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Seroprevalence of *Toxoplasma gondii* in newly diagnosed HIV seropositive patients

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***Background & objectives*: Immunocompromised individuals mainly HIV infected patients are at a great risk for developing toxoplasmosis. The presence of toxoplasmosis among HIV-infected patients directly correlates with the prevalence of anti-*Toxoplasma gondii* antibodies and the degree of immunosuppression (measured by CD4 counts). The data regarding the seroprevalence of toxoplasmosis in HIV-infected patients are scarce in India. Therefore, this study was initiated to find out the seroprevalence of toxoplasmosis in treatment-naïve HIV seropositive patients and to determine its association with CD4 counts, if any.**

***Methods*: Four hundred newly diagnosed antiretroviral therapy (ART) naïve adult HIV positive patients coming for CD4 count estimation were tested for the presence of anti-*Toxoplasma* IgG antibodies. Risk factors for acquisition of toxoplasmosis as well as the age, gender and CD4 counts of the patient were noted down.**

***Results*: *Toxoplasma* IgG was positive in 292 (73%) patients, and the positivity was not related to their CD4 counts. The proportion of anti-*Toxoplasma* IgG positivity showed no significant association with age, gender and risk factors of the patients.**

***Interpretation & conclusions*: In the absence of any specific vaccine or prophylaxis for toxoplasmosis, it is pertinent to screen all HIV-positive patients for *Toxoplasma* IgG at diagnosis, irrespective of their CD4 counts, and sensitize them about the means to prevent either acquisition or activation of infection to avert the development of toxoplasmic encephalitis.**

Key words ART - CD_4 count - HIV - immunocompromised - seroprevalence - *Toxoplasma gondii* - toxoplasmosis

The global seroprevalence of toxoplasmosis has been reported to be 46.1 per cent, and in India, it has been found to be between 16.3 and 30.8 per cent[1,2]. In immunocompetent patients, 80-90 per cent of toxoplasmosis is asymptomatic[3]. However, in the immunocompromised patients, the disease may be severe. Acute toxoplasmosis in patients with HIV infection principally affects the central nervous system[4].

Immune response to *Toxoplasma gondii* is highly heterogeneous and complex due to heterogeneity in the genetic background of hosts. Despite the robust

immune response, tissue cysts develop leading to latent infection. This latent infection may reactivate under immunocompromised status[5]. Prolonged immune activation leads to activation-induced cell death of CD4+ T lymphocytes which are the primary immune cells for protection against toxoplasmosis[6]. CD8+ T lymphocytes, another important component of defence against toxoplasmosis is also affected by HIV[6]. Human CD4+ and CD8+ T lymphocytes are both cytotoxic to *T. gondii* infected cells therefore, HIV infection increases susceptibility to *T. gondii* infection [not necessarily with toxoplasmic encephalitis (TE)]. Studies have shown that the risk of developing TE increases to 30 per cent when CD4 count is less than 100 cells/µl[7].

In immunocompromised patients, reactivation of chronic infection is the most common manifestation of toxoplasmosis[8]; therefore, the British HIV Association recommends an initial assessment of anti-*Toxoplasma* IgG antibodies in these patients[9]. Majority of patients with AIDS have IgG antibody to *T. gondii* in serum[8]. Although IgG titres do not correlate with active infection, serologic evidence of infection always precedes the development of TE[9]. The HIV Medicine Association of the Infectious Disease Society of America also recommends that when and if the anti-*Toxoplasma* IgG levels are known and a patient comes with the signs and symptoms of TE, empirical treatment can be started[10].

Singh *et al*[11] reported the association between HIV infection and toxoplasmosis in 1996. Due to the need to identify the people at risk of toxoplasmosis and initiate necessary steps to decrease the morbidity and mortality associated with it, the present study was conducted in antiretroviral therapy (ART) naïve HIV-positive patients to determine the seroprevalence of toxoplasmosis and its association with their CD4 counts, if any.

Material & Methods

This cross-sectional study was initiated in the department of Microbiology of Seth G.S. Medical College & K.E.M. Hospital in Mumbai, India, from March 2015 to July 2016. Four hundred consecutive newly diagnosed ART naïve adult HIV positive patients coming for CD4 count estimation for the first time were enrolled for participation in the study after taking their written informed consent. The study was approved by the Institutional Review Board. Patients receiving primary prophylaxis for toxoplasmosis or pneumocystis were excluded from the study. Risk factors for the acquisition of toxoplasmosis were noted along with other demographic factors such as age and gender of the patient along with their CD_4 counts.

The sample size was derived by using the earlier reported seroprevalence of 67 per cent from the same institute with a precision of 0.05 and level of confidence 95 per cent[2]. Using the formula, $n = \frac{(Z\alpha)^2 P(1-P)}{d^2}$, where, n=sample size, (1−α) per cent=level of confidence, Zα=value of standard normal variant, P=expected prevalence, and d=absolute margin of error.

Four millilitres of blood sample was collected aseptically from each patient. The sample was collected along with the blood sample for CD4 count estimation. The serum was separated and the aliquot was stored in a sterile storage vial at −20°C till testing. Samples were evaluated for anti-*Toxoplasma* IgG levels using E-TXG-K18 *Toxoplasma* IgG ELISA kit (Ratio Diagnostics, Germany).

Statistical analysis: The statistical analysis was done doing Chi-square test and Karl-Pearson correlation test.

Results & Discussion

Of the 400 patients enrolled, 292 (73%) were positive for *Toxoplasma* IgG. Only 39 of the 292 (13.35%) *Toxoplasma* IgG-positive patients provided a definite history of risk factor exposure (blood transfusion, n=20, raw meat intake, n=11, contact with cats n=8). In the remaining 253 patients, exposure to risk factor remained obscure. The sex-specific proportions of *Toxoplasma* IgG in males and females were 74.6 and 71.56 per cent (Table I), respectively, and there was no significant difference between the two. The age-specific proportions of *Toxoplasma* IgG in the different age groups ranged from 68.35 per cent (≥45 yr) to 76.58 per cent (18-34 yr) (Table II) suggesting no significant difference of anti-*Toxoplasma* IgG antibodies between the different age groups.

Though the *Toxoplasma* IgG positivity increased as CD4 counts decreased (Table III), Karl-Pearson correlation coefficient (*r*) was −0.0305077, indicating that *Toxoplasma* IgG levels and CD4 counts were not related. The regression line's equation (y=482.401−0.116313x and the *P*=0.55) also

Table I. Comparison of *Toxoplasma* IgG positivity with gender (n=400)

Gender	Positive	Negative	Total	Per cent proportion positive
Males	151	60	211	71.56
Females	141	48	189	74.60
Total	292	108	400	73.00

Table II. Comparison of *Toxoplasma* IgG positivity with age (n=400)

Age (yr)	Positive	Negative	Total	Per cent proportion positive
18-34	157	48	205	76.58
35-44	81	35	116	69.82
≥45	54	25	79	68.35
Total	292	108	400	73.00

Table III. Comparison of *Toxoplasma* IgG positivity with CD4 counts (n=400)

CD4 counts (no. of cells/µl)	Total	Positive	Per cent proportion
Normal ≥500	169	112	66
Immunocompromised (≤499)	231	180	77.92

indicated that *Toxoplasma* IgG levels and CD4 counts were not related. Eleven patients had CD4 <200 cells/µl with *Toxoplasma* IgG levels >150 IU/ml.

Toxoplasmosis is a ubiquitous disease, caused by the coccidian parasite, *T. gondii*. Seroprevalence of *Toxoplasma* IgG in HIV-positive patients varies geographically from 7 to 80 per cent, such as 27 per cent reported by Holliman[12] in the United Kingdom, 44.8 per cent by Nissapatorn *et al*[13] in Malaysia, 15.43 per cent by Sucilathangam *et al*[14] in Tamil Nadu, 34.78 per cent by Anuradha and Preethi[15] in Telangana, India. Some studies have reported a high prevalence such as 67.8 per cent (Mumbai, India) by Meisheri *et al*[2], 58 per cent by Osunkalu *et al*[16] (Malaysia), and 76.5 per cent by Muluye *et al*[17] (Ethiopia).

In the present study, the seroprevalence was found to be 73 per cent, which was higher when compared to other parts of India like Tamil Nadu and Telangana[14,15]. This could be due to the difference in the geographical distribution of the agent and infection being more common in warm climates and at lowers altitudes than in cold and mountainous regions.

Toxoplasmosis mainly spreads through the ingestion of raw and undercooked meat, contact with cats (whose faeces are infected with oocysts of *Toxoplasma),* and blood transfusion[18]. In our study, only 13.3 per cent (39/292) positive cases were found to have associated risk factors, the most common being blood transfusion followed by a history of intake of raw meat and history of contact with cats. In a study by Walle *et al*[18] majority reported having contact with cats and the habit of eating raw or undercooked meat. Yohanes *et al*[19] also found a significant association with eating of raw meat and history of contact with infected cats with positivity. Both the above studies reported little association with blood transfusion[18,19].

Bhattacharyya *et al*[20] have reported the level of anti-*Toxoplasma* IgG to be inversely correlated with CD4+ levels, which was not seen in the present study. Deroiun *et al*[7] showed that incidence of TE was significantly higher in patients with IgG titres ≥150 IU/ml than in patients with titres <150 IU/ml (with a relative risk, 3.1) when the CD4 counts fall below 200 cells/µl. Hellerbrand *et al*[21] also noted that high titres of *Toxoplasma* IgG were observed several months prior to the first clinical and radiological signs of TE. In the present study, 11 of 48 (22.9%) patients who had CD4 <200 cells/µl were found to have *Toxoplasma* IgG levels ≥150 IU/ml.

The present study had several limitations. This being a cross-sectional study, the patients were not followed up for the development of TE and there was no control group. Only 13.3 per cent patients could give a history of exposure to risk factors. Since the cases were not followed up, further questioning of exposure to risk factors could not be done which otherwise would have added value to the study. Further, the positive cases could not be confirmed by other tests such as enzyme-linked fluorescence assay or immunofluorescence assay.

In India, ART is started as soon as the patient is diagnosed with HIV/AIDS, and trimethoprim-sulphamethoxazole (TMP-SMX) is given as a prophylaxis for *Pneumocystis jiroveci* pneumonia when CD4 counts are less than 200 cells/µl[22]. TMP-SMX has been shown to offer some benefit against toxoplasmosis also, but it may not be protective in all cases[22]. Furthermore, often opportunistic disease is the first manifestation of HIV infection[5]. Such patients will

be ART-naïve and will not be receiving TMP-SMX prophylaxis. Therefore, in the absence of any specific vaccine or prophylaxis for toxoplasmosis, it is pertinent to screen all HIV-positive patients for *Toxoplasma* IgG at diagnosis, irrespective of their CD4 status to prevent either acquisition or activation of infection, to limit the development of TE.

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***Conflicts of Interest*:** None.

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