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Research Article
Toxoplasmosis in Schizophrenic Patients: Immune-diagnosis and Serum Dopamine Level

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Abstract

Background and Objective: *Toxoplasma gondii* is an obligate intracellular protozoan parasite widely distributed all over the world. It has been associated with various psychiatric conditions as schizophrenia. This study aiming to evaluate the association between *T. gondii* infection and schizophrenia and to estimate the effect of *T. gondii* infection on the serum dopamine level among schizophrenic patients. **Materials and Methods:** A case-control study was conducted over 45 schizophrenic patients and 44 normal controls. Serum IgM and IgG anti-*T. gondii* antibodies were detected by a commercial ELISA Kit. The immunoblotting method was performed for the detection of IgG anti-*Toxoplasma* dopamine was detected by the human dopamine ELISA kit. **Results:** Anti-*T. gondii* IgM was negative in all the 90 studied individuals. However, anti-*T. gondii* IgG was positive in 25 schizophrenic patients (55.6%) and 13 normal healthy controls (28.9%). Immunoblotting showed stronger specific reaction to proteins with molecular weights 50 and 60 kDa by +ve IgG schizophrenic patients. The serum dopamine level among schizophrenic patients was increased as compared to healthy controls (47.22 and 25.79%, respectively; $p < 0.001$). In addition, the dopamine levels in +ve IgG were higher than those of –ve IgG schizophrenic patients. **Conclusion:** These results suggest that chronic *T. gondii* infection causes high dopamine levels that may lead to schizophrenia. About 55% of schizophrenic patients showed positive IgG reactions to *Toxoplasma* within this population, the dopamine levels were higher than seronegative population and revealed both 50 and 60 kDa proteins band specific to *Toxoplasma*.

Key words: Toxoplasmosis, schizophrenia, IgG, IgM, immunoblotting, dopamine

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Parasitic diseases affecting the Central Nervous System (CNS) are important sources of morbidity and mortality worldwide[1]. *Toxoplasma gondii* (*T. gondii*) is one of the neurotropic intracellular protozoan parasites that infect the brain, affects a large number of the population in world[2]. Humans and most of the warm-blooded mammals become infected through the ingestion of environmentally resist sporulated *T. gondii* oocysts or ingest the infected intermediate host tissues containing *T. gondii* tissue cysts[3].

Toxoplasma gondii tachyzoites are the rapidly replicating stage of the parasite. They can be distributed all over the body through the blood stream and can invade different tissues. The rapid replication and release of tachyzoites from host cells cause a strong inflammatory response and tissue damage. Therefore, tachyzoites are responsible for the clinical manifestations of toxoplasmosis[4]. They gain access to the brain through the invasion of various brain cells, including astrocytes and neurons, where it forms cysts and it can then establish a continuous infection within CNS. In some infected individuals, it causes neurological and psychiatric symptoms[5].

Schizophrenia is a neuropsychiatric disorder with a worldwide prevalence. This disorder is characterized by delusions, hallucinations, disturbances in thinking and communication[6]. The main substances responsible for the development of these symptoms are dopamine, serotonin, GABA and glutamate[7]. The protozoan *T. gondii* has been repeatedly associated with schizophrenia as it modulates the secretion of many neurotransmitters including dopamine which is responsible for altering human behavior[8-10]. The potential link between *Toxoplasma* infection and schizophrenia have been detected by several epidemiological, neuropathological, serological and neurophysiological studies carried out on both schizophrenic and non-schizophrenic populations[11].

The pathogenesis of *Toxoplasma* infection in the brain can be explained by different mechanisms including the production of pro-inflammatory cytokines that have a neuromodulatory effect[12]. Other mechanisms include direct and selective toxic impairment of neurons and neuronal cells, especially the excessive inhibition of glutamatergic and nicotinergic neurotransmission[13]. *Toxoplasma gondii* synthesizes tyrosine hydroxylase that converts tyrosine amino acid, which was found in neurons into L-DOPA and also it produces the DOPA decarboxylase enzyme which converts L-DOPA into dopamine[9].

Diagnosis of *T. gondii* infection by molecular technique was performed by taking biological samples from different body sites which can be difficult or maybe impossible[14]. Accordingly, diagnosis of toxoplasmosis is based mainly on serology like IgG and IgM detection by the high sensitivity and good specificity expected tests such as; ELISA[15] and followed by immune diagnosis as western blot developed on blood samples[16].

Generally, toxoplasmosis has a benign course but it can be a cause of certain neuropsychiatric disorders including schizophrenia, so the first purpose of this study is to detect *T. gondii* infection in patients with schizophrenia in comparison with normal individuals as a control group. The second purpose is to estimate the effect of *T. gondii* infection on the serum dopamine level.

MATERIALS AND METHODS

Study settings and sampling: A case-control study was carried out from November, 2018 to September, 2019 on 90 individuals (both sexes) with age above 15 years. The study populations were divided into two groups, the first group was schizophrenic patients who visited outpatient clinics of Beni-Suef University, Beni-Suef psychiatric hospital or admitted in the internal department of the hospital (No. = 45). The patients had been diagnosed clinically by psychiatrists. The second group included age and sex-matched healthy non-schizophrenic individuals (controls, No. = 45). A pre-designed structural questionnaire sheet was utilized to collect the subjects socio-demographic characteristics and clinical data.

ELISA for detection of serum *T. gondii*-specific IgM and IgG: All serum samples (case and control) were serologically assayed for detection of *Toxoplasma*-specific IgM and IgG by a commercial ELISA Kit (Precheck Bio, Kyunggi-Do, Korea). Optic Density (OD) was read at 450 nm using ELISA reader and the Cut Off value (CO) was calculated as mean negative control×2.1 and the optimal concentration of antigen, antibody and conjugate were estimated after the checkerboard titrations[17]. The OD values below the cut off value were considered as negative.

Parasite maintenance: Virulent RH strain of *T. gondii* was obtained from Zoonotic Diseases Department, National Research Center (NRC) and was used to prepare whole soluble tachyzoite antigen for use in Western blot. *Toxoplasma gondii*

infective stages had been isolated from pooled meat, heart and diaphragm samples obtained from slaughtered sheep as the method carried out by Shaapan and Ghazy[18].

Antigen preparation: The *T. gondii* maintained by serial passage in mice and antigen was prepared from *T. gondii* tachyzoites according to the method described by Elfadaly *et al.*[19]. The protein content of antigen was assayed using a total protein assay kit (Giesse Diagnostics Inc., Roma, Italy) where protein estimation should not be less than 350 μg mL^{-1}. They were stored in small tubes at -20°C until used in western blot.

Immunoblotting for detection of *T. gondii* specific proteins to human IgG: Immunoblotting was carried out as previously described[20]. *Toxoplasma gondii* tachyzoites antigen preparation was separated in SDS-10% polyacrylamide gels and transferred to nitrocellulose membranes (0.45 μm, Heidelberg, Serva Electrophoresis GmbH, Germany) by electroblotting. From each of the human populations (Schizophrenic and non-schizophrenic), positive and negative samples (n = 13) based on the OD values of IgG ELISA were selected. The immune complexes were detected by horseradish peroxidase-labeled goat anti-human IgG antibody (1: 5000; KPL, Maryland, USA). After 2 h of incubation at RT, bands were developed by adding substrate (50 mg 3,3-Diaminobenzidine tetrahydrochloride and 100 μL H_2O_2 in 100 mL PBS).

Serum dopamine levels: Dopamine was detected by the human dopamine ELISA kit (Bioassay Technology Laboratory, Shanghai, China). The procedure was performed according to the instructions provided.

Statistical analysis: The SPSS (version 20) statistical program (SPSS Inc., Chicago, IL) was used to carry out a one-way analysis of variance (ANOVA) and Chi-Square (χ^2) on the data. When significant differences by ANOVA were detected, analysis of differences between the means of human subjects were performed by Dunnett's test.

RESULTS

Demographic characteristics: patients (N = 45) and healthy subjects (N = 45) were matched in age, sex and residence distribution without a statistically significant difference. Healthy control subjects were 40 males and 5 females with an average age of 37.35±12.1 (SD) years, while schizophrenic patients were 43 males and 2 females with an average age of 39.82±11.3 (SD) years. A family history of schizophrenia did not differ significantly between schizophrenia patients and healthy controls (13.3 and 4.4%, respectively) (p-value = 0.133). Cat exposure as a risk factor for Toxoplasmosis was more frequent among the non- schizophrenic controls as compared with schizophrenic patients (77.8 and 68.9%, respectively) with no statistical significance (p-value = 0.238) (Table 1).

Psychiatric disorders: Schizophrenic patients consisted of not drug-naïve (N = 10) and drug naïve cases (N = 35). The symptoms of schizophrenia among the studied population were summarized in Table 2.

Detection of *T. gondii*-specific IgM and IgG: *Toxoplasma gondii* IgM antibody was negative in all the 90 studied individuals (patients and normal), while *T. gondii* IgG antibody was positive in 13 (28.9%) and 25 (55.6%) of normal healthy

Table 1: Demographic data of the studied populations

	Normal controls (%)	Schizophrenic patients (%)	Total	p-value*
Age (years)				
Mean±SD	37.35±12.1	39.82±11.3	38.59±11.7	0.319[a]
Minimum	18	18	18	
Maximum	61	61	61	
Sex, N (%)				
Male	40 (88.9)	43 (95.6)	83 (92.2)	0.217[b]
Female	5 (11.1)	2 (4.40)	7 (7.80)	
Residence, N (%)				
Urban	23 (51.1)	24 (53.3)	47 (52.2)	0.500[b]
Rural	22 (48.9)	21 (46.7)	43 (47.8)	
Family history, N (%)				
Negative	43 (95,6)	39 (86.7)	82 (91.1)	0.133[b]
Positive	2 (4.4)	6 (13.3)	8 (8.9)	
Cat exposure, N (%)				
No	10 (22.2)	14 (31.1)	24 (26.7)	0.238[b]
Yes	35 (77.8)	31 (68.9)	66 (73.3)	

*p-value ≤0.05 is considered significant, [a]Analyzed by independent sample t-test, [b]Analyzed by Chi-Square (χ^2) test, N = 45

Table 2: Psychiatric disorders among studied population

	Normal controls (%)	Schizophrenic patients (%)	Total	p-value*
Behavioral change, N (%)				
No	45 (100.0)	0 (0.00)	45 (50.0)	<0.001*
Yes	0 (0.00)	45 (100.0)	45 (50.0)	
Personality change, N (%)				
No	45 (100.0)	0 (0.00)	45 (50.0)	<0.001*
Yes	0 (0.00)	45 (100.0)	45 (50.0)	
Impaired learning, N (%)				
No	45 (100.0)	0 (0.00)	45 (50.0)	<0.001*
Yes	0 (0.00)	45 (100.0)	45 (50.0)	
Impaired memory, N (%)				
No	45 (100.0)	0 (0.00)	45 (50.0)	<0.001*
Yes	0 (0.00)	45 (100.0)	45 (50.0)	
Mental retardation, N (%)				
No	45 (100.0)	44 (97.8)	89 (98.9)	0.500
Yes	0 (0.00)	1 (2.20)	1 (1.10)	
Increased activity, N (%)				
No	45 (100.0)	0 (0.00)	45 (50.0)	<0.001*
Yes	0 (0.00)	45 (100.0)	45 (50.0)	
Hallucination, N (%)				
No	45 (100.0)	0 (0.00)	45 (50.0)	<0.001*
Yes	0 (0.00)	45 (100.0)	45 (50.0)	
Mania, N (%)				
No	45 (100.0)	43 (95.6)	88 (97.8)	0.247
Yes	0 (0.00)	2 (4.40)	2 (2.20)	
Suicidal attempt, N (%)				
No	45 (100.0)	40 (88.9)	85 (94.4)	0.028*
Yes	0 (0.00)	5 (11.1)	5 (5.60)	

*p-value $\leq$0.05 is considered significant by Chi-Square (χ^2) test, N = 45

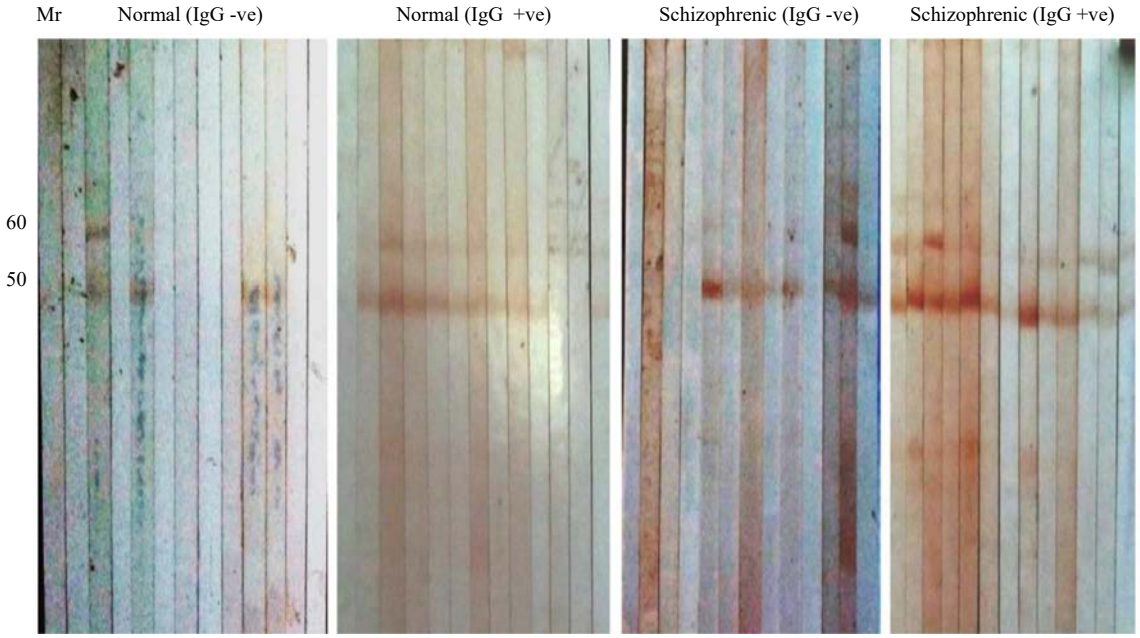

Fig. 1: Immunoblotting (10% SDS-PAGE): more schizophrenic (-ve IgG) individuals (n = 10) were recognize a band at 50 kDa in comparison to normal (IgG -ve) individuals. Similarly, recognition of 50 and 60 kDa by schizophrenic (+ve IgG, n = 13) was more common and stronger than normal (+ve IgG, n = 8). The comparison between two schizophrenic sub-populations (-ve and +ve IgG) indicated stronger reactions to 50 and 60 kDa proteins by +ve IgG schizophrenic

Table 3: *T. gondii*-specific IgM and IgG among schizophrenic patients and control

	Normal controls (%)	Schizophrenic patients (%)	Total	p-value*
IgM, N (%)				
No	45 (100.0)	45 (100.0)	90 (100.0)	ND[a]
Yes	0 (0.00)	0 (0.00)	0 (0.00)	
IgG, N (%)				
No	32 (71.1)	20 (44.4)	52 (57.8)	0.009*
Yes	13 (28.9)	25 (55.6)	38 (42.2)	

*p-value ≤0.05 is considered significant by Chi-Square (χ^2) test, [a]Not determined, N = 45

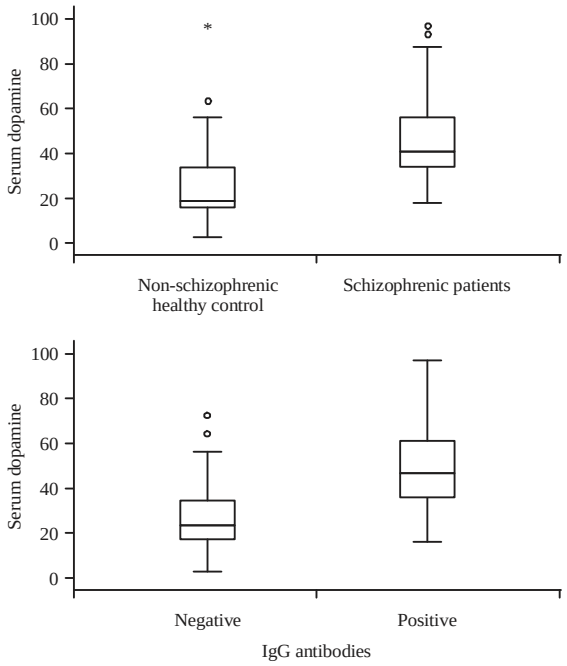

Fig. (2a-b): (a) Dopamine level in the serum among non-schizophrenic (healthy control) and schizophrenic patients. Dopamine level in schizophrenic patients was statistically higher than non-schizophrenic control individuals and (b) Serum dopamine levels in -ve and +ve IgG schizophrenic patients. Dopamine level in +ve IgG schizophrenic patients was statistically higher (p<0.001) than -ve IgG patients

controls and patients with schizophrenia, respectively with statistical significance (p-value = 0.0009) (Table 3). Among the schizophrenic patients, 35 cases were drug naïve (Dnai) and 10 cases were not drugged naïve (nDnai). There was no statistically significant correlation (p = 0.481) between *T. gondii* IgG antibodies and drug naïve patients.

Detection of IgG-specific proteins in *T. gondii* antigen preparation: Both of two human populations (normal and schizophrenic) were divided into two sub-populations according to the negative and positive IgG levels in ELISA. When both of negative IgG sub-populations were compared for the commonly recognized protein bands, schizophrenic recognized commonly bands at 50 and 60 kDa. Similarly, when both positive IgG sub-populations were compared, recognition of 50 and 60 kDa by schizophrenic was more common and strong. Finally, the comparison between the two schizophrenic sub-populations indicated stronger reactions to 50 and 60 kDa proteins by +ve IgG (Fig. 1).

Dopamine levels: The mean level of serum dopamine was significantly higher among schizophrenic patients as compared to normal controls (47.22 and 25.79%, respectively; p<0.001). Moreover, when the mean level of serum dopamine was compared between cases with positive and negative IgG cases, a higher significant (p<0.001) increase was found in the former cases (49.75 and 26.83, respectively) (Fig. 2a, b). However, there was no statistically significant difference between the dopamine levels in Dnai and nDnai cases (48.24 and 45.65, respectively).

DISCUSSION

The current study focused on the detection of *T. gondii* infection in patients with schizophrenia in comparison to the control group and the estimation of the effect of *T. gondii* infection on the serum dopamine level. The present study revealed that the majority of schizophrenic patients were male (43 from 45) (95.6%) with a mean age of 39.82±11.3 (SD) years while the mean age of normal subjects were 37.35±12.1 (SD). This could be a result of the high exposure of males to stress than females. Moreover, low educational habits might prevent the female from seeking medical advice in such psychiatric disorders. The schizophrenic disorder prevalence was higher among males[21]. The geographical distribution of schizophrenic patients were more in urban areas than rural areas without statistical significance and this could be referred to highly expose urban people to stress. The studied cases with schizophrenia were either first attack (N = 10) or drug naive cases (N = 35). Schizophrenic patients under treatment

with antipsychotic drugs were excluded from the study as antipsychotic medications are immunomodulatory agents and can inhibit the replication of *T. gondii* tachyzoites[22].

Detection of chronic infection is based mainly on serology as the cyst in humans is not detectable, except postmortem [10]. Anti-*T. gondii* IgM antibody was negative in all the studied individuals (N = 90). However, anti-*T. gondii* IgG antibody was positive in 25 schizophrenic patients (55.6%) and 13 normal healthy controls (28.9%) with statistical significance between the two groups (p-value = 0.0009). Higher IgG seropositive cases than IgM indicated the chronic infection of schizophrenic patients with toxoplasmosis[23]. Similarly, the adult patients suffering from schizophrenia were reported to have no or few positive cases (4.1%) of IgM antibodies indicated an acute infection[24]. Previous studies reported higher rates of *T. gondii* infection in schizophrenic patients compared to normal cases[25,26]. Likewise, the current study could showed a higher number of positive anti-*T. gondii* IgG (55.6%) in schizophrenic cases compared to 28.9% in healthy control. These results confirmed the previous reports which indicated that *Toxoplasma* infection is a risk factor for the genesis and symptomatology of schizophrenia[27]. The mechanism of chronic infection-induced schizophrenia was attributed to neuromodulator disturbances, together with morphological and functional alterations, e.g., low-grade neuro-inflammation, which is likely to induce psychopathological symptoms[24].

The schizophrenic patients with toxoplasmosis exhibited different dopamine levels in serum[28]. This was previously attributed to two genes of *Toxoplasma* encoding thyroxin hydroxylase, a rate-limiting enzyme in dopamine synthesis[8]. However, the current results showed an increase of dopamine levels in seropositive compared to seronegative schizophrenic cases. Such changes in the dopamine levels could be responsible for behavioral changes[7]. Experimental studies had shown that the chronic infection with *T. gondii* elevates the local brain dopamine concentrations, as in patients suffering from schizophrenia[7,28]. It was proposed that the presence of the parasite cysts in the brain is the reason for increased dopamine levels. The data of western blotting showed specific recognition of two proteins at 50 and 60 kDa by schizophrenic patients. However, the reaction was more common and stronger in patients who showed +ve IgG in ELISA. This showed that the chronic infection-induced schizophrenia was related to IgG specific reactions to 50 and 60 kDa in tachyzoites. However, late toxoplasmosis was associated with immunosuppression[29].

The positive correlations observed between dopamine levels in schizophrenic patients and toxoplasmosis suggested to analyze the ability of other pathogens associated with schizophrenia and other neurological disorders to directly alter dopamine metabolism.

CONCLUSION

The psychiatric disorders were associated with an immunosuppressive state during late toxoplasmosis, increased dopamine levels 55% of schizophrenic patients were showed positive IgG reaction to *Toxoplasma*. Within this population, the dopamine levels were higher than seronegative population and showed a specific reaction to both 50 and 60 kDa proteins of *Toxoplasma*. This might highlight the worthiness for further experiments on the potential role of these proteins as diagnostic agents. In addition, the identification of those proteins is necessary.

SIGNIFICANCE STATEMENT

Toxoplasmosis has been associated with various psychiatric conditions as schizophrenia. This disorder is a neuropsychiatric disorder with worldwide prevalence and characterized by delusions, hallucinations, disturbances in thinking and communication. During the late or chronic stage of toxoplasmosis, the psychiatric disorders like schizophrenia were associated especially with immunosuppressive individuals. Schizophrenic patients with positive anti-toxoplasma-IgG revealed a higher dopamine level than non-schizophrenic and schizophrenic with the negative anti-toxoplasma-IgG population.

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