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Original Article

***Toxoplasma* Infection in Schizophrenia Patients: A Comparative Study with Control Group**

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Abstract

Background: Schizophrenia is a serious, chronic, and often debilitating neuropsychiatric disorder. Its causes are still poorly understood. Besides genetic and non-genetic (environmental) factors are thought to be important as the cause of the structural and functional deficits that characterize schizophrenia. This study aimed to compare *Toxoplasma gondii* infection between schizophrenia patients and non-schizophrenia individuals as control group.

Methods: A case-control study was designed in Tehran, Iran during 2009-2010. Sixty-two patients with schizophrenia and 62 non-schizophrenia volunteers were selected. To ascertain a possible relationship between *T. gondii* infection and schizophrenia, anti-*Toxoplasma* IgG antibodies were detected by indirect-ELISA. Data were statistically analyzed by chi-square at a confidence level of 99%.

Results: The sero-positivity rate among patients with schizophrenia (67.7%) was significantly higher than control group (37.1) ($P < 0.01$).

Conclusion: A significant correlation between *Toxoplasma* infection and schizophrenia might be expected.

Keywords: Schizophrenia, *Toxoplasma gondii*, ELISA, Iran

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Introduction

About 30-60% of the population in both developed and developing countries are infected with the parasitic protozoan *Toxoplasma gondii*. *Toxoplasma gondii* is an intracellular protozoan that is widespread globally. Its final hosts are felids, but its intermediate hosts are almost all the warm-blooded animals (1).

Humans become infected in 3 ways: 1- ingesting *T. gondii* tissue cysts (containing bradyzoites) presented in the undercooked meat (especially lamb and pork) of infected food animals; 2- ingesting highly infectious oocysts (containing sporozoites) presented in water, garden soil, children's sandboxes, etc, contaminated by infected cat feces; 3- congenital trans-placental transmission of rapidly replicating tachyzoites from mothers who become infected during pregnancy (2). It can exist chronically in tissues and organs such as the brain of an immunocompetent host in the form of cysts. The host does not show any physical symptoms or signs in such latent infections (3).

Besides host's behavior and psychomotor skills, *T. gondii* might change the personality as well (1, 3-7).

Torrey et al. (8) found that cat ownership before age 13 was a risk factor for the later development of psychoses and speculated that the transmission of some zoonotic agent such as *T. gondii* between pets and human beings may be a possible mechanism for schizophrenia. Brown et al. (9) suggested that maternal toxoplasmosis increased the risk of adult schizophrenia in the offspring.

Schizophrenic patients infected with *Toxoplasma* encompass more levels of antibodies than the same group of non-schizophrenic group (10-12). Moreover, level of IgG, IgM, or IgA antibodies to *T. gondii*, is higher in patients with first-episode schizophrenia (13-15). Some medications that had

been used for treatment of schizophrenia could inhibit the replication of *T. gondii* in cell culture (16).

There are some risk factors for developing the disorder in later of life including winter or spring birth, urban birth, and prenatal and postnatal infections (17). Hence, environmental studies have rekindled interest in the possible role of infectious agents in schizophrenia (18).

To explore further the association between *Toxoplasma* infection and schizophrenia, this study was established to compare the amount of anti-*Toxoplasma* IgG antibodies between patients with schizophrenia and non- schizophrenia control group by ELISA.

Materials and Methods

This case-control study was carried out during 2009 and 2010 in Tehran, Iran. This study was approved by the Ethical Committee of Tehran University of Medical Sciences, Iran.

Participants

Sixty-two patients with schizophrenia were recruited from Roozbeh University Hospital, Tehran, Iran. The diagnosis was made by academic psychiatrists according to DSM-IV-TR classification. To evaluate the positive and negative symptoms the PANSS (positive and negative symptoms scale) was used. All patients had no family history of schizophrenia, no history of head trauma and brain surgery. Blood samples were obtained from the patients and control groups in the morning.

Control group consisted of 62 healthy volunteers. They were evaluated to rule out any medical and psychiatric disorders. The patient and control groups were matched as possible on socioeconomic status; dietary

habits (especially with regard to eating or drinking uncooked/undercooked meat, milk, or eggs); and age (average of 37.54 ± 9.75 year in schizophrenic patients and 37.24 ± 10.24 year in healthy volunteers). The factors of urban or rural areas were considered as well. There were no significant differences between two groups with respect to these factors ($P > 0.05$). Duration of illness in schizophrenia patients was from 2 to 37 years.

Based on clinical features the schizophrenic patients were divided to three forms including paranoid, undifferentiated, and disorganized types.

Serological Technique

Serum was separated from whole blood shortly after collection, and stored at -20°C . Tachyzoites of *T. gondii*, RH strain were collected from peritoneal cavity of mice infected 3 days earlier. The organisms were centrifuged at 2000 rpm for 20 min, washed three times in phosphate buffer saline (PBS) pH 7.2, and disrupted by sonication. Lysed cells were centrifuged at 12000g for 1 hour at 4°C . The supernatant was collected and used as the soluble *T. gondii* antigen. Protein determination was performed using the Bradford method (19).

To establish the ELISA method the 96 well microtitre plates (Nunc, Roskilde, Denmark) were coated with $5\mu\text{g/ml}$ of soluble *T. gondii* antigen in carbonate buffer (pH 9.6). Plates were incubated at 4°C for 24 hours and washed three times with PBST (PBS+20% tween 20) blocked with skimmed milk 1% (Merck, Darmstadt, Germany) in PBST and washed three times. Sera were diluted serially from 1:10 up to 1:6400 (1:10, 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200 & 1:6400) and added to each antigen wells in duplicate runs. Positive and negative samples were used in each experiment to confirm the accuracy of the method. Control

samples were the sera collected previously tested and confirmed by IFA and ELISA methods. After incubating and washing, anti human IgG conjugated with horseradish peroxidase (HRP) enzyme (Dako, Produktionsvej, Denmark) diluted 1:1000 in PBST was added; then orthophenylen diamidin (OPD) (Merck, Darmstadt, Germany) was added to each well as substrate. The reaction was stopped by adding the sulfuric acid (2N) and the optical density was read by an automated ELISA reader (BioTek, USA) at 490 nm (20).

Statistical Analysis

The cut-off was determined as the mean plus two times the standard deviation ($M \pm 2SD$) of the optical density obtained for negative samples. Then the optical density for schizophrenia patients and non-schizophrenia individuals were compared with the cut-off, separately. All data were analyzed by chi-square at a confidence level of 95% and 99% by SPSS version 13.5.

Results

In this study, 62 cases with schizophrenia and 62 control individuals were compared for anti- *Toxoplasma* antibody by ELISA. The difference of anti *T. gondii* antibodies between schizophrenia patients (42 out of 62) and control group (23 out of 62) were statistically significant ($P < 0.01$) (Table1). According to the ELISA test, the mean of optical density in sera from schizophrenia group was higher (0.58) in comparison with control group (0.22) (Table1).

The sero-positivity rate for anti- *T. gondii* IgG antibodies in patients compared with control group (Fig.1) showed possible relationship between *Toxoplasma* infection and schizophrenia.

There was no significant difference between patients and control groups when socioeconomic status, dietary habits, and age were compared.

The schizophrenia patients were consisted of 16 paranoid, 45 undifferentiated and 1 disor-

ganized types. Although the number of different types of disease was small, there was no statistically difference between type of schizophrenia and anti- *T. gondii* antibodies.

Table 1: Distribution of anti-*Toxoplasma* antibodies by ELISA in schizophrenic and non-schizophrenic individuals

Group	No.	Gender		Age (year) (M±SD)	Mean of OD	ELISA ⁺	
		Male	Female			No.	%
Schizophrenia patient	62	39	23	37.54±9.75	0.58	42	67.7
Control group	62	26	36	37.24±10.24	0.22	23	37.1

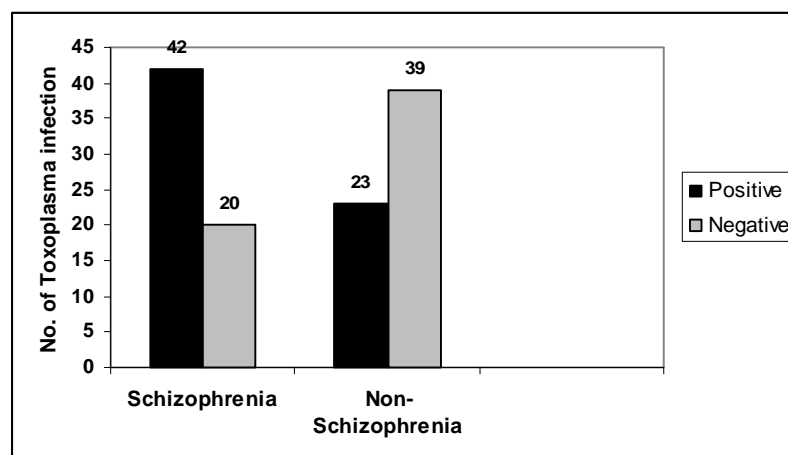


Fig. 1: Comparison of anti- *T. gondii* antibodies between schizophrenic patients and non-schizophrenia as control group

Discussion

In the present study, the sero-prevalence of anti- *T. gondii* antibodies was higher in patients with schizophrenia in comparison with control group.

In recent years, serological studies on patients with schizophrenia have been carried out showing that anti- *T. gondii* antibodies were higher in patients than control groups (14, 15). In this study, patients with schizophrenia had significantly elevated levels of IgG antibodies to *T. gondii* compared with controls ($P < 0.01$). This is in accordance with recent studies, which have suggested that infectious diseases could play a role in developing schizophrenia (14, 15).

In humans, proliferating tachyzoites have been detected in glial cells in patients who had developed toxoplasmic encephalitis (2, 21). In another presentation of toxoplasmic encephalitis, *T. gondii* bradyzoites were observed in Purkinje cells in the cerebellum (2, 22). *T. gondii* cysts have also been reported in astrocytes in humans (2, 23).

Postmortem investigations of brains from individuals who had schizophrenia have reported many glial abnormalities (18, 24), including decreased numbers of astrocytes (18, 25). Neurotransmitters such as dopamine, norepinephrine might be affected by toxoplasmosis, whom are affected in schizophrenic people as well (18, 26).

The role of antibodies in psychotic patients infected with *Toxoplasma* was shown for the first time in 1953 (27). Torrey et al. reported antibodies in 495 (52%) of 961 psychiatric inpatients compared with 170 (25%) of 681 controls.

The prevalence of antibodies to *T. gondii* was higher in individuals with schizophrenia than in control groups and the infection with *Toxoplasma* may confer a risk for schizophrenia (28).

Yazar et al. (18) compared 100 schizophrenic patients with two control groups. In their study, 66% of schizophrenic patients and 23% of controls were positive for IgG titers. Leweke et al. (15) in Germany compared 113 schizophrenic patients with 102 normal people and reported antibodies in 34% of cases compared with 16% of controls.

Saraei et al. compared 104 Iranian schizophrenic patients with 114 normal people and reported *T. gondii* antibodies in 55.3% of cases and 50.9% in control group (29). It is postulated that patients' brain plays the most important role in the perception of the relation between toxoplasmosis and schizophrenia disease (3, 30, 31).

In the present study, the seropositivity rate for anti-*Toxoplasma* antibodies in patient group (67.7%) indicates that chronic *Toxoplasma* infection is greater compared to control group (37.1%) ($P < 0.01$).

In conclusion, a significant correlation between *Toxoplasma* infection and schizophrenia might be expected.

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