



A Study of Association of *Toxoplasma gondii* Infection With Schizophrenia in Mashhad Area, Khorasan Razavi Province, Iran

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Abstract

Background and aims: Congenital toxoplasmosis is assumed to play a role in developing schizophrenia in human. This study aimed to estimate the relationship between *Toxoplasma* infection and schizophrenia by using enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR).

Methods: In this case-control study, 46 schizophrenic patients forming our case group as well as 40 healthy relatives of schizophrenic patients forming our control group were selected from among 456 inpatients with schizophrenia in Ibn Sina and Hejazi hospitals in Mashhad from June 2016 to February 2017. The blood samples from both groups were collected for serological and molecular tests. Different statistical methods such as Chi-square, independent *t* test, and logistic regression models were used in the present study.

Results: In the present study, the seroprevalence of *T. gondii* was 54% in case group and 45% in control (OR=1.44, 95% CI: 0.62- 3.40, *P*=0.38). There was no significant association between the seroprevalence of *T. gondii* infection and age, gender, and season as the risk factors in the case group. Furthermore, a poor agreement was observed between the microscopy and PCR methods. Non-significant differences were found between the mean levels of interferon gamma (IFN- γ), C-reactive protein (CRP), and white blood cell (WBC) in two groups.

Conclusion: Contrary to the reports from some studies, no association was found between *Toxoplasma* infection and the schizophrenia. In order to better understand the effect of *Toxoplasma* on schizophrenia, it is necessary to develop laboratory methods to differentiate acquired toxoplasmosis from congenital one.

Keywords: Case-control study, *Toxoplasma gondii*, Schizophrenia, Serology, PCR, IFN- γ

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Introduction

Toxoplasma gondii is an obligatory intracellular protozoan in the phylum Apicomplexa that infects a wide variety of warm-blooded vertebrates as intermediate hosts.¹ It is transmitted to human by raw and partly-cooked infected meat, oocyst ingestion, as well as congenital and blood products.² Many human cases of toxoplasmosis are asymptomatic, but it may cause serious complications for infants born from infected mothers and for people with weakened immune systems.^{3,4} Some experimental studies have reported the formation of *Toxoplasma* cysts in the brains of rats, causing a decrease in neophobic behaviors among rats and producing an increase in risky actions among them when facing cats. These behavior types can be expressed to make easy *Toxoplasma* transmission to the cat as a definitive host.^{5,6} Based on this experimental finding, many studies have been conducted to examine the association of chronic toxoplasmosis with several mental

and behavioral disorders such as schizophrenia, bipolar disorder, depressive disorder, and obsessive-compulsive disorder, as well as with personality changes including suicidality and involvement in road traffic accidents.⁷⁻⁹ However, the mechanisms with which *T. gondii* alters brain function remain largely unclear. It seems that *Toxoplasma* infection can affect the host behavior via three possible mechanisms. First, cerebral cysts directly cause multilocular lesions and histopathological changes in the host brain. Few studies have reported a reduced grey matter volume in *T. gondii* positive schizophrenia patients compared to that of *T. gondii* negative patients.¹⁰ Second, latent toxoplasmosis causes a sustained elevation in circulating levels of interferon gamma (IFN- γ) and chronic activation of Indoleamine-pyrrole 2,3-dioxygenase (IDO). This enzyme alters the levels, turnover, and efficiency of many neuromodulators, including dopamine, glutamate, and serotonin. Third, the parasite itself may actually be

a source of dopamine production.^{11,12} Dopamine is the main neurotransmitter involved in schizophrenia, and increasing the function of dopaminergic neurons has been proven.^{13,14} Several seroepidemiological studies have shown the potential association of the *T. gondii* infection with schizophrenia in Iran and other countries¹⁵⁻²⁵. In addition, the level of proinflammatory cytokines may be changed in the schizophrenia patients when infected with *T. gondii*. This study aimed to compare the frequency of *Toxoplasma* infection and the IFN- γ , C-reactive protein (CRP), and white blood cell (WBC) levels in patients suffering from schizophrenia (case group) and in healthy volunteers (control group) using serological and molecular methods.

Materials and Methods

Study Design

In this case-control study, 46 schizophrenic inpatients (i.e., the case group) were selected from among 456 schizophrenic inpatients in Ibn Sina and Hejazi hospitals in Mashhad, Iran from June 2016 to February 2017. The mean ages of patients were 45.19 ± 15.6 years and 45.72 ± 15.1 years in control group (Table 1). Two experienced psychiatrists were clinically diagnosed by means of the Structured Clinical Interview based on the criteria defined in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV).²⁶ At the time of admission, none of the patients showed evidence of immunosuppressive diseases, mental retardation, other psychiatric disorder, history of neurologic and ophthalmic disease, and addiction to motivation drugs (amphetamines). The control group included 40 healthy volunteers from the psychotic inpatients' families demographically comparable to patients group.

Sample Collection

As for the sample collection, 10 mL of venous blood was taken from each person in case and control groups by syringe. The Blood samples were divided into parts under sterile conditions. One part (7 mL) of each blood sample was placed in EDTA coated vacutainer tube, and the remaining part (3 mL) was placed in a serum separator tube. First, 1 mL of uncoagulated sample was used for WBC count by an automated hematology analyzer (Sysmex KX-21N™, United States). Then all EDTA and non-EDTA blood tubes were centrifuged at 3500 rpm for 5 minutes to obtain Buffy coats and serum samples. The separated sera and buffy coat samples were stored at -20°C for later use.

Serology

Sera was analyzed for specific IgG *T. gondii* antibody detection by using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Pishtaz Teb Diagnosis, Tehran, Iran). The optical density (OD) was read at 450 nm in an ELISA plate reader (ELX800 absorbance reader, Bio Tek, USA). Antibody concentrations (IU/mL) were determined by using a standard curve. The results were

described based on the cut off 10 IU/mL where results upper than cut off were determined positive and results below that were considered negative.

IFN- γ , CRP Measurement

Commercially available ELISA kits (Biotechnology laboratory, China) were used for IFN- γ measurement according to the manufacturer's instructions. Results were read at 450 nm OD by Elisa Reader (ELX800 absorbance reader, Bio Tek, USA). Thus, a Latex Agglutination CRP test (OMGA, Scotland, the United Kingdom) was performed as the qualified test in order to quantify CRP analysis and, then, the positive qualified serum samples were measured by Immunoturbidimetric assay (Mindray BS800M1 chemistry analyzer, China).

Polymerase Chain Reaction

DNA of buffy coat samples was extracted using a commercial kit – DNA Molecular Biological System Transfer (MBST, Iran), according to the manufacturer's recommendation. Subsequently, a nested-polymerase chain reaction (PCR) assay was carried out to detect the *T. gondii* B1 gene as previously described by Burg et al.²⁷

Statistical Analysis

Statistical comparisons were carried out using SPSS 22.0 statistical software (version 22.0. Armonk, NY: IBM Corp). Socio-demographic data including age, birthplace, residence, and season of birth were obtained from all patients. All possible associations of *T. gondii* infection with schizophrenia were identified using chi-square statistical tests at a significant level of 5%. Logistic regression models were used to evaluate the association between *T. gondii* seropositivity and potential risk factors using odds ratios (ORs), and 95% confidence intervals (CIs) was taken after adjustments. The agreement between serological and PCR methods was shown as kappa-value. No agreement was detected when kappa-value was less than zero, a slight agreement was observed for kappa-values between 0-0.2, a poor agreement was seen for kappa-values between 0.2 and 0.4, a moderate agreement was found for kappa-values between 0.4 and 0.6, a substantial agreement was demonstrated for kappa-values between 0.6 and 0.8, and a good agreement was detected when kappa-values exceeded 0.8 and 1.3. Two independent sample *t* tests were used for comparing the IFN- γ , CRP, and WBC levels in case and control groups.²⁸

Results

A total of 86 serum samples – 25 (54%) of serum samples of case group and 18 (45%) of serum samples control groups, had antibodies against *T. gondii* (OR=1.4, 95%CI: 0.62- 3.04, $P= 3.40$) (Table 1). Seroprevalence of *T. gondii* infection and its relations to socio-demographic characteristics of the studied populations are shown in Table 2. The seroprevalence of *T. gondii* infection in patients group compared to the control group was not

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significant regarding gender, age, and season as risk factors (Table 3). Results of logistic regression analysis showed no association between the seroprevalence of *T. gondii* and different risk factors such as gender, age, season and type of schizophrenia in case group (Table 4). The result of nested-PCR indicated that 21% (7/33) and 55.5% (10/18) of the seropositive in case and control groups had *Toxoplasma* DNA in blood samples ($P < 0.02$). No agreement was observed between the serology and PCR methods (Kappa= -0.42) (Table 5). Non-significant differences were found between the mean levels of IFN- γ , WBC, and CRP in two groups (Table 6) ($P > 0.05$).

Discussion

The seroprevalence of *Toxoplasma* infection in schizophrenia patients was higher than that in control group, but the difference was not statistically significant. Similar results had been also reported in previous studies in Iran¹⁹⁻²¹ and other countries.^{22,23} Contrary to our study, many studies had shown a significant difference of seroprevalence of *T. gondii* infection in the case and control groups.^{15-18,24,25} However, the obtained results regarding the association of infection with schizophrenia are doubtful. The high seroprevalence of patients with schizophrenia may be related to insufficient hygiene and self-care skills, and the serological examination had not led to the differential diagnosis of acquired and congenital toxoplasmosis in the patients. In the present study, the presence of active *T. gondii* was evaluated in the case and control groups by molecular technique. The nested-PCR results showed a higher frequency of *Toxoplasma* infection in seropositive control group compared to seropositive

Table 3. Results of Logistic Regression Analysis for Comparison of *Toxoplasma* Seropositivity in Schizophrenia Patients With Control Group

Characteristics	OR	95% CI	P Value
<i>Toxoplasma</i>	1.55	0.64-3.75	0.32
Sex	0.72	0.30-1.70	0.45
Age	1.44	0.41-2.83	0.42
Season	1.03	0.09-1.54	0.87

Table 4. Results of Logistic Regression Analysis for Comparison of *Toxoplasma* Seropositivity in Schizophrenia Patients

Risk Factors	OR	95% CI	P Value
Age	3.41	0.24-1.56	0.152
Sex	1.22	0.37-2.40	0.635
Season			
Spring	0.48	0.04 -4.1	0.47
Summer	0.19	0.01-1.87	0.16
Autumn	0.14	0.01-2.01	0.15
Winter	-	-	-
Type of schizophrenia	0.641	0.700-2.23	0.423

Table 5. The PCR Results on the Seropositive Blood Samples in the Case and Control Groups

Groups	PCR-Positive		PCR-Negative		Total	P Value	Kappa
	No.	%	No.	%			
Case	7	21.2	26	78.7	33	<0.02	-0.42
Control	10	55.5	8	44.4	18		
Total	17	33.3	34	66.6	51		

Table 1. Comparison of *Toxoplasma* IgG Levels in Case and Control Groups

Serology	Case Group (n=46)		Control Group (n=40)		OR	95% CI	P Value
	No.	%	No.	%			
Positive	25	54.3	18	45	1.44	0.62-3.40	0.38
Negative	21	45.6	22	55			

Table 2. Socio-demographic Characteristics and Seroprevalence of *Toxoplasma* Seropositivity in the Case and Control Groups

Variable	Case group		Prevalence of <i>Toxoplasma</i> Infection		Control group		Prevalence of <i>Toxoplasma</i> Infection		P Value
	No.	%	No.	%	No.	%	No.	%	
Age									
20-50	29	63	13	44.8	23	57.5	9	39	0.78
50-80	17	37	12	70.6	17	42.5	9	61	0.48
Gender									
Female	22	47.8	11	50	22	55	11	50	1
Male	24	52.2	14	58.3	18	45	7	38.9	0.34
Season									
Spring	16	34.8	8	50	15	37.5	11	61	0.27
Summer	17	37	10	58.8	8	20	2	11	0.20
Autumn	7	15.2	5	71.4	10	25	1	5.9	0.03
Winter	6	13	2	33.3	7	17.5	4	22.2	0.50

Table 6. The Mean Level of WBC, CRP and INF- γ in Seropositive and Seronegative Case and Control Groups

Groups	WBC ($\times 1000$ (mm ³) ⁻¹) (Mean \pm SD)	CRP (mg L ⁻¹) (Mean \pm SD)	INF- γ (IU mL ⁻¹) (Mean \pm SD)	P Value
Case (seropositive)	7.24 \pm 2.07	10.80 \pm 7.43	61.58 \pm 22.15	>0.05
Case (seronegative)	7.31 \pm 2.02	9.50 \pm 2.12	58.14 \pm 27.9	
Control (seropositive)	6.22 \pm 1.49	8.50 \pm 2.12	65.84 \pm 45.02	
Control (seronegative)	6.36 \pm 1.78	9.67 \pm 2.52	54.68 \pm 23.25	

schizophrenic patients. The results could be attributed to the long-term consumption of anti-schizophrenia drugs that are able to inhibit the growth of *T. gondii*.²⁸⁻³⁰

In this study, no significant difference was detected between the seroprevalence of *T. gondii* infection in case group and that in control group regarding age and gender as risk factors. Our study results were consistent with the results from several studies³¹⁻³⁵ but they were inconsistent with the findings from other studies, showing that the frequency of *Toxoplasma* infection was significantly associated with age and gender.^{18,24,36} Some studies have shown that the rate of schizophrenia in spring and winter births were higher than general population.^{37,38} In our study, the frequency of *Toxoplasma* infection of a schizophrenic patient born in autumn was found to be also higher than that in the same season in the control group; but no association was found between *Toxoplasma* seropositivity and seasonal birth in schizophrenic patients.

Some researchers believe that IFN- γ as the main mediator of the immune response against *T. gondii* infection could play an important role in inducing schizophrenia.^{11,12} Some studies have reported an association between the high antibody titers against *Toxoplasma* and increased INF- γ , CRP, and WBC as markers of inflammatory activity in seropositive patients with schizophrenia.^{10,13,14} In this study, the level of INF- γ , CRP, and WBC in case group was found to be almost the same as in control group. Some individuals in control group refused to fully cooperate with us in taking blood samples during the study, which caused a decrease in the number of required samples in the control group. Our investigations showed that despite the high frequency of *Toxoplasma* infection in schizophrenic patients, *T. gondii* infection could not be considered as a risk factor to schizophrenia. In order to confirm the presence or absence of the association between *T. gondii* and schizophrenia, it is necessary to compare the prevalence of congenital toxoplasmosis in the case group and that in control group. The major limitation of this study was the selection of control group. Moreover, some individuals in control group were not willing to fully cooperate with us in taking blood samples and, therefore, only the blood samples of seropositive participants in two groups were examined by molecular technique.

Ethical Approval

This study was approved by the local Research Ethics Committee of the Medicine University of Mashhad before initiating the study procedure. License reference number was IR.MUMS.

REC.1395.128. The procedures and purposes of the study were explained to all patient and healthy volunteers before receiving a written informed consent. All participants' samples were also coded and examined as blind study.

Conflict of Interest Disclosures

None declared.

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