Research Paper

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Comparative Study of Elisa and Lat Techniques For the Serological Prevalence of Anti-Toxoplasma Antibodies Among Hiv Infected Patients

Medical Science

KEYWORDS : Toxoplasma gondii, IgG, IgM, HIV, Seroprevalence, Latex agglutination test, Enzyme Linked Immuno Sorbent Assay, Toxoplasmosis

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ABSTRACT

Toxoplasmosis, a zoonotic disease distributed worldwide, is an infection caused by the ubiquitous obligatory intracellular coccidian protozoan organism, Toxoplasma gondii. It is a major public health concern because the disease is serious in terms of mortality or physical and /or psychological sequellae in patients with HIV disease. Different methods like Latex Agglutination Test (LAT) and Enzyme Linked Immuno Sorbent Assay (ELISA) are available for the detection of anti-Toxoplasma antibody. The study was planned to conduct a comparative evaluation of ELISA and LAT techniques to assess the seroprevalence of Toxoplasma gondii IgG and IgM antibodies and associated risk factors in HIV infected patients. Venous blood samples were collected from 72 HIV infected pre anti-retroviral therapy patients at ART centre of Tirunelveli Medical college hospital. Serum samples were analyzed for anti-Toxoplasma gondii IgG and IgM antibodies using a commercially available ELISA kit and Latex Agglutination Test (LAT) kit. Socio-demographic and associated risk factors for Toxoplasmosis from each individual were also obtained and the data was analyzed using SPSS version 18. Of the examined HIV seropositive individuals by ELISA, 23.6% (17/72) and 5.6% (4/72) were positive for anti-T.gondii IgG and IgM antibodies, respectively. By LAT, 2.8 %(2/72) and 1.4 %(1/72) were positive for anti-T.gondii IgG and IgM antibodies, respectively. Comparatively, ELISA was found to be a more sensitive test. Strategies are required to prevent risk of spread of toxoplasmosis at institutional level. Public health education should be implemented regarding this disease and prevention, risk factors, and its negative influence-a significant contribution

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite with a global distribution in humans and other warm-blooded animals. Transmission to humans occurs through ingestion of *T. gondii* oocysts shed into the environment by cats, or by eating meat of infected animals. Under normal immune conditions, *T. gondii* infection is frequently asymptomatic, but in individuals who are immunocompromised, such as in patients with AIDS, the parasites can become widely disseminated, causing severe toxoplasmosis and encephalitis. Primary infections acquired during pregnancy may also result in severe damages to the fetus, manifested as mental retardation, seizures, blindness, and death [1].

In the immunocompromised patients, diseases due to *T. gondii* are generally considered to represent reactivation of latent infection if their CD4+ T-cell count decreases below 200 cells/µL [2-4]. Detection and monitoring of Anti-*Toxoplasma* antibodies are of a great interest in HIV-infected patients. In Tamil Nadu, various studies have done in northern districts [5, 6] but so far not reported in southern districts about serologic status and risk factors of Toxoplasmosis among HIV-infected patients except our earlier recent report.[7] Therefore, this study was aimed to conduct a comparative evaluation of ELISA and LAT techniques for the seroprevalence of anti-*T. gondii* IgG and IgM antibodies in HIV infected individuals.

Materials and Methods

Study population

This prospective study was conducted at Tirunelveli Medical College Hospital, Tirunelveli, 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 72 peripheral blood samples for this study were collected from HIV patients at ART centre, Tirunelveli Medical College Hospital.

Sample collection

Collected blood samples were transported immediately to Microbiology Laboratory. In the laboratory serum was separated, labelled and stored at −20°C until use. Using a pretested questionnaire, data on sociodemographic characteristics and associated risk factors were collected from all study participants.

Serological assays

Serum samples were screened for anti-*T. gondii* IgG and IgM antibodies by ELISA using "Toxoplasma IgG and IgM" kit (AB Diagnostics, NewDelhi)) and Latex Agglutination Test (LAT) IgM and IgG by using the Latex agglutination kit (Tulip Diagnostics) according to the manufacturers' instructions. Positive and negative controls were used with each series of anti *T. gondii* IgG/IgM test.

Results

Various socio demographic risk factors like age, gender, residence, water source, diet and animal contact, associated with the seropositivity of toxoplasmosis by ELISA presented in **Table 1**. Out of 72 samples (46 males and 26 females), 3 samples and 21 samples gave positive results to *Toxoplasma* antibodies by Latex agglutination test (LAT) and ELISA respectively. The test results showed higher incidence of *Toxoplasma* antibodies in males (32.6%) than females (23%) by using ELISA. LAT was positive in 4.2 % and ELISA was positive in 29.2% of cases for anti-*Toxoplasma* antibodies.**(Table-2)** All the samples positive by LAT were also positive by ELISA method. 25% of samples negative by LAT were positive by ELISA. Of the examined HIV seropositive individuals by ELISA, 23.6% (17/72) and 5.6% (4/72) were positive for anti-*T.gondii* IgG and IgM antibodies, respectively. By LAT, 2.8 %(2/72) and 1.4 %(1/72) were positive for anti-*T.gondii* IgG and IgM antibodies, respectively.

Table - 1 Analysis of socio demographic risk factors

Variables	Total	Positive by ELISA
Age		
20-30	12	2
31-40	36	10
41-50	22	7
>50	3	2
Gender		
Male	46	15

Female	26	6
Rural / Urban		
Rural	56	13
Urban	16	8
Water source		
River	53	14
Well	19	7
Food habits		
Vegetarian	48	15
Non-vegetarian	24	6
Contact with domestic animal		
Cow	19	2
Goat	8	1
Chicken	3	1
Cat	3	0

Table-2 Comparison between ELISA and LAT

Tests	TOXO-IgM		TOXO-IgG		Total (%)
	Positive	%	Positive	%	
LAT	1	(1.4)	2	(2.8)	3(4.2)
ELISA	4	(5.6)	17	(23.6)	21(29.2)

Discussion

The prevalence of toxoplasmosis in the human population is associated with exposure to risk factors. Serological surveys indicate that about 80% of the primary infections are asymptomatic, due to the effective immune system.[8] Serological tests are used to diagnose toxoplasmosis, and these tests are often helpful for the diagnosis of active infection, as well as indicating previous exposure.[9] The seroprevalence of toxoplasmosis is persistently evolving globally, with respect to regional socioeconomic status and habits.[10]

Seropositivity levels vary widely among different regions of India from low to high rates. In TamilNadu, various studies have done in northern districts but so far not reported in southern districts about serologic status and risk factors of Toxoplasmosis among HIV-infected patients except our earlier recent report in which the overall seropositivity of toxoplasmosis in and around Tirunelveli District of Tamil Nadu was 13.14 (46/350) per cent based on IgG ELISA.[5-7]

In the present study, Toxoplasma specific IgG and IgM antibody levels were analyzed using two different methods like screening test LAT and ELISA, one of the standard procedures for detection of antibodies to increase the accuracy of diagnosis and the seropositive rates from each assay were 4.2% and 29.2%, respectively. Detection of both IgG and IgM simultaneously helps establishing exposure to *T. gondii* and the chronological status of such exposure.

Present study revealed seroprevalence of 29.2% *T. gondii* in HIV positive individuals tested by ELISA. Meisheri *et al*. conducted a study of seroprevalence of Toxoplasmosis in general population and in HIV/AIDS patients in Bombay and reported the overall seroprevalence was 30.9 per cent (51/165) in the immunocompetent adult (34 per cent in men and 26.2 per cent in women) and in HIV infected hosts the seroprevalence was 67.8 per cent. [11] LAT is an efficient method for routine serologic screening for anti-*Toxoplasma* antibodies. The current study revealed that the prevalence rate of anti-*T. gondii* IgG antibodies in HIV positive pre-ART individuals was significantly higher (23.6%) by ELISA than LAT (2.8%). In this comparative study, ELISA was seen to be more sensitive technique than LAT. This variation between the two techniques could be due to the fact that the antibody levels in these individuals could be low hence not detected by LAT.

In this study, most of the *Toxoplasma* seropositive patients had $CD4^+$ T cell counts <100/µl which was similar to the observation made by Kasper and Kumarasamy *et al*.[12,13]

There are certain advantages of LAT over ELISA, including a low individual test cost, ability to obtain semi quantitative results, and a relatively short time to obtain results. Semi quantitative results can be obtained by performing 2 to 10-fold dilutions. At the same time; the disadvantages include the need to carefully interpret marginal results and problems with specificity due to interfering substances. These disadvantages are overcome by ELISA technique since its specificity is better than LAT, as in our study where 25% samples negative by LAT were positive by ELISA.

In conclusion, the present findings showed a high seroprevalence of anti-*T. gondii* antibodies in HIV infected patients. Consumption of undercooked or raw meat and contact with cat might greatly contribute towards acquiring *T. gondii* infection. It may be appropriate to include a routine serological screening test for determination anti-*T. gondii* antibodies

in HIV infected individuals. Those HIV infected individuals, positives for anti-*T. gondii* antibodies should be considered for chemo prophylactic treatment. In addition, health education particularly not to eat undercooked and raw meat, and avoiding contact with cats should be considered.

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