

EVALUATION OF THE TOXOCELL LATEX AGGLUTINATION TEST AS SCREENING TEST AND DISTRIBUTION OF TOXOPLASMA ANTIBODIES AMONG SEXUALLY ACTIVE PEOPLE IN NAJAF CITY

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Abstract:

The present study was intended to reveal the validity of Toxocell (Biokit, Spain) latex agglutination test as screening test. The rate of toxoplasma antibodies distribution in randomly selected subjects was estimated. The sensitivity and specificity of the test were conducted in comparison to the "gold standard" reference test Enzyme-linked Immunosorbent Assay (ELISA, BioCheck, Inc).

Fifty two adult persons (31 females and 21 males) were enrolled in this study. Twenty (38.4%) serum samples out of 52 subjects were positive for toxoplasma antibodies by direct latex agglutination test (DLA). The prevalence of toxoplasma antibodies in females and males were 54.8% and 14.28%, respectively. Among twenty DLA sera positive, only 5(25%) serum samples were positive with toxoplasma IgG ELISA test, three females and two males. However, the results of IgM ELISA assay were positive for only two (10%) female serum samples. None of negative DLA serum samples gave positive results with neither IgG nor IgM ELISA assay.

The sensitivity and specificity and positive predictive value (PPV) of DLA test (in comparison to IgM ELISA assay) were 100%, 64% and 10%, respectively. We concluded that in spite of low specificity of latex agglutination test, it was probably more suitable for laboratories in remote area as screening test where ELISA facility was unavailable.

الخلاصة:

ان الهدف من الدراسة الحالية هو أظهار مدى مرجعية اختبار تلازن اللاتكس لشركة (Biokit) الأسباني كإختبار مسحي. حيث تم تقدير معدل انتشار الأجسام المضادة للمقوسات لدى مجموعة من الأشخاص المختارين بصورة عشوائية. كما قدرت حساسية وخصوصية الفحص بالمقارنة مع الفحص المرجعي القياسي "اختبار الأليزا لشركة (BioCheck).

تضمنت الدراسة جمع عينات دم من ٥٢ (٣١ اناث، ٢١ ذكور) من الأشخاص البالغين. اظهرت نتائج التحري ٢٠ (١٠%) حالة موجبة للجسام المضادة للمقوسات باختبار تلازن اللاتكس من اصل ٥٢ عينة مصل. وكان معدل انتشار الأجسام المضادة للمقوسات لدى الأناث و الذكور ٥٤،٣٨% و ١٤،٢٨% على التوالي. بينما اظهر

أختبار التحري عن الأجسام المضادة من نوع (G) بتقنية الأليزا ان خمسة (25%) حالات موجبة فقط من مجموع الأمصال الموجبة لفحص تلازن اللاتكس، ثلاثة منها للإناث وإثنان للذكور. ولكن نتائج التحري عن الأجسام المضادة نوع (M) باختبار الأليزا كانت موجبة لحالتين (10%) من النساء فقط. علماً أن جميع عينات الأمصال السالبة لإختبار تلازن اللاتكس، كانت سالبة أيضاً لإختبار التحري عن الأجسام المضادة نوع (G) و (M) باستخدام تقنية الأليزا.

كانت النسب المئوية للحساسية، الخصوصية والقيمة التنبؤية الموجبة لإختبار تلازن اللاتكس 100%، 64% و 10% على التوالي، مقارنة بالأختبار المرجعي لفحص الأجسام المناعية نوع (M) باستخدام تقنية الأليزا. نستنتج انه بالرغم من الخصوصية المنخفضة لفحص تلازن اللاتكس، فإنه غالباً ما يكون أكثر ملائمة لإجراء الفحوصات المسحية في المختبرات البعيدة عن مراكز المدن لعدم توفر تقنية التحري بالأليزا.

Introduction:

Toxoplasma gondii is an obligate intracellular protozoan parasite that causes toxoplasmosis in many species of mammals including humans and birds throughout the world; the infection is cosmopolitan in distribution (1). Domestic cats are the definitive hosts of the parasite and are the main source of the infection via oocysts passed in their feces. Man, livestock and even rodents may act as intermediate hosts for the parasite (1, 2). The two primary ways in which humans acquire toxoplasmosis are consumption of raw or undercooked meat and contact with cats (*Toxoplasma's* definite host) which shed oocysts in their feces during acute infection (3, 4, 5).

Toxoplasmosis is a very common disease with prevalence ranging any where from 5% to 95% among young adults living in different parts of the world. Serological surveys of humans indicate that as much as one-third of the world's population has been infected by *T. gondii* (Frenkel,1971). In the United States alone, 50% of the population has antibodies to *T. gondii*, and may reach to 93% in Parisian women and 50% in their children (1).

In Iraq, prevalence range between 41-51% in Basra (6) and less then 1% in Duhok (7), and reach to 18% in Baghdad (8).

Despite this high prevalence in the world, the diagnosis of Toxoplasmosis is only rarely regarded as the cause of illness. However, the fact that it may mimic a variety of clinical syndromes, it may cause severe and even fatal infection in the immunocompromised host (9,10), like patients receiving cytotoxic therapy, transplant recipients, chronic renal failure and AIDS patients, such seropositive patients may develop toxoplasmic encephalitis(11).

Aim of the study: The aim of this study is to detect the distribution of *Toxoplasma*

gondii antibodies among randomly selected blood specimens of sexually active peoples (male and female) in Najaf city, and to evaluate the sensitivity, specificity and predictive value of Commercial latex agglutination test (toxocell latex kit_Biokit/spain) in comparison with ELISA technique.

Materials and methods:

Collection of Blood Samples:

Fifty two blood samples were collected randomly from (31) women and (21) men with age ranging between 16 to 45 years old in private laboratory in Al-Meylad area and Al-Sadder hospital (in Najaf governorate), three ml of venous blood was drawn using a 5 ml size disposable syringe then transferred to 10 ml disposable sterile serum tube. The blood samples were left to clot then centrifuged at 3000 rpm for 5 minutes to separate the serum. Serum samples transferred to eppendorf tubes and stored at 4 - 8 °C for 24 hrs. If long period of storage is required, the sera will be kept frozen at - 20 °C until use.

Serological Tests:

Two serological tests were used for detection of toxoplasma antibodies; direct latex agglutination test (DLA) and enzyme-linked immunosorbent assay (ELISA) IgG & IgM test.

Latex Agglutination:

Commercial toxocell latex kit (Biokit/spain) was composed of Toxoplasma reagent, positive and negative controls and with black ground plastic disposable slides. Kits were stored in refrigerator at temperature 2-8°C until needed. The antigen reagent is a suspension of polystyrene particles of uniform size coated with soluble toxoplasma antigen reaction. Latex particles allow visual observation of antibody-antigen reactions.

ELISA test:

The test was down in Duaa Al-Salama laboratory (private laboratory) in Najaf city, by using ELISA BioCheck, Inc kit for IgG and IgM antibodies according to manufacturer's instructions.

Sensitivity, Specificity and predictive value were calculated as following:

- Sensitivity=true positive / total (true positive + false negative) × 100.
- Specificity=true negative / total(true negative + false positive) × 100.
- Positive predictive value (PPV) = $\frac{\sum \{ \text{True Positive} \}}{\sum \{ \text{Test outcome Positive} \}} \times 100$.
- Negative predictive value (NPP) = $\frac{\sum \{ \text{True negative} \}}{\sum \{ \text{Test outcome negative} \}}$

Results:

Twenty samples (38.4%) gave positive results to toxoplasma antibodies by direct latex agglutination test (DLA). The test results show higher incidence of toxoplasma antibodies in females (54.8%) than in males (14.28%) as shown in Table 1. Out of 20 DLA sera positive, only 5 (25%) were positive with toxoplasma IgG ELISA test, 3(17.6%) and 2(66.6%) for Females and males respectively. two samples (10%) gave positive result with toxoplasma IgM ELISA test, both of them were Females samples. None of negative DLA sera gave positive results with either IgG or IgM ELISA test.

Table 3 shows the positive and negative results and also the sensitivity, specificity and positive predictive value of DLA (Toxocell latex kit, Biokit/spain) as 100%,64% and 10% respectively.

DISCUSSION:

The present study showed that the sero-prevalence of Toxoplasma in Al-Najaf area was 38.4% which is higher than reported in Baghdad city (18%) (Abdul Ridha, 2005) and lower than that found in Basra (6) and Duhok (7,12),

Despite of high seropositivity by DLA test, positive result to IgM by ELISA technique was 3.8%, that is lower than what recorded in Baghdad (22.22%) by Abdul Ridha,M.A.2005,and higher than the result of similar study in Duhok (0.97%) (7).

The high level of sero-positive women through using direct latex agglutination test will give false impression about the true infection levels about the true infection levels because of high level of false positive rate, this result was in agreement with many previous studies (13).

Although, the ELISA test results for detection of IgG antibodies reveal that five cases (25%) were positive among all latex agglutination test positive, this result is lower than found by Dawood et. al. (2010), they reported that about 80%. The IgM ELISA test results were 10% of all DLA positive cases, which merely similar to that found by Dawood.

The presence of toxoplasma antibody in the serum is regarded as the important criterion for the diagnosis of toxoplasmosis. Currently, several laboratories utilize different techniques such as indirect hemagglutination, ELISA, indirect immunofluorescence and latex agglutination (14). Perfectly, the screening test is supposed to be specific, sensitive and easy to perform. Commercial toxocell latex kit(Biokit/spain) is widely used in Iraqi laboratories. Therefore, we are attempting to evaluate its efficiency as screening test. We found that the toxocell latex kit(Biokit/spain) to be 100% sensitive ,64% specific, with a positive predictive value of 10%. The high sensitivity is similar to previous reports (15), but the low specificity and low positive predictive value unmatched previous reports (13). As the sensitivity of latex agglutination method is 100%, therefore, the sero-negative patients could be excluded, whilst sero-positive cases should be confirmed by other serological tests like ELISA or IFAT. Moreover, the test is commercially available and is very simple to carry out, making it suitable for laboratories in remote areas.

Our finding of positive predictive value (PPV) of toxocell latex kit(Biokit/spain) was 10% which lower that found by (13), the drop in PPV indicates a drop in the prevalence of the disease.

Table 1: Distribution of positive *Toxoplasma gondii* sera according to gender using DLA test

<i>Gender</i>	<i>Number of subjects</i>	<i>Number of sera positive by DLA (%)</i>
Male	21	3 (14.28)
Female	31	17 (54.8)
Total	52	20 (38.4)

Table 2: Distribution of toxoplasma IgG, and IgM antibodies results among DLA positive sera

<i>Gender</i>	<i>No. of DLA positive sera</i>	<i>No. of IgG Ab. positive sera using ELISA test</i>	<i>No. of IgM Ab. positive sera using ELISA test</i>
Male	3	2 (66.6)	0
Female	17	3 (17.6)	2 (11.8)
Total	20	5 (25)	2 (10)

Table 2: the positive and negative results of toxoplasma antibody tested by the gold standard test (ELISA test) and DLA test (A and B)

A- IgG ELISA DLA

Screening test	ELISA test IgG	Total
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			Gold Standard		
			Positive	Negative	
Direct Latex agglutination test (DLA)	Positive		True positive (TP) = 5	False Positive (FP) = 15	20
	Negative		False Negative (FN) = 0	True Negative (TN) = 32	32
Total			5	47	52

Sensitivity of DLA = $5/5 \times 100 = 100\%$

Specificity of DLA = $(32/15+32) \times 100 = 68\%$

PPV for IgG = $5/20 \times 100 = 25\%$

B- IgM ELISA and DLA

Screening test			ELISA test IgM Gold Standard		Total
			Positive	Negative	
Direct Latex agglutination test (DLA)	Positive		True positive (TP) = 2	False Positive (FP) = 18	20
	Negative		False Negative (FN) = 0	True Negative (TN) = 32	32
Total			2	50	52

Sensitivity of DLA = $2/2 \times 100 = 100\%$

Specificity of DLA = $(32/18+32) \times 100 = 64\%$

PPV for IgM = $2/20 \times 100 = 10\%$

Reference:

1-John DT, Petri WA (2006). *Markell and Voges Medical parasitology*. 9th ed. Saunders Elsevier.

2- Heinemann D, Goldsmith R(1992). Toxoplasmosis infection. *Trop. Med. and parasit* 1992; 35: 942.

3-Dawood AK, Abdulrazaq AM, Ahmad JN(2010). Detection of Toxoplasmosis in human and cats immunologically. *Kufa Journal for Veterinary Medical Sciences*, 1:65-71.

4-El-Sebai MM(1992). Study on Toxoplasmosis in Qasseem, *Saudi J Soc parasit* , 21: 273-275.

5-Frenkel JK, Dubey JP(1972). Toxoplasmosis and its prevention in cats and man. *J Infect Dis* ; 126: 664-673.

6-Yacoub AA-H, Bakr S, Hameed AM(2006). Seroepidemiology of selected zoonotic infections in Basra region of Iraq. *Eastern Mediterranean Health Journal* 12:112-118.

7-Razzak AH, Wais SA, Saeid AY(2005). Toxoplasmosis: the innocent suspect of pregnancy wastage in Duhok, Iraq . *Eastern Mediterranean Health Journal*, 11(4):624-632.

8-Abdul Ridha MA(2005).Toxoplasmosis and Urinary Tract Infection Among Immunocompromised Patients. (MSc. thesis) College of Medicine, University of Tikrit.

9-Sabian AB. Toxoplasmosis(1994). In: Desanic AG ed. *Advances in pediatrics*, New York Interscience publisher Inc, 1-56.

10-Desmots G, and Couvreur J(1974). Congenital Toxoplasmosis. *N Eng J Med* ,290: 1110-1116.

11-Abdülgani F(2004). Prevalence of IgM and IgG antibodies to toxoplasma gondii in blood donors in the North Region of Jordan. *İnönü Üniversitesi Tıp Fakültesi Dergisi* 11:143-146.

12-Al-Doski BD(2000). Seroepidemiological study of toxoplasmosis among different groups of population in Duhok city by using latex agglutination test and indirect hemagglutination test (M.Sc.thesis). Duhok, Iraq, University of Duhok,.

13-Sukthana Y, Chintana T,SupatanapongW, Siripan C,Lekkla A, and Cheabchalrad R(2001). Predictive value of latex agglutination test in serological screening for Toxoplasma gondii. *Southeast Asian J Trop Med Public Health* ; 32:314-318.

14-Woldemicheal T, Fontanet AL, Sahlü T(1998). Evaluation of the Eiken latex agglutination test for anti-Toxoplasma antibodies and seroprevalence of

Toxoplasma infection among factory workers in Addis Ababa, Ethiopia. *Trans R Soc Trop Med Hyg* ; 92: 401-3.

15-Guruz AY, Ok UZ, Korkmar M(1996). Assessment of latex indirect agglutination test (Toxolax Fumouze) for the detection of Toxoplasma specific antibody in human sera in Turkey. *J Egypt Soc Parasitol* ; 26: 376-84.