)
Eraky et al.[15]	Egypt	Child age group	40	8 (20.00)	20	2 (10.00)
Khatab et al.[16]	Egypt	Child age group	30	12 (40.00)	20	2 (10.00)
Zibaei et al.[17]	Iran	All ages	85	12 (14.12)	85	4 (4.71)
Babaie et al.[10]	Iran	18-64 age	262	94 (38.88)	63	24 (38.09)
Yazar et al.[18]	Turkey	All ages	50	27 (54.00)	50	9 (18.00)
Akyol et al.[19]	Turkey	11-60 age	100	31 (31.00)	50	10 (20.00)
This study	Turkey	All ages	200	31 (15.50)	164	47 (28.66)

N: total number of individuals.
n: number of *T. gondii* seropositive individuals.
CE: Cryptogenic epilepsy

While seven studies (Table 3)[13-19] revealed *T. gondii* seropositivity in CE patients to be higher than in the control group, Babaie et al.[10] study and this study showed seropositivity to be high in the control group. We believe that the relationship between *T. gondii* infection and CE will be more clearly understood when larger patient populations are studied and well-matched control groups are selected. Having found the seropositivity rate to be higher in the control group than in epileptic patients in Iran, Babaie et al.[10] also suggested conducting prospective studies with large sample sizes consisting of well-matched control groups to clarify the effect of *T. gondii* on the occurrence of epilepsy. The sample size included in this study was higher than that of the other eight studies (Table 3),[13-19] and attention was paid to ensure that the region where the individuals selected for the control group lived was compatible with the patient group.

It should be examined the mechanisms of the occurrence of epilepsy and how *T. gondii* infection affects the neurological system to interpret the toxoplasmosis-CE relationship. Epileptic activity is particularly caused by repetitive synchronous hyperactivity of cortical and hippocampal neurons. Epileptiform discharges are caused by decreased extracellular magnesium, increased extracellular potassium (K) concentration, inhibition of the sodium pump, or antagonism of GABA receptors.[3] *T. gondii*, as a carbon source, may lead to epileptic seizures by using GABA, the neurotransmitter primarily responsible for preventing the onset of seizures.[20] Brooks et al.[20] reported that glutamic acid decarboxylase 67 (GAD67), a key enzyme catalyzing GABA synthesis in the brain, was affected in mice infected with the type 2 ME49 *T. gondii* strain, but not in mice infected with the type 3 CEP strain. The study highlighted that the regulation of GABAergic synapses may be due to polymorphic parasitic factors, and the brains of mice also had tachyzoite and activated microglia and astrocytes as well as numerous cysts.

It is also known that epileptic activity may result from changes in the brain dopamine level. The genome of *T. gondii* contains two regions of aromatic amino acid expiration. These amino acids provide the synthesis of the L-DOPA enzyme and thus affect the biosynthesis of dopamine and serotonin. It increases the parasite K^+ ion level in dopaminergic cells, thereby causing dopamine to be released three times more. Dopamine levels were also found to be high in brain tissue with *T. gondii* cysts. Dopamine levels and concentrations increase when the parasite settles in the amygdala region.[3]

To sum up, studies have reported that neurological disorders of toxoplasmosis depend on the localization of tissue cysts in the CNS, *T. gondii* infections may not be associated with all types of epilepsy but only some types of epilepsy[9] and that only some strains, not all *T. gondii* strains,[20] may lead to epilepsy. Thus, we believe that the relationship between *T. gondii* and CE may vary from region to region, to different communities within the same region, and from individual to individual within the same community. In addition, the higher *T. gondii* seropositivity in the control group can be partially explained by the fact that the control group was older.

Because *T. gondii* seroprevalence increases with age as a result of cumulative seropositivity. In a study, it was determined that *T. gondii* seroprevalence increased at a rate of 1.09% with each year of age.[21]

Study Limitations

The main limitation of the study is that it was conducted in a single hospital; therefore, the patient population was from a single region. In addition, the fact that *T. gondii* IgM levels were not measured in the patients is also a limitation of the study.

CONCLUSION

Toxoplasma gondii seropositivity in CE patients included in the study was lower than that in the control group. According to these results, toxoplasmosis did not increase the risk of CE in the region where this study was conducted. However, for a better understanding of the CE-*T. gondii* relationship, the role of different *T. gondii* strains in the etiology of CE, the importance of the immune system of individuals infected with *T. gondii* in the occurrence of CE, and the *T. gondii*-CE-dopamine relationship should be examined. In addition, seroprevalence studies should be conducted in larger populations and in different countries.

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Ethics

Ethics Committee Approval: The ethical approval for this study was obtained from the Non-invasive Ethics Committee of Van Yüzüncü Yıl University (09/07/2021-2021/08-15).

Informed Consent: Consent form was filled out by all participants.

Author Contributions

Concept: Z.T.C., S.A., M.T.A., Ö.S.T.A., Design: S.A., Z.T.C., H.Y., Data Collection or Processing: Ö.S.T.A., M.K., C.B., Analysis or Interpretation: S.A., M.T.A., H.Y., Literature Search: S.A., M.T.A., Writing: S.A., Z.T.C.

Conflict of Interest: No conflict of interest was declared by the authors.

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