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IMMUNOBLOT ASSAY FOR TOXOPLASMOSIS IN SCHIZOPHRENIC PATIENTS

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ABSTRACT
Background: *Toxoplasma gondii*, is a common intracellular protozoan parasite, it can infect most nucleated cells, including astrocytes and neurons. According to many studies, Toxoplasmosis is one of the infectious causes of schizophrenia which is a severe mental illness. **Patients and Methods:** The study included hundred patients with symptoms and signs of schizophrenia and hundred normal persons as a control group. Detection of *Toxoplasma* IgG by ELISA and immunoblotting was done for all groups. **Results:** Out of one hundred schizophrenic patients *Toxoplama gondii* IgG was positive in 47 (47 %), negative in 51 (51%) of cases and equivocal in 2 (2 %) cases by ELISA. *Toxoplasma* IgG assay by immunoblotting for these cases were (52.0 %) in which seroconversion of five cases were detected (2 cases from equivocal plus 3 new cases who were negative by ELISA). **Conclusion:** There is association between toxoplasmosis and schizophrenia. Immunoblot assay is a sensitive method for detecting *toxoplasma gondii* IgG seroconversion and confirming the equivocal results.

KEYWORDS: Immunoglobulin G (IgG), *Toxoplasma gondii,* Schizophrenia, immunoblot and ELISA.

INTRODUCTION

Toxoplasma gondii is an important intracellular protozoan parasite widely infecting humans and animals. *T. gondii* is known to infect muscle and brain tissue.[1]

Toxoplasma gondii, is a common brain-tropic intracellular protozoan parasite, it can infect most nucleated cells, including astrocytes and neurons. In a recent study *Toxoplasma* shows a strong tendency for interacting with neurons through CNS infection.[2]

The risk of schizophrenia is increased in those with prenatal exposure to *Toxoplasma gondii* infection. There is a relationship between infection and the disease progression.[3]

Diagnosis of toxoplasmosis is mainly based on detection of specific anti-*Toxoplasma* IgG and IgM antibodies. When the amount of specific IgG are too low or close to the cut-off value of the test a confirmatory test is highly needed.[4]

The dye test is not commercially available and unsuitable for routine use, so that it is only used by very few laboratories. The qualitative detection of toxoplasma IgG by Western blot test was recently developed and reported to be a good alternative as a confirmatory test for sera with low or border-line titers. [5]

AIM OF THE WORK

Assessment of *Toxoplasma gondii* immunoglobulin G (IgG) in schizophrenic patients by ELISA and immunoblot method.

PATIENTS AND METHODS

1. Study population

The study was carried out at the Parasitology department and the Psychiatry Department, Al-Azhar University (New-Damietta) from March 2015 until January 2016. The study included hundred patients with symptoms and signs of schizophrenia. Hundred normal persons were included as a control group.

2. Clinical Evaluation

All the studied persons were subjected to clinical evaluation for manifestations of schizophrenia including hallucinations, disorganized speech and behavior, agitation and delusions that are often paranoid. History of exposure to risk factors of toxoplasmosis (such as contact with felids, eating undercooked meat and contact with the soil).

3. Detection of *Toxoplasma* IgG by ELISA

Toxoplasma immunoglobulin G was measured by using microwell ELISA *Toxoplasma* IgG immunocapture kits from (Chemux bioscience, San Francisco, ca, USA). The separated serum was stored frozen at (-20°C) until used

for estimation of *Toxoplasma* specific IgG. We dispensed 100 µL of diluted sera, calibrators and controls into the appropriate wells. Incubate for 30 minutes at room temperature. We removed the liquid from all wells and repeat washing three times with washing buffer.

We dispensed 100 µL of enzyme conjugate to each well and incubate for 30 minutes at room temperature. Repeat washing three times with washing buffer. We dispensed 100 µL of TMB chromogenic substrate to each well and incubate for 15 minutes at room temperature. We added 100 µL of stop solution to stop reaction. Read O.D at 450 nm with a microwell reader.

Cut-off ≥1 will be considered positive for IgG antibodies to *Toxoplasma gondii*. Cut-off ≤ 0.9 will be considered negative for anti-*T. gondii* IgG. Cut-off between 0.9 and 0.99 will be considered equivocal.

5. Detection of *Toxoplasma* IgG by Immunoblot assay

LDBIO TOXO II IgG is a qualitative test for serological IgG diagnosis by Immunoblot assay of toxoplasmosis. In Western Blot technique: the antigens of *Toxoplasma gondii*, once separated by electrophoresis, are bound by electroblotting to the surface of a nitrocellulose membrane cut into 24 strips numbered from 1 to 24. Each sera specimen to be tested is separately incubated with a strip. The *anti-Toxoplasma* antibodies potentially present in the sample selectively bind themselves onto the antigens of *T. gondii*. The alkaline phosphatase-anti human IgG conjugate then binds itself to the bound anti-*Toxoplasma* antibodies. Finally, the immune complexes react with the substrate. The antigens recognized by the anti-*Toxoplasma* antibodies of type IgG present in the samples are revealed as purple transversal bands.

We prepare a distribution plan for the samples and C+ positive control (R10). We distribute 1.2 ml of sample buffer (R2) in each channel according to the established plan. Let the strips rehydrate themselves for approximately 1 minute, with the number visible at the top, by gently shaking the tray to totally immerse them in the buffer. We distribute the samples and positive control according to the distribution plan, at a rate of 10 µl per channel. Gently shake the tray after each dispense. Place the tray on a rocking platform. Incubate for 90 min ± 5 min at 18-25 °C.

Wash step: Empty the contents of the channels with a Pasteur pipette or by turning the incubation tray over. We dispense 2 to 3 ml of diluted wash buffer in each channel. Incubate on the rocking platform for 3 min. Repeat 2 times, then empty the contents of the channels. Dispense 1.2 ml of anti IgG conjugate (R3) into each channel. Place the tray on the rocking platform. Incubate for 60 min ± 5 min at 18-25 °C. Then washing step. Distribute 1.2 ml of NBT/BCIP substrate (R5) into each of the channels. Place on the rocking platform and protect from direct light. Incubate for 60 min ± 5 min at 18-25 °C.

Stop the reaction by aspirating substrate with a Pasteur pipette or by turning the incubation tub over and dispensing 2 ml of distilled water in the channels. Repeat this last washing step one more time. The color of the strips will naturally lighten while drying. Interpretation must only be performed after drying is complete.

A positive sample can present numerous bands located between 15 and 200k kilodaltons (kDa). Search for the presence of specific bands in the 30 - 45 kDa area for each of the tested samples. The presence on the strip of a minimum of 3 bands out of specific bands 30, 31, 33, 40 and 45, allows the assay to be interpreted as positive and to conclude that anti-*T. gondii* IgG antibodies are present in the tested sample.

6. Statistical analysis

The collected data were tabulated and statistically analyzed using statistical package for social science (SPSS) version 19 (SPSS Inc. USA). Quantitative data were represented as the mean and standard deviation (SD) and for comparison between two groups, the independent samples student (t) test was used. For interpretation of results, p value ≤ 0.05 was considered significant.

RESULTS

The present study was carried out at the parasitology department and the Psychiatry department, Al-Azhar University hospital (New-Damietta) and included 100 patients with symptoms and signs of schizophrenia as well as 100 normal persons of the same age and sex, without schizophrenia or symptoms and signs suggesting toxoplasmosis as a control group. In the cases group there were 70 (70%) males and 30 (30%) females, while in the controls there were 50 (50%) males and 50 (50%) females, with no statistical significance between cases and controls as regard sex distribution.

Among cases, age distribution was 49 (49%), 34(34%), 12(12 %) and 5(5%) in third, fourth, fifth and sixth decades respectively. While in the control group it was 50(50%), 26(26 %), 12(12%) and 12(12%) in third, fourth, fifth and sixth decades respectively. There was no statistical significance of age in cases when compared to controls (31.25±6.51 vs. 32.20±8.11 respectively). It ranged from 22 to 63 years with a mean of 33.92±8.91 years for all studied cases and controls. In addition, the third decade was the most common age in cases and controls (49.0% of cases and 50.0% of controls).

Regarding positive family history of schizophrenia, it was positive in 31 (31%) and negative in 69 (69%) of cases compared to 0 (0.0%) of control group with statistically significant positive family history in cases when compared to controls.

Among cases 13(13%), 39 (39 %), and 48(48%) were in contact with cats, eating under cooked meat and contact with soil respectively, compared to none in controls for

all risk factors. There was statistical significance in studied risk factors in cases when compared to controls, table (1).

Table 1: Relation between cases and controls as regard to risk factors.

	Cases		Controls		Statistics	
	n	**%**	**n**	**%**	**Test**	**P value**
Contact with cats	13	13.0%	0	0.0%	61.4	<0.001
Eating undercooked meat	39	39.0%	0	0.0%	11.03	<0.001
Contact with soil	48	48.0%	0	0.0%	41.24	<0.001

Toxoplama gondii IgG was positive in 47 (47 %), negative in 51 (51%) of cases and equivocal in 2 (2 %) cases by ELISA, all the 47 positive cases were positive by immunoblot assay plus new 5 cases not detected by ELISA (2 cases from equivocal plus 3 cases from negative group). In control group it was positive in 10 (10%) , negative in (89%) and equivocal in one case by ELISA while by immunoblotting it was positive in (11%) and negative 89 % .There is statistical significance of *Toxoplasma gondii* IgG detection by ELISA when compared by immunoblotting with 100% sensitivity table (2) , table (3) and figure (1).

Table 2: Comparison between IgG assay by ELISA and immunoblot in cases.

		Cases				Statistics
		ELISA		Immunoblot		
		n	**%**	**n**	**%**	
IgG	Positive	47	47.0%	52	52.0%	P value <0.01*
	Negative	51	51.0%	48	48.0%	
	Equivocal	2	2.0%	0	0.0 %	

Table 3: Comparison between IgG assay by ELISA and immunoblot in control.

		Control				Statistics
		ELISA		Immunoblot		
		n	**%**	**n**	**%**	
IgG	Positive	10	10.0%	11	11.0%	P value 0.01
	Negative	89	89.0%	89	89.0%	
	Equivocal	1	1.0%	0	0.0%	

The majority of positive IgG cases were males; but the difference is statistically insignificant.

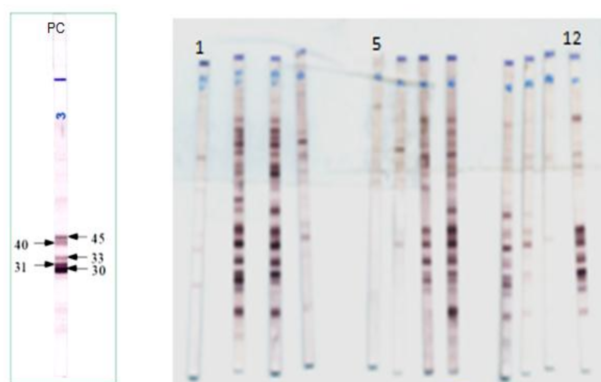

Fig. 1: Profiles with positive and negative blots in Toxo II IgG kit LDBIO®. PC: positive control with 5 bands: 30, 31, 33, 40, and 45 kDa; 12, positive blot with 5 bands (30, 31, 33, 40 and 45 kDa); 5, negative blot without bands; 1, negative blot showing 2 bands with low intensity (30 and 45 kDa).

DISCUSSION

Toxoplasmosis is considered as one of the infectious causes of schizophrenia. Seropositive schizophrenic patients had significantly reduced gray-matter volume as compared with seronegative patients.[1]

In the present study as regard the exposure to risk factors for toxoplasmosis among one hundred schizophrenic patients, 13(13%), 39 (39 %), and 48(48%) cases were in contact with cats, eating under cooked meat and contact with soil respectively, compared to none in controls for all risk factors. There was statistical significance in studied risk factors in cases when compared to controls. These results are in agree with other studies in different countries.[6,7,8]

Regarding positive family history of schizophrenia, it was positive in 31 (31%) and negative in 69 (69%) of cases compared to 0 (0.0%) of control group with statistically significant positive family history in cases when compared to controls. These results are in agree with a study by The London Child Health and Development Study (CHADS) for identifying premorbid risk markers for schizophrenia.[9]

In the present work, out of one hundred schizophrenic patients *Toxoplama gondii* IgG was positive in 47 (47 %), negative in 51 (51%) of cases and equivocal in 2 (2 %) cases by ELISA. By performing *Toxoplasma* IgG immunoblot for this cases seroconversion of five cases

were detected in the form of all the 47 positive cases were positive by immunoblot plus new 5 cases (2 cases from equivocal plus 3 cases from negative group). While in control group it was positive in 10 (10%), negative in (89%) and equivocal in one case by ELISA and on performing immunoblot assay it was positive in (11%) and negative 89 % with no equivocal results. There is statistical significance of positive IgG by ELISA when compared to IgG by immunoblot with p value < 0.01.

These results are in agree with many studies in which the serum of schizophrenic patients were assayed for *Toxoplasma* IgG ,it was positive in 40%, 46% and 67.7% by Tamer et al. (2008) in Turkey, Celik et al.(2015) and Alipour et al. (2011) in Iran respectively. The geographical distribution of studied cases and controls, sensitivity and specificity of the used materials may explain the differences of percent in each study.[10,11,12]

In contrast to the results of the present study, Karabulut et al. (2015) found that there is no association between toxoplasmosis and schizophrenia in area with a high prevalence of *T. gondii*.[13]

But in a study by Li Y et al. (2013) concluded that certain patterns of *Toxoplasma* IgG antibodies were predictive of developing schizophrenia, with the magnitude of association rising when the level of antibodies increased to two or more.[14]

In the present study out of 100 schizophreni patients *Toxoplama gondii* IgG was positive in 47 cases (47 %) by ELISA and 52 cases (52%) by immunoblot. These results indicate that immunoblot assay is more sensitive in detecting seroconversion and confirming the equivocal results.

The results of this study are in agree with a study by Khammari et al. 2013 to estimate the proportion of equivocal and discordant results between the enzyme immunoassay, fluorescent antibody test (FAT) and immunoblot as a confirmatory test and found that immunoblot appears to be an excellent confirmatory technique for equivocal results in routine-used techniques as immunoblot method is sensitive, specific, easy to perform and interpret.[15]

Also the results of the present study is in accordance with results of other studies in different countries concluded the useful value of immunoblot assay as a confirmatory test.[16,17,18,19]

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