Seroprevalence of *Toxoplasma gondii* in Schizophrenic Patients in Iraq using ELISA test

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Abstract

Toxoplasmosis is a widespread infection of great importance, and that the disease does not show any clinical specific signs . this study was performed on 200 patients with schizophrenia were collected from :Al-Rashad Teaching Hospitals and 100 healthy individuals, considered as control group, the samples were collected during the period of December 2012 until the end of February, 2013. Antibodies against *T. gondii* parasite in the serum were detected by using latex agglutination test (LAT) and showed a percentage of positive antibodies in schizophrenic patients and healthy individuals (control) 143(71.5%) and 45(45%) respectively.While using Enzyme Linked Immunosorbent Assay (ELISA) the positive blood sera in LAT test of the schizophrenic patients showed 114(79.7%) IgG against *T. gondii* compared to 33(73.3%) of the healthy individuals with no significant differences. Also detection of IgM in the sera of the schizophrenic patients showed 6(4.19%) compared to0 (0 %) of healthy individuals with significant differences between them.

Keywords : *Toxoplasma gondii*, schizophrenia .

الانتشار المصلي لطفيلي المقوسات الكوندية في مرضى انفصام الشخصية في العراق باستخدام اختبار الاليزا .

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الخلاصة :

داء المقوسات الكوندية مرض واسع الانتشار وله أهمية كبيرة ولايظهر هذا المرض اي اعراض سريرية متخصصة . هذه الدراسة أجريت على 200 عينة لاشخاص مصابين بمرض انفصام الشخصية جمعت العينات من مستشفى الرشاد التعليمي و 100 عينة لاشخاص اعتياديين اعتبرت مجموعة سيطرة . تم جمع العينات من شهر كانون الاول 2012 نهاية شهر شباط 2013 . الاجسام المضادة لطفيلي المقوسات الكوندية الموجودة في مصول العينات حددت باستعمال اختبار لاتكس حيث اظهرت النسب الايجابية للاصابة بين مرضى المصابين بمرض انفصام الشخصية والاشخاص الاعتياديين هي143(71.5 %) و45(45%) على التوالي ،بينما باستعمال اختبار الاليزا لمصول الدم الموجبة بأختبار LAT للمرضى اظهر نسبة114 (79.7 %) اجسام مضادة من نوع IgG ضد المقوسات الكوندية مقارنة بنسبة 33(73.3 %) للاشخاص الاعتياديين مع عدم وجود فروق معنوية بين النسب . وحددت ايضا الاجسام المضادة من نوع IgM حيث كانت بنسبة 6(4.19 %) لمرضى الانفصام مقارنة بنسبة 0(0%) للاشخاص الاعتياديين مع وجود فرو ق معنوية بين النسب .

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Introduction:

Schizophrenia is a serious neuropsychiatric disease of uncertain etiology. Epidemiological and neuropathological studies have indicated that some cases of schizophrenia may be associated with environmental factors, such as exposure to infectious agents. However, specific infectious agents associated with the development of schizophrenia have not been identified [1]. In humans, acute infection with *Toxoplasma gondii* can produce psychotic symptoms similar to those displayed by persons with schizophrenia [2]. A study by Mortensen *et al*. [3] revealed that newborns who have antibodies to *T. gondii* have an increased risk of later being diagnosed with schizophrenia. *Toxoplasma gondii* is a protozoan parasite found worldwide [4] that infects all kinds of mammals, including cats, livestock, and human beings. In its life cycle, cats and other felids are the definitive hosts and the other warmblooded vertebrates are intermediate hosts [5]. Human response to *T. gondii* is related to immune status of the infected person, strain of *T. gondii* and course of infection [6].In immunocompetent subject, primary infection is usually asymptomatic or associated with self limited symptoms such as fever, malaise, and cervical lymphodenopathy [5]. Infection acquired during pregnancy is frequently associated with transmission of tachyzoite *T.gondii* to their fetus, resulting in congenital disease. In immunocompromised women ,reactivation of latent infection can cause life threatening complications [7]. If a pregnant woman contracts toxoplasmosis, it may be passed through the placenta to the fetus, resulting in congenital toxoplasmosis, which is a cause of mortality and malformation [8]. Infection in human generally occurs through consuming food or drink contaminated with oocysts and tissue cysts from undercooked meat. [9]. Most of *Toxoplasma* infections are asymptomatic to mild and in some infected persons cervical lymphadenopathy, ocular disease [4]. central nervous system manifestation [10] and brain abscess may occur [11]. And human studies revealed that latent toxoplasmosis may cause personality changes [12].Hence this study was aimed to determine the prevalence of anti toxoplasmosis antibodies (IgG and IgM) among local schizophrenic patients using Latex (LAT) and Enzyme Linked Immunosorbent Assay (ELISA) tests.

Methods:

This study, concerned schizophrenic patients performed among different groups of men and women, the samples were collected from :Al-Rashad Teaching Hospitals during the period of December 2012 until the end of February, 2013. Samples were used for detection of specific antibody to *T . gondii* . In this study, 300 samples were collected, 200 of them from schizophrenic patients(100 men and 100 women) and 100 of them from ordinary healthy people (50 men and 50 women) for comparison . Avolume (5 ml) of venous blood were collected from each subject . Detection of parasite antibody was achieved by using Latex (LAT)kit Linear Company, Enzyme Linked Immunosorbant Assay (ELISA - IgG and ELIZA- IgM)kits BioCheck Company .

Statistical Analysis: Chi-square test was used to significant compare between percentage of this study.

Results and Discussion:

The results of the LAT(Abs) test showed a percentage of positive *T. gondii* antibodies in sera of schizophrenic patients and healthy individuals (control) 143(71.5%) and 45(45%) were positive in LAT test respectively showed in figure (1), the statistical analysis showed significant differences at ($P<0.01$, $p<0.05$) . And results of ELISA - IgG\IgM Abs. tests for 143 serum samples of schizophrenic patients and 45 from healthy individuals are showed in figures (2), (3) .The number of positive sera for IgG ELISA was 114(79.7%) in schizophrenic patients and 33(73.3%) in healthy individuals while the number of positive test in IgM ELISA was 6(4.19%) in schizophrenic patients and Zero in healthy individuals. The statistical analysis revealed that there were no significance differences ($P>0.05$) in seropositive individuals but found significant differences in seronegative ones . In this study,the prevalence of *T. gondii* in schizophrenic patients and healthy individuals was 79.7%, 73.3% (IgG) and 4.19%, 0% (IgM) respectively and the difference was not statistically significant.The results of this study coniforms the result of Saraei- Sahnesaraei *et al.* [13] and Daryani *et al*.[5] studies in Iran, that showed seropositive for IgG specific antibodies to *T. gondii* and the differences were not statistically significant. Prevalence of *T. gondii* in both studies carried out in Iran showed the same results in both schizophrenic patients and control group. Some researchers such as Boronow *et al*. [15] in the United States, Torrey and Yolken [16] in Ireland ;Emelia *et al.* [17] in Malaysia reported that the differences between the two groups (schizophrenic patients and healthy individuals) were not statistically significant. In contrast, other researchers showed that the differences between the two groups were

statistically significant Alvarado-Esquivel *et al*., [4] in a northern Mexican city; Jassam [19] in Iraq; Alipour *et al*. [20] in Iran .

IgM antibodies in this study showed no significant differences between the groups. This is due to the fact that IgM is an indicator of recent infection and becomes negative within 4–12 weeks, hence presumably is not associated with the increased risk of schizophrenia [21]. Generally IgM antibodies are detected within the first 2 weeks of infection and reduce to negligible levels within 6 months after exposure. However, in toxoplasmosis, IgM titres can remain elevated up to a year or even more. Thus, the mere presence of IgM antibodies is not diagnostic of an acute toxoplasmosis infection. However, a negative IgM antibody test rules out recently acquired infection unless the serum is tested too early after exposure so that antibodies have not as yet developed. A single positive IgG antibody test indicates chronic infection, which might have been acquired before conception, thus posing no risk to the fetus [22] . Because antibody titers to *Toxoplasma* IgG may remain elevated for significant periods of time, an increase in IgG antibody may reflect an active primary infection, reactivation of infection, or a persistent immune response to a dormant infection [23]. Increased IgG titers to *Toxoplasma* have been associated with both severe and subtle neuropsychiatric abnormalities [24]. Different results in various studies may be happened due to many reasons which include geographical conditions, using only serological tests with no DNA detection, selection of control group, source of infection (oocyst or tissue cyst), differences in genetic susceptibility, timing of the infection, different strains of *Toxoplasma* and consumption of antischizophrenia drugs, in some studies there was an association between *T. gondii* infection and schizophrenia. It may be because of certain circumstances how the infection occurs or because of secondary manifestation [5]. In Spain, schizophrenia patients showed a high rate of seropositivity to *T. gondii* because they worked in the hospital garden that had been faecally contaminated by the hospital's cats [14]. On the other hand, institutionalized schizophrenia patients may be fed undercooked meat, thereby increasing their exposure to *T. gondii* [5]. Alternatively, in some instances increased *T. gondii* antibodies in schizophrenia patients are secondary to immune system abnormalities, such as in individuals infected with HIV, a pathogen which is a primary agent of schizophrenia, causing reactivation of *T. gondii* tissue cysts in different organs and generation of antibody to *T. gondii* which is a secondary manifestation [25]. Torrey *et al.* [26], in their meta-analysis of 11 studies, showed that individuals with schizophrenia have an increased prevalence of antibodies to *T. gondii* and suggested that this, as well as genetic and environmental factors, could be associated with a large number of cases of schizophrenia. One study showed that mothers having antibodies to *T. gondii* late in pregnancy, even though the infection was not necessarily recent, had an increased risk of giving birth to offsprings who later were diagnosed with a schizophrenia spectrum disorder [3].

In this study no significant association revealed between the precence of *T. gondii* antibodies and schizophrenia, some possible reasons including the fact that more than 95% of patients in the present study received anti-schizophrenia treatment. Leweke *et al*. [18] did a study on three groups including schizophrenia patients receiving anti-schizophrenia treatment, those who had never received any drug and those of the control group, they showed that the antibody levels for the treated group were intermediate between the levels of the never-treated group and those of the control group, it suggests that anti-schizophrenia medication may have decreased the antibody levels. It is noted that in terms of the possible effect of medications some of the therapeutic agents commonly employed for the treatment of schizophrenia and bipolar disorder have the ability to inhibit the replication of *T. gondii* tachyzoites in cell culture [27].

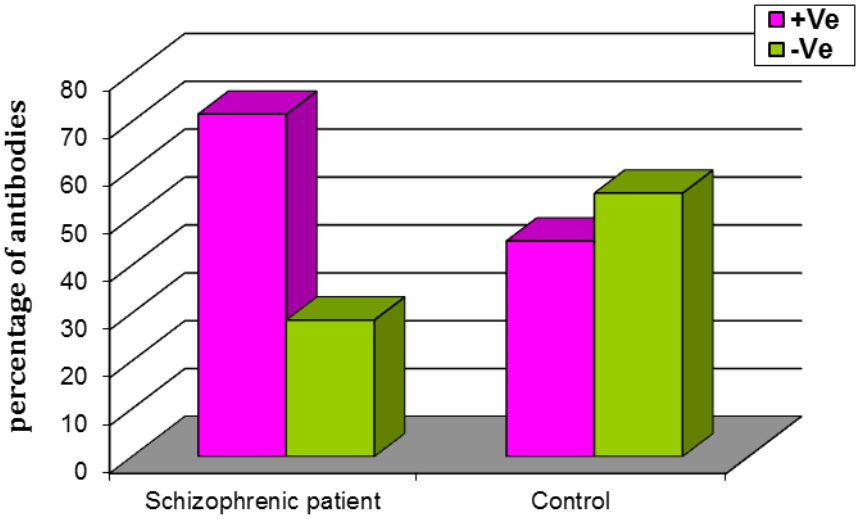

Figure 1- The percentage distribution of anti-T.gondii antibodies in 200 sera of Schizophrenic patients and 100 sera of healthy individuals (control) measured by LAT (Abs) test.

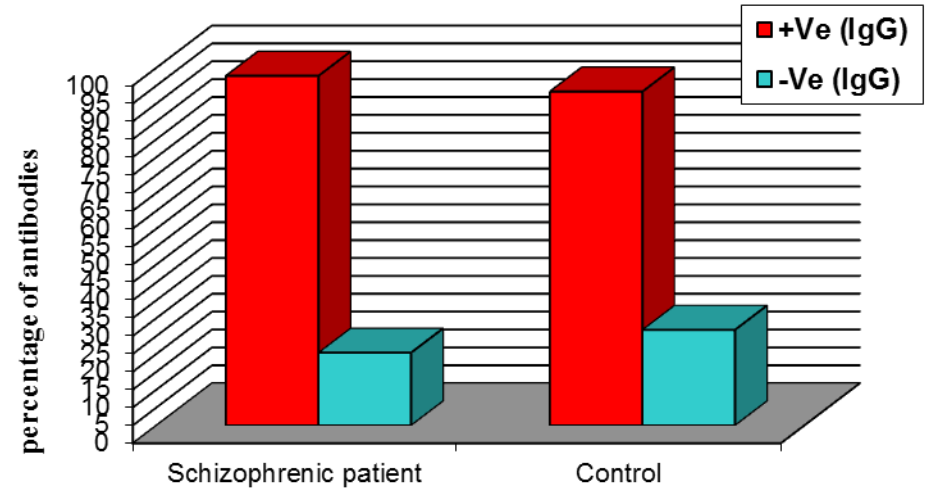

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Figure 2- The percentage distribution of anti-T.gondii antibodies in sera of Schizophrenic patients and healthy individuals (control) measured by ELISA (Abs) test.

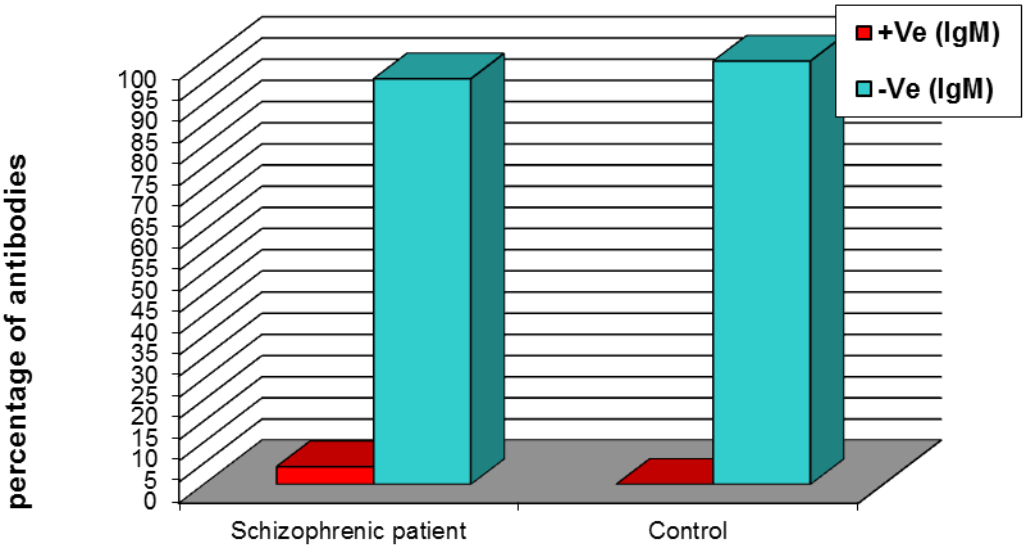

Figure 3- The percentage distribution of anti-T.gondii antibodies in sera of Schizophrenic patients and healthy individuals (control) measured by ELISA-lgM (Abs) test.

Table 1- The percentage distribution of anti-*T.gondii* antibodies in 200 sample sera of Schizophrenic patients and 100 sample of healthy individuals (control) as measured by LAT test:

Sample	Test - LAT					Chi-square value
	Total	+Ve		-Ve		
		No.	Percentage (%)	No.	Percentage (%)	
Schizophrenic patient	200	143	71.5	57	28.50	10.54 **
Control	100	45	45.00	55	55.00	4.092 *
Total	300	188	---	112	---	---
Chi-square value	---	---	7.946 **	---	7.946 **	---
* (P<0.05), ** (P<0.01).						

Table 2- The percentage distribution of anti-*T.gondii* antibodies in sera of Schizophrenic patients and healthy individuals (control) in measured by ELISA-IgG test:

Sample	Test - ELISA-IgG					Chi-square value
	Total	+Ve (IgG)		-Ve (IgG)		
		No.	Percentage (%)	No.	Percentage (%)	
Schizophrenic patien	143	114	79.7	29	20.3	12.783 **
Control	45	33	73.3	12	26.7	11.471 **
Total	188	147	---	41	---	---
Chi-square value	---	---	1.583 NS	---	1.583 NS	---
** (P<0.01).						

Table 3- The percentage distribution of anti-*T.gondii* antibodies in sera of Schizophrenic patients and healthy individuals (control) measured by ELISA-IgM test:

Sample	Test - ELISA-IgM					Chi-square value
	Total	+Ve (IgM)		-Ve (IgM)		
		No.	Percentage (%)	No.	Percentage (%)	
Schizophrenic patien	143	6	4.19	137	95.81	13.012 **
Control	45	0	0.00	45	100	14.500 **
Total	188	6	---	182	---	---
Chi-square value	---	---	0.873 NS	---	0.873 NS	---
** (P<0.01).						

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