Possible Association Between *Toxoplasma Gondii* Infection and Schizophrenia

Egyptian Study

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Background: *Toxoplasma gondii* is an intracellular parasite that in most individuals can persist in multiple tissues where the latent stage of the parasite is mainly found in the central nervous system. Schizophrenia is a serious neuropsychiatric disease of uncertain etiology, and recent studies have focused on *T. gondii* as a possible causal factor in schizophrenia. Soluble intercellular adhesion molecule 1 (sICAM-1), which represents a circulating form of ICAM-1, has been implicated in the development of many diseases.

Aim: The present study aimed to investigate the frequency of *T. gondii* infection among schizophrenia patients and to determine the usefulness of sICAM-1 as an indicator of *Toxoplasma* role in the etiopathogenesis of schizophrenia.

Methods: Sixty patients with schizophrenia, 30 with depressive disorder, and 20 healthy volunteers were subjected to determination of anti–*T. gondii* immunoglobulin G (IgG) antibody seropositivity and sICAM-1 serum level using commercially available enzyme-linked immunosorbent assay kits.

Results: The results showed that the seropositivity rate of anti–*T. gondii* IgG antibodies among schizophrenia patients (56.7%) was higher than among patients with depressive disorder (40%); despite this, the difference was not statistically significant. It was significantly higher in schizophrenia patients than in the healthy volunteers group (30%). Regarding the serum level of sICAM-1, it was significantly higher in the anti–*T. gondii* IgG–seropositive schizophrenia subgroup compared with those of the seropositive healthy volunteers and seropositive depressive disorder subgroups. Moreover, there was a significantly higher level of sICAM-1 in the seropositive schizophrenia subgroup compared with that of the seronegative schizophrenia subgroup. Concerning the seropositive depressive disorder subgroup, there was a significantly higher level of sICAM-1 compared with that of the seronegative depressive disorder subgroup.

Conclusions: These statistically significant results support the association between *T. gondii* infection and schizophrenia and suggest the usefulness of sICAM-1 as an indicator for the possible role of *Toxoplasma* among other factors in the etiopathogenesis of schizophrenia.

Key Words: schizophrenia, *Toxoplasma gondii*, anti–*T. gondii* IgG, sICAM-1, enzyme-linked immunosorbent assay

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Toxoplasma gondii is an intracellular parasite of the phylum Apicomplexa that multiplies in almost every tissue of the body at the fast-dividing tachyzoite stage before transforming into the slowly dividing bradyzoite stage that forms long-lived cysts in skeletal muscle and the central nervous system. Although approximately 30% of the world's population have *T. gondii* infection and harbor cysts in the brain, overt disease symptoms such as encephalitis are only evident during immune suppression.[1] However, an increasing number of studies are providing evidence that toxoplasmosis is associated with subtle changes in behavior in animals and humans, and it has been noted that the incidence of mental diseases such as schizophrenia is greater in *T. gondii*–infected individuals.[2]

Schizophrenia is a serious neuropsychiatric disease of uncertain etiology. Numerous hypotheses have been proposed to explain schizophrenia. Regarding genetic and environmental factors, some studies have rekindled interest in the role of infectious agents in schizophrenia as possible etiologic agents, perhaps in persons who also have an increased genetic susceptibility.[3]

Several epidemiological studies suggested that schizophrenia patients have a higher prevalence of anti–*T. gondii* antibodies than those in the control groups.[4–6] It was documented that *Toxoplasma*-seropositive people may have psychiatric changes even with clinically unapparent toxoplasmosis.[7] Regarding the neuropathology, in vitro studies on *T. gondii* have shown that glial cells, especially astrocytes, are selectively affected.[8,9] Furthermore, postmortem studies of brains from individuals who had schizophrenia have reported many glial cell abnormalities,[10] including decreased numbers of astrocytes.[11] It has also been shown that *Toxoplasma* infections may affect levels of dopamine, norepinephrine, and other neurotransmitters, which are known to be affected in people with schizophrenia. The possibility exists that the excess dopamine thought to occur in individuals with schizophrenia might be mediated by *T. gondii* rather than made by the affected individuals.[12]

In addition, endocannabinoids that are produced by everyone act as homeostatic regulators of all body systems, including the nervous system. Imbalances in the endocannabinoid system have been considered as possible causes of various forms of mental illness and abnormal behavior. It was suggested that an undefined subset of schizophrenia is caused by an excess of endocannabinoids that are produced to protect the brain in response to infections by agents such as *T. gondii*.[13]

There is an increasing attention toward the role of adhesion molecules in the pathogenesis and immune responses to parasitic infections. Large numbers of adhesion molecules are important for the transmigration of leukocytes to the sites of inflammation and for the recognition between leukocytes and arriving target cells.[14] Intercellular adhesion molecule 1 (ICAM-1) is expressed on a variety of cells, including B and T lymphocytes, granulocytes, fibroblasts, keratinocytes, and endothelial cells at sites of inflammation. It has been suggested that a

circulating soluble form of ICAM-1 (sICAM-1) that is detected in human serum is an early marker of immune activation and response.[15,16] The expression of ICAM-1 was up-regulated on the surface of brain endothelial cells after infection with *T. gondii* tachyzoites.[17] Furthermore, up-regulation of sICAM-1 increased in acute and reactivated toxoplasmic encephalitis.[18] This up-regulation was believed to be a specific response to the interaction between host cells and the parasite at different stages of parasite infiltration, parasite intracellular products.[19]

To ascertain the possible association between *T. gondii* and schizophrenia, the present study aimed to investigate the frequency of *T. gondii* infection among Egyptian schizophrenia patients and to determine the usefulness of sICAM-1 as an indicator of *Toxoplasma* role in the etiopathogenesis of schizophrenia.

MATERIALS AND METHODS

Subjects

Sixty schizophrenia patients (40 males and 20 females, with the mean age of 38 ± 10 years) diagnosed by 2 independent psychiatrists according to *Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition* diagnostic criteria (G1) were selected from patients admitted to the Institute of Psychiatry, Ain-Shams University Hospital. The patients either were experiencing a first episode of schizophrenia and had never been medicated with neuroleptics or had had a washout period of treatment of at least 4 months. All selected patients had normal brain magnetic resonance imaging scans, no history of head trauma or brain surgery or previous meningitis/encephalitis, and no history of alcoholism or substance abuse. The patients did not have any known immunological abnormalities, a comorbid medical condition, or a neurological disease.

Two control groups were composed of 30 pretreated patients with depressive disorder (G2) (another psychiatric disorder) with a mean age of 37 ± 11 years (19 males and 11 females) and 20 healthy volunteers (G3). The healthy volunteer group was chosen from among health care workers and from among the relatives/visitors of the patients. They were screened for the absence of physical and psychiatric disorders and matched to patients according to sex (18 males and 12 females) and age (mean, 37.76 ± 10.50). After matching, we verified that the case and control groups did not differ significantly with respect to these factors ($P > 0.05$). Five milliliters of blood was taken under sterile conditions from all subjects included in this study. The blood samples were then centrifuged at 1000 rpm, and the sera were stored at −20°C until the analysis for anti–*T. gondii* immunoglobulin G (IgG) antibodies positivity and determination of the sICAM-1 serum level.

Determination of the Anti–*T. Gondii* IgG Antibody Positivity

Serum samples were analyzed for anti–*T. gondii* IgG antibodies by a commercially available quantitative enzyme-linked immunosorbent assay (ELISA) kit (UBI MAGIWEL ToxoIgG, Catalog No. IA-101; United Biotec Inc, Mountain View, Calif). The test was performed in the Laboratory of Parasitology Department, Research Institute of Ophthalmology, Giza, Egypt, following the manufacturer's instructions. Serum samples were diluted in sample diluent at 1:100. Then, 100 μL of reference calibrators, positive control, negative control, and diluted serum samples, was added to wells of microtiter plate coated with purified *T. gondii* RH strain antigen, incubated for 30 minutes at room temperature, and followed by washing 5 times. Then 100 μL of enzyme conjugate (anti–human IgG conjugated to horseradish peroxidase) was added to each well, except the blank well, and incubated for 30 minutes at room temperature. After washing, 100 μL of tetramethyl benzidine substrate was added to each well, including the blank well, and incubated for 15 minutes at room temperature, followed by addition of 50 μL of the stop solution. The optical densities were read at 450 nm with a microwell reader. According to the results of ELISA, each group enrolled in this study was subdivided to subgroups *a* and *b* for positive and negative anti–*T. gondii* IgG antibodies, respectively.

Determination of sICAM-1 Serum Level

Soluble ICAM-1 serum levels were detected using a commercially available ELISA (Quantikine Human sICAM-1/CD54 Immunoassay R&D Systems) according to the manufacturer's instructions. This assay uses the quantitative sandwich enzyme immunoassay technique. Serum samples were diluted in calibrator diluents at 1:20. One hundred–microliter standards, samples, controls, and conjugate are pipetted into the appropriated wells of microtiter plate coated with monoclonal antibody specific for sICAM-1, incubated for 1.5 hours at room temperature, and any sICAM-1 present is sandwiched by the immobilized and the enzyme-linked monoclonal antibody specific for sICAM-1. After a wash to remove any unbound substances and/or antibody-enzyme reagent, 200 μL of substrate solution was added to each well and incubated for 30 minutes at room temperature, with protection from light, and the reaction was terminated by a stop solution (50 μL). The final adsorbance was determined at 450 nm using a microplate reader. Values of samples were calculated from a standard curve generated from standards of known concentrations.

Ethical Considerations

An informed consent was taken from all participants after the study purpose was explained. The study was approved by Research Ethics Committee, Faculty of Medicine, Ain Shams University.

Statistical Analysis

Collected data were coded, tabulated, and introduced to a PC using the Statistical Package for Social Science for Windows version 11.0. The χ^2 test was used to analyze the frequency of anti–*T. gondii* IgG positivity in the studied groups. The serum level of sICAM-1 in the studied groups was expressed as mean ± SD. These means were compared between groups using the Student *t* test to clarify statistically significant differences. A value of $P < 0.05$ was considered statistically significant, and a value of $P < 0.001$ was considered statistically highly significant.

RESULTS

In the present study, 34 (56.7%) of the 60 cases of schizophrenia group (G1), 12 (40%) of the 30 cases of depressive disorder group (G2), and 6 (30%) of the 20 healthy volunteers group (G3) were positive for anti–*T. gondii* IgG antibody. The percentage of the anti–*T .gondii* IgG antibody positivity in the schizophrenia group (56.7%) was significantly higher ($P < 0.05$) than in the healthy volunteers group (30%) (Table 1).

Regarding the level of the sICAM-1 in the schizophrenia anti–*T. gondii* IgG–seropositive subgroup (G1a), there was a significantly higher level compared with those of the healthy volunteer anti–*T. gondii* IgG–seropositive subgroup (G3a) and the depressive disorder anti–*T. gondii* IgG–seropositive subgroup (G2a) ($P < 0.05$). Meanwhile, there was a significantly higher level of sICAM-1 in the schizophrenia anti–*T. gondii* IgG–seropositive subgroup (G1a) compared with that of the schizophrenia anti–*T. gondii* IgG–seronegative subgroup (G1b)

TABLE 1. Result of the Anti–*T. Gondii* IgG Antibody Positivity in the Studied Groups

Studied Groups	N	Anti–*T. Gondii* IgG	
		Positive	Negative
Schizophrenia group (G1)	60	34 (56.7%)*	26 (43.3%)
Depressive disorder group (G2)	30	12 (40%)	18 (60%)
Healthy volunteer group (G3)	20	6 (30%)	14 (70%)

*Significant difference as compared with anti–*T. gondii* IgG positive in G3 ($P < 0.05$).

($P < 0.001$). Concerning the depressive disorder anti–*T. gondii* IgG–seropositive subgroup (G2a), there was a significantly higher level of sICAM-1 compared with that of the depressive disorder anti–*T. gondii* IgG–seronegative subgroup (G2b) ($P < 0.05$) (Table 2).

DISCUSSION

There has been long-standing interest in investigating a possible association between exposure to protozoan parasite *T. gondii* and the development of severe psychiatric disorders, including schizophrenia,[20] and there is increasing evidence that schizophrenia has a neurodevelopmental etiology, and several prenatal infections, including toxoplasmosis, have been associated with the risk of schizophrenia.[21]

Toxoplasma gondii has emerged as a possible cause of schizophrenia for a variety of reasons: (1) many studies have reported that individuals with schizophrenia, compared with controls, have a higher prevalence of antibodies to *T. gondii*; (2) some individuals with adult toxoplasmosis develop psychotic symptoms (delusions and hallucinations) similar to those of schizophrenia; (3) epidemiologically, there are many similarities between toxoplasmosis and schizophrenia; (4) antipsychotic drugs known to be effective in schizophrenia also inhibit some parasites, including *T. gondii*; (5) *Toxoplasma* has been shown to induce elevated levels of dopamine in experimentally infected animals (elevated dopamine is commonly seen in individuals with schizophrenia); and (6) studies have shown that individuals with schizophrenia, compared with controls, have had greater exposure to cats in childhood.[22]

Infection with *T. gondii* results in the invasion of the brain and the formation of tissue cysts that persist throughout the life of the host without causing symptoms because immunocompetent hosts control this chronic infection with a T lymphocyte–driven defense.[23] Activated T-helper cells secrete interferon-γ (IFN-γ), which induces the enzyme indoleamine 2,3-dioxygenase. This enzyme degrades the tryptophan that is needed for the tachyzoitic phase of *T. gondii*. Consequently, activated parasites die by tryptophan depletion,[24] and tryptophan degradation products may result in excess dopaminergic tone. Thus, the host defense system might produce a lack of serotonin and an accumulation of dopaminergic activity. Furthermore, *T. gondii* genome is known to contain 2 aromatic amino acid hydroxylases that potentially could directly affect dopamine and/or serotonin biosynthesis.[2] Psychiatrically, this suggests depressive and psychotic syndromes.[25] Therefore, this parasitic chronic infection, which shifts between silent and microactivated states in conjunction with the host defense system, presents an attractive theoretical schema for increased frequencies of this infection in patients with affective and psychotic syndromes.

The results of the present study showed that the frequency of anti–*T. gondii* IgG antibody positivity was greater in schizophrenia patients (56.7%) than in patients with depressive disorder (40%); despite of this, the difference was not statistically significant. However, the frequency was significantly higher in schizophrenia patients (56.7%) than that in the healthy volunteer group (30%), in accordance with results of Tamer et al,[26] Hamidinejat et al,[27] Ahmad et al,[28] Alvarado-Esquivel et al,[29] and Tedla et al.[30] This higher seropositivity supports an association between *T. gondii* infection and schizophrenia. In this study, the frequency of anti–*T. gondii* IgG seropositivity in Egyptian schizophrenia patients (56.7%) was lower than those reported from Turkey (66%)[4] and Ireland (60%),[3] although it was higher than those reported in Iran (35%),[28] Germany (34%, 42%),[5,6] and China (14%).[31] The difference in seropositivity rates among the previous studies and our study might be explained by differences in terms of the neuropathogenicity of strains of *Toxoplasma* prevalent in different areas of the world,[32] differences in the genetic susceptibility of different human populations, and the route and timing of the infection either before or after the onset of the disease.[33] Each of these factors is known to lead to different disease outcomes, and the study of these factors will be important in further defining the relationship between *T. gondii* and schizophrenia.

Under healthy conditions, the endothelial cells of the blood-brain barrier (BBB) express very low levels of adhesion molecules that could be used by leukocytes for transendothelial migration.[34,35] The ICAM-1 is constitutively expressed on the cell surface of different cell lines after activation by proinflammatory signals like IFN-γ, and during inflammation, it is involved in the adhesion and penetration of immune cells from the blood into target organs through barriers such as the BBB. In the CNS, ICAM-1 is expressed on astrocytes, microglial cells,

TABLE 2. Serum Level of sICAM-1 in the Studied Subgroups

Studied Groups		n	Mean ± SD, ng/mL	Range, ng/mL
Schizophrenia group (G1)	G1a: anti–*T. gondii* IgG positive	34	262.1 ± 127.8 *†	14.5–450
	G1b: anti–*T. gondii* IgG negative	26	63.7 ± 41.8	15.2–145
Depressive disorder group (G2)	G2a: anti–*T. gondii* IgG positive	12	145.8 ± 47.6‡	94–250
	G2b: anti–*T. gondii* IgG negative	18	90.4 ± 41.8	30.8–165
Healthy volunteer group (G3)	G3a: anti–*T. gondii* IgG positive	6	133.1 ± 44.6	65–185
	G3b: anti–*T. gondii* IgG negative	14	88 ± 72.5	10–250

*Significant difference as compared with G2a and G3a ($P < 0.05$).

†Highly significant difference as compared with G1b ($P < 0.001$).

‡Significant as compared with G2b ($P < 0.05$).

and also neurons, [36,37] and these cells in the CNS are sources of circulating ICAM-1.[38] Soluble ICAM-1 represents a circulating form of ICAM-1 that resulted principally from proteolytic cleavage (shedding) of membrane ICAM-1.[39] The ICAM-1 and its circulating form had been implicated in the development of many diseases.[40]

The results of this study showed that sICAM-1 exhibited a significantly higher level in anti–*T. gondii* IgG–seropositive schizophrenia subgroup compared with those in healthy volunteers and depressive disorder subgroups. Meanwhile, there was a significantly higher level of sICAM-1 in the anti–*T. gondii* IgG–seropositive schizophrenia subgroup compared with that in the seronegative schizophrenia subgroup. Concerning the anti–*T. gondii* IgG–seropositive depressive disorder subgroup, there was a significantly higher level of sICAM-1 compared with that of the seronegative depressive disorder sugroup. These results suggested that the higher sICAM-1 is more linked to anti–*T. gondii* IgG–seropositive schizophrenia patients. This suggestion might be acceptable because many investigators have reported a possible role of ICAM-1 in infection with *T. gondii*.[41,17] *Toxoplasma gondii* crosses nonpermissive biological barriers, such as the BBB, thereby gaining access to tissues where it most commonly causes severe pathology.[42] This ability of crossing the BBB was documented to be through up-regulation in ICAM-1 expression on the surface of brain endothelial cells after infection with *T. gondii* tachyzoites.[43,17] Infection with *T. gondii* elicits a T_H1-type immune reaction with a prominent production of cytokines such as IFN-γ, which is responsible for up-regulation of the ICAM-1 expression.[44] Barragan et al[41] found that ICAM-1 and sICAM-1 antibodies have inhibited transmigration of parasite across cellular barriers, implicating this receptor in the process of transmigration. Furthermore, Silva et al[45] found that the severity of toxoplasmic encephalitis in mice was associated with increased ICAM-1 expression in the central nervous system and higher BBB permeability.

Soluble ICAM-1 is considered as a marker of inflammatory BBB impairment, and it seems to be valid for discrimination between immunology-induced BBB impairment and BBB impairment of other reasons.[46,47] A discrete disturbance of the BBB has been described in about 20% to 30% of schizophrenia patients, especially in schizophrenia negative symptoms[48] and during treatment with antipsychotic medication.[49,50]

Schwarz et al[51] showed that the serum concentrations of sICAM-1 were significantly lower in unmedicated and medicated schizophrenia patients compared with those of the healthy controls, and the comparison of unmedicated and medicated patients in pairs showed a trend to increased levels during antipsychotic treatment. Moreover, they found that sICAM-1 was higher in individuals with BBB impairment than in those with intact BBB and concluded that reduced sICAM-1 levels in schizophrenia indicate a decreased activity of the cellular immune system because signs supporting this are the reduced lymphocyte stimulation to specific antigens in the acute phase of schizophrenia,[52] reduced production of IFN-γ (a cytokine of cellular immune activation) in lymphocyte cultures of acutely ill schizophrenia patients,[53] and the clearly reduced immune response to hepatitis B vaccination in schizophrenia patients compared with that of the control subjects.[54]

Considering that ICAM-1 and its soluble form are induced on the cell surface of different cell lines after activation by proinflammatory signals like IFN-γ, which is produced by activated T-helper cells resulting from chronic *Toxoplasma* infection,[23,24,44] and the documented reduced production of IFN-γ in lymphocyte cultures of acutely ill schizophrenia patients,[53] the significantly higher levels of sICAM-1 in seropositive schizophrenia patients obtained in this study suggest that this higher level in these patients is linked to *Toxoplasma* infection and also suggest the usefulness of sICAM-1 as an indicator of *Toxoplasma* role in the etiopathogenesis of schizophrenia.

In conclusion, toxoplasma seropositivity frequency was significantly higher in schizophrenia patients than in the healthy volunteer group, and sICAM-1 exhibited a significantly higher level in the toxoplasma-seropositive schizophrenia subgroup compared with healthy volunteers and the seronegative schizophrenia subgroup, suggesting that the higher sICAM-1 is more linked to *Toxoplasma*-seropositive schizophrenia patients. These statistically significant results support the association between *T. gondii* infection and schizophrenia and suggest the usefulness of sICAM-1 as an indicator of the possible role of *Toxoplasma* among other factors in the etiopathogenesis of schizophrenia.

As most of the studies related to toxoplasmosis and schizophrenia are based on serological tests, not on the direct detection of *T. gondii* organism or its DNA in infected body fluids, therefore, additional studies should focus on serological and molecular methods in the sera and cerebrospinal fluid of individuals with recent-onset psychosis, and this would be important in better defining the relationship between *T. gondii* infection and schizophrenia. Further studies are needed to evaluate the effect of toxoplasmic treatment on improvement of psychotic symptoms in patients with early-onset psychosis with *Toxoplasma* seropositivity and higher sICAM-1 levels.

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