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Latent Toxoplasmosis in Immunocompetent Patients Suffering from Some PsychiatricDisorders

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ABSTRACT

Background: Relation between toxoplasmosis and psychiatric disorder can be explained by many factors include abnormal neurochemical transmitter, hormonal alternation, immunological change, dysregulated tryptophan metabolism, and induce abnormalities in specific region of the brain.The aim of the present study was to evaluate the role of latent toxoplasmosis in immunocompetent patients suffering from some psychiatric disorders.**Patients and methods:**This is case control study included 132 patientswhich conducted at Zagazig University Hospitals (Tropical Medicine, and Psychiatry Departments), Psychiatry outpatient, and Clinic Immunology Research Laboratory, Microbiology, and Immunology Department. They were grouped according to The Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria their final diagnosis, clinically by psychiatrist into two groups. The first group included 66 patients with schizophrenia. The second group included 66 patients with bipolar disorders.**Results:**There was a statistically significant difference between two studied groups as regard sex, place of residence and marriage respectively but there was insignificant difference between two studied group as regard age. About 48.5% of schizophrenia patients had history of contact with cats versus 24.2% of bipolar disorders patients, with statistically significant difference between them, while 24.2% of bipolar disease patients gave history of soil contact versus only 3 % of schizophrenia patients with high statistically significant difference. While there was no significant difference among both groups regarding other risk factors.There was a statistically significant relation between contact with a cat and serofrequency in patients with Schizophrenia, 84.4% of patients contact with a cat have Schizophrenia with titre $\geq$ 0.042. There was significant difference in serofrequency and serosenstivitybetween two studied groups**Conclusion:**Toxoplasmosis should be considered as a potential cause for neuropsychiatric disorders for example schizophrenic and bipolar patients.

Keywords: T. gondii; Psychiatric Disorders;Latent Toxoplasmosis

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INTRODUCTION

Toxoplasmosis is an important zoonosis caused by an obligate intracellular parasitic protozoan **(1).**It is usually spread by eating poorly cooked food that contains cysts, exposure to infected cat fecesand from an infected mother to her baby during pregnancy. Rarely, the disease may be spread by blood transfusion**(2).**Toxoplasma gondii is widespread and capable of infecting many mammalian species**.** Up to third of the world's population is infected by toxoplasmosis, but have no symptoms**(3)**. In the United States, approximately 11% of people are infected, while higher prevalence reported in other areas of low socioeconomic countries **(2).**

Due to its asymptomatic nature, it is easy for a host to become infected with Toxoplasma gondii and develop toxoplasmosis without knowing it **(4).** Although mild, flu-like symptoms occasionally occur during the first few weeks following exposure, infection with T. gondii produces no readily observable symptoms in healthy human adults **(3).**In most immuno-competent people, toxoplasma infection enters a latent phase, during which only bradyzoites (tissue cysts) are present**.**These tissue cysts and even lesions can occur in the retina, heart, skeletal muscle, and the central nervous system (CNS), including the brain **(5),** Cysts form in the CNS (brain tissue) upon infection with T. gondii and persist for the lifetime of the host**(6)**

T. gondii has a particular tropism for muscle and brain tissues, where it remains localized in the form of cysts throughout life and establishes a chronic infection stimulating the production of a variety of cytokines by microglia, astrocytes and neurons **(7)**, T. gondii infection is incriminated in the etiopathogenesis of several neuropsychiatric disorders. The role of this infectious agent in the emergence of psychiatric disorders has been documented **(8)**, some studies revealed possible links between latent toxoplasmosis and several psychiatric disorders **(9).**

Therefore, this study aimed to evaluate the role of latent toxoplasmosis in immunocompetent patients suffering from some psychiatric disorders.

PATIENTS AND METHODS

This study was conducted at Zagazig University Hospitals (Tropical Medicine, and Psychiatry Departments), Psychiatry outpatient, and Clinic Immunology Research Laboratory, Microbiology, and Immunology Department from January 2020 to July 2020.This study was designed as a case control study included 132 patients with age ranging between 18 to 45 years. They were 66 with schizophrenia and 66 with bipolar disorders.Informed consent was taken from all participants in the study and IRP approval was taken.

Inclusion Criteria:

All Patients suffering schizophrenia and bipolar disorder aged between18-45years old admitted in psychiatry department or in outpatient psychiatric clinic has been diagnosed clinically by psychiatrist according to The Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria. Patientswere divided into two groups:

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- **Group I:**included 66 patients with schizophrenia.
- **Group II:**included 66 patients with bipolar disorders.

Exclusion Criteria:

- Patients with history of alcoholism or drug abuse.
- Immunocompromised patients.
- The patients with any known immunological abnormalities or a serious physical illness, or a neurological disease.

Methods:

1. Full medical history (full history taking include: animal contact, contact with cats feces, consumption of raw or undercooked meat, unpasteurized milk unwashed vegetables, fruits, and untreated water),with socio demographic characteristics include (age, gender, birth place, residence, occupation, education level, cat ownership).
2. Clinical data explored in patients will include (type, course, and evaluation time of psychiatric disorder, treatment response, and presence of concomitant disease, blood transfusion, and surgical history).
3. Thorough clinical examination (general and local).
4. Diagnosis of psychiatric disorders will be done according to The Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria.
5. **(Anti toxoplasma IgG) by enzyme-linked immunosorbent assay (ELISA), (Stat Fax ® 303 Plus).**

Colorimetric assay: the optical density was read at 450 nm.Using indirect ELISA principle to detect ToxoplasmaIgG. Purified Toxoplasma antigen Toxoplasma antigen is adsorbed in solid phase to the polystyrene reaction microplate. If there is Toxoplasma IgG antibody in test sample, it binds to Toxoplasma antigen coated in microplate, forms antigen-antibody complex, and then binds to the enzyme labeled anti-antibody and forms antigen-antibody-antiantibodycomplex on surface of the microplate, and display blue color in corresponding well via the action of substrate. Therefore, it can detect specifically the Toxoplasma IgG in human serum/plasma.

Specimen Collection and Preparation:

3 ml venous blood was taken from every patient in plain vacuum tube to clot for 20 minutes at 37c then centrifuged for 5 minutes on 3000 rpm, sera were separated and stored at -20°C till used.

Procedure:

All reagents should be allowed to reach room temperature for 15 minutes before use.Dilute the wash buffer at the rate of 1:40 dilution with distilled water before use. Add 100µL Sample Dilution Liquid in the corresponding hole, each plate should be provided with negative control 2 holes, positive control 1 hole and blank control 1 hole. Add 10µL sample in the corresponding hole, mix by using the pipette, add 100µL negative control and positive control to negative control holes and positive

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control hole.Shake gently to mix for 30 s. Incubate at 37 ℃ for 20 minutes with the sealing plate membrane sealing plate. At the end of the incubation, remove and discard the plate cover. Take out, add wash buffer to each well for 20 seconds. Repeat 5 times. Respectively adding Conjugate 50μL, Incubate at 37 ℃ for 20 minutes with the sealing plate membrane sealing plate.Repeat the wash step for 5 times as in step 6. Add Substrate A (50μL) and Substrate B 1 drop (50μL), Incubate at 37 ℃ for 10 minutes with the sealing plate membrane sealing plate.Add 50μL Stop Solution to each well, mix gently by shaking, read the absorbance within 10 minutes after stopping the reaction. Calibrate the plate reader with the Blank well and read the absorbance at 450nm.

interpretation:

Cut off = 0.042; Positive Results: ≥ 0.042.; Negative Results: < 0.042.

Statistical analysis:

Data collected and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) software for analysis. According to the type of data qualitative represent as number and percentage, quantitative continues group represent by mean±SD. Differences between quantitative independent multiple by ANOVA or Kruskal Wallis. P value was set at <0.05 for significant results &<0.001 for high significant result.

RESULTS

The present study showed a statistically significant difference between two studied groups as regard sex , place of residence and marriage (P <0.001, 0.005, 0.019) respectively but there was insignificant difference between two studied group as regard age **(Table 1)**.

About 48.5% of schizophrenia patients had history of contact with cats versus 24.2% of bipolar disorders patients, with statistically significant difference between them, while 24.2% of bipolar disease patients gave history of soil contact versus only 3 % of schizophrenia patients with high statistically significant difference. While there was no significant difference among both groups regarding other risk factors**(Table 2)**.

There was a statistically significant relation between contact with a cat and serofrequency in patients with Schizophrenia, 84.4% of patients contact with a cat have Schizophrenia with titre ≥ 0.042**(Table 3)**.

There was significant difference in serofrequency and serosenstivity between two studied groups**(Table 4)**.

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Table 1: Demographic data of the studied groups

Items	Schizophrenia (N=66)		bipolar disorders (N=66)		test	p-value
• Age mean± SD	34.24±10.58		32.31±8.26		t.test 1.16	0.24
• Sex	NO	%	NO	%	χ^2	
Male	27	40.9	48	72.7	13.61	<0.001**
Female	39	59.1	18	27.3		
• Place						
Urban	39	59.1	23	34.8	7.78	0.005*
Rural	27	40.9	43	65.2		
• Marriage						
single	18	27.3	31	47.0	5.48	0.019*
Married	48	72.7	35	53.0		

SD: Standard deviation, t: Independent t test, χ^2: Chai square test. NS: Non significant (P>0.05)
**: highly significant (P<0.001)

Table 2: Bivariate analysis taking patients with Schizophrenia and bipolar disorders as dependent variable

Variable		Schizophrenia (N=66)		bipolar disorders (N=66)		χ^2	P
		NO	%	NO	%		
Dealing with animals	Yes	53	80.3	58	87.9	1.41	0.23
	No	13	19.7	8	12.1		
Contact with a cat	Yes	32	48.5	16	24.2	8.38	0.004*
	No	34	51.5	50	75.8		
Contact with a dog	Yes	20	30.3	24	36.4	0.54	0.46
	No	46	69.7	42	63.6		
Consumption of raw meat	Yes	5	7.6	4	6.1	0.11	0.73
	No	61	92.4	62	93.9		
Consumption of un boiled milk	Yes	4	6.1	3	4.5	0.15	0.95
	No	62	93.9	63	95.5		
Contact with soil	Yes	2	3.0	16	24.2	12.6	<0.001**
	No	64	97.0	50	75.8		
Blood transfusion	Yes	3	4.5	6	9.1	1.07	0.3
	No	63	95.5	60	90.9		

NS: Non significant (P>0.05) **: highly significant (P<0.001

Table 3: Relation between toxoplasma gondii and serofrequency in Schizophrenia

Variable		Schizophrenia				P
		< 0.042		≥0.042		
		NO	%	NO	%	
Contact with a cat	Yes	5	15.6	27	84.4	0.02
	No	14	41.2	20	58.8	
Contact with a dog	Yes	6	30.0	14	70.0	0.8
	No	13	28.3	33	71.7	
Consumption of raw meat	Yes	2	40.0	3	60.0	0.5
	No	17	27.9	44	72.1	
Consumption of un boiled milk	Yes	0	0.0	4	100.0	0.19
	No	19	30.6	43	69.4	
Contact with soil	Yes	0	0.0	2	100.0	0.36
	No	19	29.7	45	70.3	

NS: Non significant (P>0.05)

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Table 4: Serofrequancy and serosenstivity between the studied groups

Variable	N	Titre <0.042		Titre ≥0.042		χ^2	P
		NO	%	NO	%		
Schizophrenia	66	19	28.8	47	71.2	4.53	0.03*
bipolar disorders	66	9	13.6	57	86.4		

* Significant difference

DISCUSSION

Toxoplasmosis is a common disease caused by Toxoplasma gondii infection in various ways, such as according to environment, eating habits, contact with this obligate intracellular protozoan occurs through direct ingestion of food or water contaminated with cat faeces containing oocysts, ingestion of tissue cysts in uncooked meat, transplacental infection of the foetus, blood transfusion or organ transplantation (**10**).

This study was a case control study which conducted in the period from January 2020 to July 2020 at Zagazig University Hospitals (Tropical Medicine, and Psychiatry Departments), Psychiatry outpatient, and Clinic Immunology Research Laboratory, Microbiology, and Immunology Department.

In this study, we included 132 patients, 66 with Schizophrenia and 66 with bipolar disorder. The mean age was 34.24±10.58 and 32.31±8.26 for schizophrenia and bipolar patients,respectively with statically insignificant between schizophrenia and bipolar disorder groups this results agreed with **Maremmani et al.(11)**found no statically significant between bipolar and schizophrenia according to age.

Our study included 27 males (40.9%) and 39 females (59.1%) in schizophrenia patient this result agree with**Yuksel et al. (12)**found female are predominant in schizophrenic group, while in bipolar disorder 48 (72.7%) male and 18 (27.3%) female, this agree with **Maremmani et al.(11)** found male is more predominant in bipolar group**.**

In the present study there were statically difference between schizophrenia and bipolar disorder groups according to residence, with high incidence of schizophrenia about 59.1% in patient live in urban area , while in bipolar group 65.2% of patients live in rural area, this agree with **Hamdani et al. (13)** found incidence of schizophrenia has prominent variation by urbanicity, and agree with **Hussein et al., (14)**found 68.4% of bipolar disorder patient live in rural area, also agreed with **Alvarado-Esquivel et al. (15)** reported that most psychiatric inpatients belonged to a lower socio-economic level and had lower housing conditions than the healthy populations.

The comparison between schizophrenia and bipolar disorder regarding putative environmental risk factor include dealing with animal, contact with cat,

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contact with dog, consumption of raw meat, consumption of unboiled milk, contact with soil, and blood transfusion show no statically significant except in contact with cat, which was higher in schizophrenic group, contact with soil which was higher in bipolar disorder (P = 0.004 and <0.001 respectively). There is statistically significant difference between contact with cat and serofrequency in patient with schizophrenia (P = 0.02). This agree with **Hussein et al. (14)**. This suggests that infection might be acquired by dealing with cat excrement containing the parasites. Association between T. gondiiseropositivity and cat contact was also found in studies done by **Alvarado-Esquivel et al. (15)** in which dealing with cat excrement was significantly associated with T. gondii infection (P = 0.001).

We did not find any association between T. gondii infection and consumption of raw meat, unboiled milk. This explains by the fact that Egyptian people, mostly preferred to consume different type of meat in well cocked manner. In humans, T. gondii is commonly acquired by the oral ingestion of tissue cysts containing bradyzoites, through the consumption of undercooked meat infected with T. gondii**(16,17).**

Increase the frequency of toxoplasmosis in our both study groups may be explained by some factors and different psychopathologic mechanisms can substantiate the link between T.gondii infection and neuro psychiatric disorders, these include abnormal neurotransmitters metabolism,dysregulated tryptophan metabolism immunological change,and testosteronehormonsalteration **(18,19,20)**.

Also, T.gondii infection found bradyzoites in the cerebellar Purkinje cells, T. gondiicysts in astrocytes in humans**,** can induce abnormalities in specific region in the brain (hippocampus,amygdala) **(19)**. Another possible explanation is that neurotransmitters, such as dopamine and norepinephrine, might be affected by toxoplasmosis which affects schizophrenic people as well,neuroinflammation and imbalance between pro and anti-inflammatory cytokines such as (IFNγ,CRP,TNFα,IL2, metalloproteins)seem to be the main mechanisms **(21).**This was supported by **Hussein et al., (14)**found that high level of IGg antibody in schizophrenic patients associated with high inflammatory response (IFNγ) and indolamine de oxygenase(IDO) enzyme.

Another study done on total serum sample (n= 228) patients from them 114 schizophrenic patients and 114 healthy volunteers measuring serum anti IGg level Showing interestingly, significant result in serointensty rate of anti IGgT.gondii (61.1%) compared to a psychiatrically healthy volunteers (40.8%) (P<0.05) .However, there is no significant between seropositivity rate of anti T.gondiiIGg antibody between the two groups ,analysis from demographic data reveal that the seropositivity rate of anti T.gondiiIGg antibody in schizophrenic patients was significantly associated with age group above 40 years old(P=.007), Nevertheless there is no significant relation between seropositivity rate of aniT.gondiiIGg antibody with gender (P=.897) **(22)**.

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CONCLUSION

Toxoplasmosis should be considered as a potential cause for neuropsychiatric disorders for example schizophrenic and bipolar patients.

No Conflict of interest.

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