Toxoplasma gondii Infection by Serological and Molecular Methods in Schizophrenia Patients with and without Suicide Attempts: An Age-Sex-Matched Case-Control Study

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February 19, 2021

Abstract

Introduction: The opinion that latent T. gondii infection is having a broadly asymptomatic projection has now been interrogated, in specific due to the echoed association between the latent infection and an elevated incidence of schizophrenia or even suicide attempts. Notwithstanding conducted studies aimed to understand this feasible link are restricted. Methods: In the present case-control study, we focused to illuminate the relationship between the serological and molecular presence of T. gondii and schizophrenia with or without the suicide attempts by comparing it with healthy individuals. A total of 237 participants (117 in schizophrenia; 120 in healthy control) were included in this study. Results: Overall, latent T. gondii infections were found statistically higher in 63 (53.8%) of the 117 patients with schizophrenia and in 33 (27.5%) of the 120 controls ($p < 0.001$). In schizophrenia patients, seroprevalence T. gondii was again found to be statistically higher in suicide attempters (59.6%), compared to no history of suicide attempts (48.3%) ($p < 0.05$). The molecular positivity rate of T. gondii DNA was higher in the schizophrenia group, compared to the healthy control group ($p < 0.05$), whereas the history of suicide attempts was not statistically associated ($p = 0.831$) with T. gondii DNA positivity by PCR. Conclusion: This case-control study enlightens additional demonstration to the belief that T. gondii infection would be an underlying component for the pathophysiology of schizophrenia. Regardless of the clarity results of this study, this supposition warrants further endorsement.

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T. gondii **with ELISA and PCR in Schizophrenia**

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Abstract

Introduction: The opinion that latent *T. gondii*infection is having a broadly asymptomatic projection has now been interrogated, in specific due to the echoed association between the latent infection and an elevated incidence of schizophrenia or even suicide attempts. Notwithstanding conducted studies aimed to understand this feasible link are restricted. **Methods:** In the present case-control study, we focused to illuminate the relationship between the serological and molecular presence of *T. gondii* and schizophrenia with or without the suicide attempts by comparing it with healthy individuals. A total of 237 participants (117 in schizophrenia; 120 in healthy control) were included in this study. **Results:**Overall, latent *T. gondii* infections were found statistically higher in 63 (53.8%) of the 117 patients with schizophrenia and in 33 (27.5%) of the 120 controls ($p < 0.001$). In schizophrenia patients, seroprevalence *T. gondii* was again found to be statistically higher in suicide attempters (59.6%), compared to no history of suicide attempts (48.3%) ($p < 0.05$). The molecular positivity rate of *T. gondii* DNA was higher in the schizophrenia group, compared to the healthy control group ($p < 0.05$), whereas the history of suicide attempts was not statistically associated ($p = 0.831$) with *T. gondii* DNA positivity by PCR.**Conclusion:** This case-control study enlightens additional demonstration to the belief that *T. gondii* infection would be an underlying component for the pathophysiology of schizophrenia. Regardless of the clarity results of this study, this supposition warrants further endorsement.

Keywords: Schizophrenia, Suicide attempt, *Toxoplasma gondii* , ELISA, PCR

What's already known about this topic?

Toxoplasma gondii (*T. gondii*) is a neurotropic parasite and its infection maybe take a key position in the risk of schizophrenia and also suicide attempts.

What does this article add?

Beyond the serologically based retrospective research, *T. gondii*detection was examined by molecular methods in this case-control study, and the relationship between *T. gondii* positivity in schizophrenia and suicide attempt risk was investigated for the first time with the healthy control group with similar age-gender data.

1. INTRODUCTION

Toxoplasma gondii (*T. gondii*) is a neurotropic parasite affecting warm-blooded animals including humans[1] and is found in more or less 30% of people.[2] Felines are the final host for *T. gondii* , shedding in their feces up to millions of oocyst stages daily, which become contagious in nature.[3] In people, the infection may be transmitted by the ingestion of water or food. In this way, the majority of *T. gondii* infections remain asymptomatic or maybe produce infectious mononucleosis in people who have an intact immune system. Severe toxoplasmosis may develop if *T. gondii* infects immunocompromised patients such as acquired immunodeficiency syndrome (AIDS).[4] In parallel with the achievements after the concept of microbiota, it is thought that microorganisms may be associated with non-infectious pathological changes.

The pathophysiology of schizophrenia is tangled, and biological factors are among the leading causes.[5] Thus, studies focusing to elucidate the possible agents in schizophrenia have been carried out. In many studies conducted for this purpose, *T. gondii* has been evaluated as the prominent agent in this relationship due to its tropism and persistence in the brain.[6] Several reports are describing that *T. gondii* is linked to the elevated risk of mice catching by felines,[7] to modulated behavioral change by influencing neurotransmitters in the human brain.[8]In humans, dysregulation of some neurotransmitters is significantly related to schizophrenia. *T. gondii* can synthesize tyrosine hydrolase and thus dopamine dysregulation may occur. Furthermore, *T. gondii* also modifies the level of a vast of other neurotransmitters, including glutamate, serotonin, norepinephrine, and γ-aminobutyric acid (GABA).[9] The link between schizophrenia and *T. gondii* seems to be vigorous, as evidenced by numerous seroprevalence and neurotransmitter studies.

Worldwide, almost 800.000 people die by suicide and roughly 10 million individuals attempt suicide annually. Suicide attempts are recognized to be a complex mechanism, with psychological, social and biological perspectives involved.[10] In 2009, *T. gondii* , a hitherto unforeseen agent, has been associated with the suicide attempt. The possible relationship between *T. gondii* and suicide attempts has also been shown in a current seroprevalence study. According to the author, *T. gondii* IgG positivity was statistically highly correlated with lower agreeableness, lower conscientiousness and higher neuroticism personality types.[11] Overall, topic-related and current studies provide considerable evidence for the hypothesis that *T. gondii* infections maybe take a key position in the risk of the suicide attempt.[12]

In this case-control study, it was aimed to elucidate the relationship between *T. gondii* , which was detected by serological and molecular methods, and patients with schizophrenia, and the suicide attempt by comparing it with the healthy individuals.

2. METHODS

In this case-control study, the detection and prevalence of *T. gondii* by serological and molecular methods in patients with schizophrenia were compared to the healthy control.

2.1. Study design and sample size calculation

This study was approved by the Istanbul Aydin University Clinical Research Ethics Committee with B.30.2.AYD.0.00.00-480.2/217 protocol number before the research. The statistical power analysis was showed that a minimum sample of n=73 in each group would have the assumption of 95% CI and power of 80%. A nominal α=0.05 and two-tailed tests were used in the power calculations.

We recruited a total of 117 schizophrenia patients aged 18 – 65 years as a case group. For each schizophrenia patient, one healthy control people were recruited. Individuals in the control group were randomly selected from the city where the patients lived (Istanbul, Turkey) and matched for age ($\pm$ 3 years) and gender. The workflow diagram clarifies the design of this case-control study (Fig 1).

2.2. Study participants and sampling

In this case-control study, a total of 237 volunteers between 18 and 65 years old were enrolled in the schizophrenia or the healthy control groups. One hundred seventeen patients with schizophrenia who applied to the psychiatry department of Istanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty in Istanbul were enrolled in the schizophrenia group of the study. Schizophrenia is diagnosed clinically according to the Diagnostic and Statistical Manual of Mental Disorders (DSM–5). Patients diagnosing with schizoaffective or schizophreniform disorders were excluded from this study. Also, possible suicide attempts of the patients with schizophrenia were determined with the Suicide Behaviors Questionnaire-Revised (SBQ-R) and clinical interviews. The healthy control group was consisted of 120 participants without any psychiatric disease or family history and having similar age and gender data with the schizophrenia group. Two venous blood samples from each volunteer were taken by venipuncture from a single cubital region and were collected into plain and containing dipotassium ethylenediaminetetraacetic acid (K_2EDTA) vacuum tubes. The tubes were filled and K_2EDTA tubes were immediately mixed with gentle 180° inversion. Serum samples were separated from plain blood tubes by centrifugation at 2500 rpm and stored at -80°C prior to *T. gondii* IgG analysis. Whole blood samples were also stored at -80°C for the assessment of *T. gondii* DNA by molecular methods.

2.3. Serological evaluation

Serum samples were evaluated for *T. gondii* IgG with IBL-America Toxoplasma IgG enzyme-linked immunosorbent assay (ELISA) kit (Minneapolis, MN, USA) following the manufacturers' instructions, for which the sensitivity and specificity values were more than 98%.*T. gondii* IgG titers were read at optical density 450 nm, and seropositivity was defined as a *T. gondii* IgG titer equal to or higher than 9 IU/mL.

2.4. DNA extraction

DNA was extracted using Plus Blood Genomic DNA Purification Kit (GeneMark Diagnostics, Carlsbad, CA,

USA) according to the manufacturers' instructions. NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used to measure DNA concentration and purity at 260/280 nm in extraction samples. The extracted DNA was stored at -20degC prior to testing.

2.5. Polymerase chain reaction (PCR)

For the molecular detection of *T. gondii* , the real-time PCR was performed in the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, California USA) using targeted a 529 bp repeat element and internal transcribed spacer-1 (ITS-1) region-specific *T. gondii* primers listed in Table 1. The reaction mixture contained 15 µL of dH_2O, 5 µL of 5X Hot Start Master Mix (GMbiolab, Taiwan, China), 2 µL of 10 µmol/L forward and reverse primer, and 3 µL DNA Template. The thermal cycling was performed at 37 cycles (Enzyme activation at 95°C for 5 minutes, denaturing at 95°C for 30 seconds, annealing at 56°C for 30 seconds and extension at 72°C for 1 minute). Melting curves were generated from 50 to 95°C and read every 4°C for 5 seconds.

Table 1. Nucleotide sequences of primers used in this study

Primer	**Sequence (5' - 3')**
ITS-1 Forward	ATCCCAACAGAGACACGAATT
ITS-1 Reverse	ACACGTCCTTATTCTTTATTAACCA

2.6. Detection of Amplicon

After completion of real-time PCR, the amplified products were confirmed by gel electrophoresis. Five mL of each amplicon were loaded on 2% agarose gel containing ethidium bromide (EtBr) and SYBR® Gold nucleic acid gel stain (Invitrogen Life Technologies, Eugene, Oregon, USA). The gel electrophoresis was performed at 25 V for 30 min and was visualized by Safe Imager ™ (Invitrogen Detection Technologies, Eugene, Oregon, USA).

2.7. Statistical analysis

Data analyses were evaluated with the Statistical Package for the Social Sciences (SPSS, IBM Corp.) version 25. Normal distribution and homogeneity of the results were evaluated with Shapiro-Wilk and Levene's test, respectively. Parametric and non-parametric test assumptions were done by Student's t-test and one-way ANOVA, as appropriate. A $p< 0.05$ was considered to indicate a statistically significant difference. Statistical significance is denoted by an asterisk.

3. RESULTS

Sociodemographic and other characteristics are shown in Table 2. A total of 117 patients diagnosed with schizophrenia and 120 healthy controls matched for age and gender were included in this study (Fig 1). The mean age was 47.51 years (SD=24.83) in suicide attempters of subdivided schizophrenia group, 43.96 years (SD=18.33) in no history of the suicide attempt of subdivided schizophrenia group, and 42.27 years (SD=29.11) in the healthy control group (p=0.416). Of all participants 50.2% of them were male and 49.8% were female (Table 2). As an expected and unfortunate consequence of schizophrenia, a considerably higher proportion of participants were unemployed (61.4% and 65.0% vs 20.0%; p = 0.018) and single (71.9% and 58.3 vs 34.2; p = 0.043), compared to control group. No other substantial difference was evaluated between groups regarding the education and socioeconomic levels of the participants (Table 2).

Table 2. Sociodemographic and other characteristics of the participants

		Schizophrenia n (%)	Schizophrenia n (%)	Healthy Control n (%)	*p-value*
		History of suicide attempt	No history of suicide attempt		
Gender	Male	34 (59.6)	32 (53.3)	53 (44.2)	0.619
	Female	23 (40.4)	28 (46.7)	67 (55.8)	
Marital Status	Single	41 (71.9)	35 (58.3)	41 (34.2)	**0.043**
	Married	16 (28.1)	25 (41.7)	79 (65.8)	
Education Level	None	8 (14.0)	11 (18.3)	3 (2.5)	0.138
	Primary	27 (47.4)	31 (51.7)	28 (23.3)	
	Secondary	13 (22.8)	12 (20.0)	30 (25.0)	
	University	9 (15.8)	6 (10.0)	59 (49.2)	
Socioeconomic Level *	No income	11 (19.3)	22 (36.7)	7 (5.8)	0.073
	Low	22 (38.6)	17 (28.3)	34 (28.3)	
	Intermediate	24 (42.1)	19 (31.7)	50 (41.7)	
	High	0 (0.0)	2 (3.3)	29 (24.2)	
Employment	Unemployed	35 (61.4)	39 (65.0)	24 (20.0)	**0.018**
	Employed	22 (38.6)	21 (35.0)	96 (80.0)	
Age	Age	Mean ± SD 47.51±24.83	Mean ± SD 43.96±18.33	Mean ± SD 42.27±29.11	0.416

* Low <3000[?]; intermediate 3000 – 5000 [?]; High > 5000 [?]

T. gondii IgG antibodies were found statistically higher in 63 (53.8%) of the 117 patients with schizophrenia and in 33 (27.5%) of the 120 controls ($p < 0.001$). When the cases included in the schizophrenia group are examined by dividing into two sub-groups according to their suicide attempt history, the relationship between the history of suicide attempt and seroprevalence *T. gondii* was again found to be statistically significant ($p < 0.05$). In reflection of our serological findings, it was determined that the rates of *T. gondii* seropositivity were higher in the schizophrenia group, compared to the control group. Likewise, *T. gondii* IgG antibodies positivity was found to be more in schizophrenia patients with a history of suicide attempt (Table 3).

The results in Table 3 also summarized that the molecular detection of*T. gondii* by PCR. The molecular positivity rate of T. gondii was higher in schizophrenia group, compared to healthy control group ($p < 0.05$), whereas history of suicide attempts was not statistically associated ($p = 0.831$) with *T. gondii* positivity by PCR (Table 3). This could be attributed to the fact that the *T. gondii* is less detected by the molecular method than the serological method. In this study, the amplified products were visualized by the standard gel electrophoresis and Figure 2 shows a sample image of electrophoresis.

Table 3. Comparison between schizophrenia and control group for*T. gondii* status

		Serological profile of *T. gondii* n (%)	Serological profile of *T. gondii* n (%)	*p-value*	Molecular profile of *T. gondii* n (%)	Molecular profile of *T. gondii* n (%)	*p-value*
Schizophrenia	Suicide attempt +	IgG+ 34 (59.6)	IgG- 23 (40.4)	**<0.05**	PCR+ 16 (28.1)	PCR- 41 (71.9)	0.831

		Serological profile of *T. gondii* n (%)	Serological profile of *T. gondii* n (%)	*p-value*	Molecular profile of *T. gondii* n (%)	Molecular profile of *T. gondii* n (%)	*p-value*
	Suicide attempt -	29 (48.3)	31 (51.7)		12 (20.0)	48 (80.0)	
	Total	63 (53.8)	54 (46.2)	**<0.001**	28 (23.9)	89 (76.1)	**<0.05**
Healthy Control	Healthy Control	33 (27.5)	87 (72.5)		2 (1.7)	118 (98.3)	

4. DISCUSSION

In this case-control study, we used serological and molecular techniques to elucidate a possible connection between *T. gondii* and schizophrenia patients compared to healthy people. To the best of our knowledge, this is the first case-control study conducted in schizophrenia and healthy individuals with the diagnosis of *T. gondii* by serological and also molecular methods. Overall, *T. gondii* positivity was found more common in patients with schizophrenia (53.8%), compared to healthy individuals (27.5%) by ELISA in this study ($p < 0.001$).

Conventional diagnostic approaches for *T. gondii* infection include microscopical, cultural, serological, and radiological methods. Because of the fact that natural scantness of conventional diagnostic techniques, PCR may be adopted to diagnose *T. gondii*infection.[13] As far as we know, there is only one study[14] that has limitations such as the lack of a healthy control group and targeting the SAG1 and B1 regions in the molecular diagnosis of *T. gondii* , although it is approximately similar in general concept to our study. Despite its limitations in this retrospective study, the authors highlighted that the prominence to use serological and molecular techniques for correct identification of*T. gondii* in schizophrenia patients.[14]

In recent years there has been a burgeoning concern about the influence of latent *T. gondii* infection on mental disorders, especially schizophrenia. The nature of this connection remains unclear, although it is conceivable that they manifest causality.[15] To date, numerous studies have been carried out showing the relationship between *T. gondii* and schizophrenia.[16–19]Likewise, these studies and also our study, a meta-analysis reveals a general significant relation having with *T. gondii* latent infection and schizophrenia. Also, this well-designed and highly scientific paper saying that the latent *T. gondii* acts an impact on human behavior as well.[20] After two years of this meta-analysis, a case-control study has been published to determine the association between schizophrenia and *T. gondii* conducted with 99 schizophrenia patients and 152 healthy blood donors. Although only serological methods were used in this study and using only this analysis has some limitations, the authors reported a positive relationship between *T. gondii* and schizophrenia.[21]

In contrast to various publications that declared a significant correlation between *T. gondii* and schizophrenia, some studies unable to show a significant relationship.[22–24]Nevertheless, it should not be underestimated that the quality and quantity of studies showing the percentages of *T. gondii* , which has only approximately 30% positivity in a healthy population worldwide[25], was found to be elevated especially in patients with schizophrenia. In this manner, it is clear that further research is still warranted.

Suicide is a crucial emerging health problem, commonly seen in people with mental disorders, and previous suicide attempts or even suicide ideation are the best indicators of possible suicide risk.[26] In 2019, a meta-analysis with the aim to determine if *T. gondii* is linked with suicide attempt was published.[12] In this meta-analysis, 14 studies about suicide attempts were included and the authors have calculated a statistically highly significant correlation for *T. gondii* IgG antibodies with suicide attempters (OR = 1.39; 95% CI 1.10–1.76, p = 0006). Moreover, the authors reported that latent *T. gondii*infection may play a significant role in the risk of suicide attempts and potential agents should be evaluated further.[12]

Besides the contribution of our study with preliminary results of*T. gondii* and suicidal attempt history relation in patients with schizophrenia, there are two major limitations in this study that could be addressed in

future further research. First, we enrolled a relatively small sample size (in 117 patients with schizophrenia), which would bias the results of our overall and subdivided schizophrenia group analyses toward the null hypothesis. Second, we were unable to evaluate the first-episode psychosis in stages of schizophrenia in the case group. As previously noticed[27], if patients with the first-episode psychosis of schizophrenia were possible to include in our study, these outcomes would provide an idea or even insight into how *T. gondii* behaves during the development of this surreptitious disease.

5. CONCLUSION

In conclusion, our study revealed a relation with both schizophrenia and *T. gondii* , also the suicidal attempt of these patients. Although it is difficult to suggest a direct etiological interaction, after this preliminary finding we can see the *T. gondii* and schizophrenia link more obvious. On the other side, there is currently no radical treatment for latent *T. gondii* and no effective vaccines. Simultaneously with its knotty prevalence, *T. gondii* is a considerable and sketchily realized agent. Ultimately, it will be decisive to advanced multidisciplinary study (encouraging to taken together with medical microbiology and psychiatry) to clear insight into the fiendishly mechanisms of neuropsychiatric disorders and to conduct more effective and plausible studies to stiffen for this neurotrophic links.

Funding

This work was supported by the Istanbul Aydin University Scientific Research Projects Unit (2020/13).

Conflict of Interest

The authors have declared that there are no conflicts of interest in relation to the subject of this study.

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Figure 1. Workflow diagram of patients with schizophrenia and matched healthy individuals included in this study

Figure 2. PCR amplicon of T. gondii ITS-1 region identified on 2% agarose gel electrophoresis. M, 100 bp marker; P, T. gondii positive DNA; Lane 1, T. gondii PCR positive sample; Lane 2 – 5, T. gondii PCR negative samples; N, negative control.