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A Rapid Latex Agglutination Test (Lat) for Qualitative and Semi Quantitative Detection of *Toxoplasma Gondii* Antibodies in Different Patient Categories

KEYWORDS: Toxoplasma gondii, rapid latex agglutination test, Toxoplasmosis

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ABSTRACT *This study emphasizes the use of a rapid latex agglutination test for qualitative and semi quantitative detection of Toxoplasma gondii antibodies in different patient categories. This prospective study was conducted on peripheral blood samples of HIV patients (72), pregnant women (50) and ocular chorioretinitis cases (7) those admitted at Tirunelveli Medical College Hospital, Tamil Nadu from April to September' 2012. A total of 129 samples were subjected to Rapid Latex Agglutination Test (Toxogen) for qualitative and semi quantitative detection of Toxoplasma gondii antibodies. Out of 129 samples, eight samples (6.2%) gave positive results to Toxoplasma antibodies by Latex agglutination test (LAT).The test results show higher incidence of Toxoplasma antibodies in females (3.87%) than in males (2.3%) Out of 8 LAT positive cases, 4 were pregnant women, 3 were HIV patients and 1 presented with chorioretinitis. Out of 8, 3 were Positive with Toxoplasma IgM antibodies, 5 were IgG antibodies. Out of 8 LAT positive cases, 3 showed positive reaction at titre 1:16, 3 at 1:32 and 2 at 1:64. 2 gave positive for IgM antibodies and one for IgG antibodies at titres of 1:16 and 1:32 while one for IgM and IgG at 1:64 titre. The sensitivity and specificity of LAT test were 100% and 88.4% respectively.*

Introduction

Toxoplasma gondii an obligate intracellular protozoan, is one of the most common parasites that infects warm blooded animals including man.[1] Approximately half of the world's population is predisposed to this parasitic infection. Toxoplasmosis is generally asymptomatic; however, this parasitic disease can cause symptomatic and/or life threatening conditions in neonates, pregnant women, immunocompromised patients such as HIV/AIDS patients, organ transplant recipients and cancer patients.[2-4]

The laboratory diagnosis of toxoplasmosis can be performed in several ways including histological examination, the isolation of the parasite after inoculation and several serological methods.[5] A wide range of serologic tests are available commercially. A number of problems associated with available methods for serodiagnosis of this infection have been an impetus to search for alternative methods. Recent concern about Toxoplasma infections in pregnant women, neonates and immunocompromised patients has increased the demand for test methods for Toxoplasma that is rapid, specific, and inexpensive. Diagnosis of acute infection with T. gondii can be established by the detection of the simultaneous presence of IgM and IgG antibodies to Toxoplasma in the serum. The routine methods presently available to physicians in practice are often expensive to perform, time consuming, not readily adaptable to screening programs, or not sensitive enough to be useful in early diagnosis of the infection.[6]

Under this situation, our study emphasizes the use of a rapid latex agglutination test for qualitative and semi quantitative detection of Toxoplasma gondii antibodies in different patient categories.

Materials and Methods

Study population

This prospective study was conducted at Tirunelveli Medical College Hospital, Tirunelveli, and Tamil Nadu over a 6 months period (April' 2012 to September' 2012). The study was approved by the Institutional Scientific and Ethical Committee and written informed consents were obtained from the patients.A total of 129 peripheral blood samples for this study were collected from HIV patients (72), pregnant women (50) and ocular chorioretinitis cases (7) those admitted at Tirunelveli Medical College Hospital.

Socio-demographic, Clinical and Behavioural data

The History of illness and patients information and socio-demographic data like epidemiological risk, antenatal risk, HIV risk and clinical risk assessment factors were collected in the specially designed data sheet for this purpose. Socio-demographic data including age, education, occupation, parity, residency and related risk factors including source of drinking water, obstetrical history, frequency and type of meat, vegetables, fruits and milk consumption, cooking preferences, owning cat, history of cleaning cat litter box or feeding raw meat scraps, history of blood transfusion, organ transplant etc., were collected for further analysis.

Separation of sera

About 2-5 ml of blood collected from each patient was separated after centrifugation. The sera collected were inactivated at 56°C for 30 minutes in water bath and stored in small aliquots at –20°C.

Rapid Latex Agglutination Test (Toxogen, Tulip Diagnostics (P) LTD)

The reagent and samples kept in the refrigerator was brought to room temperature before use. Samples to be tested were diluted to 1:16 with 0.9% saline.(0.1 ml of serum + 1.5 ml of 0.9% saline).

Qualitative Method

One drop of diluted serum was placed on the reaction circle

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of the glass slide using a disposable pipette provided with the kit and then one drop of well mixed latex reagent was added to the drop of diluted serum sample. Using a mixing stick the sample was mixed with the latex reagent uniformly over the entire circle. The slide was rocked gently, back and forth. Agglutination was observed for macroscopically at five minutes.

Semi Quantitative Method
Using isotonic saline, serial dilutions of the serum samples positive in the qualitative method was prepared starting from 1:32, 1:64, 1:128, and 1:256 and so on. Each dilution of the serum sample was pipetted onto separate reaction circles of the slide and then one drop of well mixed latex reagent was added to each dilution of the serum sample. Using a mixing stick, the sample and the latex reagent was mixed uniformly over the entire circle. The slide was rocked gently, back and forth. Agglutination was observed for macroscopically at five minutes.

Interpretation of Results
Qualitative Method:
Agglutination indicates presence of diagnostically significant level of antibodies to Toxoplasma gondii. No agglutination indicates absence of diagnostically significant level of antibodies to Toxoplasma gondii.

Semi Quantitative Method
The highest dilution of serum showing agglutination corresponds to the titre of antibodies to Toxoplasma gondii.

Differentiation of anti-Toxoplasma antibodies into IgG-IgM
By previous treatment of the sera with reducing agents, such as 2-Mercaptoethanol, it is possible to observe the type of immunoglobulin responsible for the reaction. Fifty µl of the 2-Mercaptoethanol solution was added to 1 ml of 1:16 diluted serum under test and was incubated for 60 minutes at 37°C.At the end of the incubation period, the semi quantitative test procedure was proceeded as outlined above. If there is a significant decrease in the reactivity and/or drop in the antibody titre after 2-Mercaptoethanol treatment, it was considered IgM positive.

Results
Various socio demographic risk factors like age, gender, residence, water source, diet and animal contact, associated with the seropositivity of toxoplasmosis by Latex agglutination test presented in Table 1. Out of 129 samples, eight samples (6.2%) gave positive results to Toxoplasma antibodies by Latex agglutination test (LAT). The test results show higher incidence of Toxoplasma antibodies in females (3.87%) than in males (2.3%).

Out of 8 LAT positive cases, 4 were pregnant women, 3 were HIV patients and 1 presented with chorioretinitis.(Table 2) Out of 8, 3 were Positive with Toxoplasma IgM antibodies, 5 were IgG antibodies.(Table 3) Out of 8 LAT positive cases,3 showed positive reaction at titre 1:16,3 at 1:32 and 2 at 1:64. 2 gave positive for IgM antibodies and one for IgG antibodies at titres of 1:16 and 1:32 while one for IgM and IgG at 1:64 titre depicted in Figure 1.

Out of 14 patients with CD4 count less than 50 cells/µl, one patient had antitoxoplasma antibodies, while two patients had antitoxoplasma antibodies among 43 patients with CD4 count between 50-100 cells/µl. Out of 20 pregnant women with bad obstetric history, 4 had seropositivity with LAT. Highest rate of seropositivity was observed in first trimester of pregnancy among the tested sera. Regarding Toxoplasma antibody titre of IgG and IgM in pregnant women with abortion as in Table-4. IgG titre was 3(6%) cases, while IgM titre was 1(2%) case as in Table-5.

Discussion
Toxoplasma gondii is an obligate intracellular parasite. Infection with T. gondii which is normally controlled by the host immune system, results in an asymptomatic chronic infection maintained by dormant tissue cysts in immunocompetent individuals.[7,8]However, toxoplasmosis may cause severe disorders in immunocompromised patients and in pregnant women because of the high risk of transplacental transmission and the occurrence of multiple congenital lesions in the fetus.[9, 10]

The presence of Toxoplasma antibody in the serum is regarded as the important criterion for the diagnosis of toxoplasmosis. At present, several laboratories utilize different techniques such as indirect hemagglutination, ELISA, indirect immunofluorescence and latex agglutination test. [11-13]

Compulsory serologic screening for T. gondii infection is performed only in a few European countries (e.g., France, Switzerland, and parts of Italy). However, in most developing countries, a single serum sample of a pregnant woman is available recently or in the distant past. Identifying susceptible women is essential so that early treatment can be offered. Prenatal screening for antibodies to T. gondii is an important tool in this process. Ideally, the test should be specific, sensitive and easy to perform.

Table - 1 Analysis of socio demographic risk factors

Variables	Total	Positive
Age		
<20	2	0
20-30	51	3
30-40	49	2
>40	27	3
Gender		
Male	45	3
Female	84	5
Rural / Urban		
Rural	96	8
Urban	33	0
Water source		
River	99	4
Well	30	4
Food habits		
Vegetarian	44	3
Non-vegetarian	85	5
Contact with domestic animal		
Cow	19	2
Goat	8	1
Chicken	3	1
Cat	3	0

Table - 2 Seropositivity of toxoplasmosis in different patient categories

Category Of Patients	Number Of Subjects	Number Of Seropositive By LAT (%)
Pregnant women	50	4(3.1%)
HIV patients	72	3(2.33%)
Chorioretinitis	7	1(0.78%)
Total	129	8(6.20%)

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Table -3 Differentiation of anti-Toxoplasma antibodies into IgG-IgM

Result- LAT	TOXO-IgM		TOXO-IgG	
	No.	%	No.	%
Positive	3	(2.33)	5	(3.88)
Negative	126	(97.67)	124	(96.12)
Total	129	(100)	129	(100)

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