Research Article

Toxoplasmosis in Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome patients in a tertiary care hospital of Pune city of Maharashtra, India

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ABSTRACT

Background: Toxoplasmosis in immunocompetent people is generally asymptomatic, but in immunocompromised patients including those suffering from human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), it leads to serious opportunistic infections (OIs), the most common being cerebral toxoplasmosis or toxoplasmic encephalitis. **Objective**: The objective of the current study was to determine the seroprevalence of *Toxoplasma gondii,* immunoglobulin G and immunoglobulin M antibodies in HIV/AIDS patients using enzyme-linked immunosorbent assay (ELISA) technique in Pune, Maharashtra, India. **Material and Methods**: The study design was a hospital–based, cross-sectional observational study. 100 HIV-positive patients were included in the study. Their serum samples were collected, and sera surveyed employing ELISA assay. Database was created in MS Excel, and appropriate descriptive and analytical statistics were calculated using Epi info statistical package. **Results:** 52% of the patients were seropositivity. Seropositivity was significantly associated with low CD4 counts. **Conclusion:** Seropositivity among HIV patients in our study group was as high as 52%. Toxoplasma infection being an important OI in HIV/AIDS patients; hence, screening for toxoplasma antibodies should continue to be stressed upon for all HIV patients when they present to the health-care provider.

KEY WORDS: Human Immunodeficiency Virus; Toxoplasma Antibodies; Immunoglobulin G; Immunoglobulin M; Seroprevalence

INTRODUCTION

Toxoplasmosis is caused by *Toxoplasma gondii*, an obligate intracellular protozoan parasite that causes generalized infection in humans and animals. The infection may be asymptomatic or may be accompanied by fever or symptoms of lung, liver, heart neurological, lymph node, or eye involvement. Toxoplasma infection affects about one-third of the world's population, but most infected individuals remain asymptomatic.[1] The initial infection is followed by the development of cell-mediated immunity, and this results in the chronic or latent phase of the infection, wherein the organism persists in various tissues of the infected individual, usually brain, skeletal muscle, and heart. However, in people who are living with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) or in those who are immunosuppressed due to other causes, there is an increased risk of reactivation of latent toxoplasma infection, and the most common manifestation is toxoplasmic encephalitis (TE)[2] which usually manifests when the CD4 counts fall below 200 cells/ul. Globally, the number of patients who die from AIDS has been declining over the years due to the introduction of highly active antiretroviral therapy (HAART), toxoplasma disease in HIV patients has also seen

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a decline due to early initiation of HAART and also primary prophylaxis against *T. gondii*.[3] However, despite the use of HAART, neurotoxoplasmosis remains the prevalent cause of neurological disorders in HIV-positive patients, especially in low-income group countries, where several patients continue to present with severe immunosuppression, and the coverage of HAART is not universal.[4] Around 95% of patients with cerebral toxoplasmosis present with detectable levels of immunoglobulin G (IgG) anti-*T. gondii* serum antibodies. Thus, it is extremely important that serology for *T. gondii* be performed, so that, with an early diagnosis, the person can receive appropriate treatment/prophylaxis to prevent the reactivation of the disease.[5] This study was carried out to study the clinical profile of HIV patients who are positive for anti-Toxoplasma IgG and immunoglobulin M (IgM) antibodies.

MATERIALS AND METHODS

Study Population

This hospital-based cross-sectional study was carried out in a tertiary care hospital of Pune with due approval from the Ethical Committee of the hospital. The study included 100 consecutive HIV-infected patients, newly diagnosed or already under follow-up, who attended the outpatient clinic and/or admitted in any of the wards at the hospital during the study period of 2 years, and informed consent was obtained. The study was carried out from March 2003 to April 2005.

Serum Samples

Approximately, 5 mL of venous blood sample was drawn from the participating HIV patients by a venipuncture into a sterile test tube. The sera were obtained after separation by centrifugation at 2500×g for 5 min and subsequently kept at –20°C until use

Detection of Anti-IgG and Anti-IgM Antibodies

The serostatus of Toxoplasma infection was screened using a standard enzyme-linked immunosorbent assay commercial kit in accordance with the manufacturer's instructions.

Statistical Analysis

Data obtained from both the questionnaire and laboratory tests were entered into a database created in MS Excel sheet. The data were checked for inaccuracies and corrected and were analyzed using Epi info statistical package. Appropriate descriptive and inferential statistics were calculated.

RESULTS

Baseline Profile of Study Participants

Table 1 shows the various age groups of the study participants. Majority of these patients were in the age group of 30-40 years followed by 40-50 years. These two groups accounted for 80% of the patients in the study. Only 4 females formed part of this study group. 95% of the study participants were married. 84% of the patients stated they were unaware as to how they contracted HIV and only 16 admitted to unsafe sexual contact.

Seroprevalence of Toxoplasmosis in HIV/AIDS Patients and Association with Other Factors

Table 2 shows the seroprevalence of Toxoplasma infection and mean CD4 cell count in each group. 52% (CI 42.32-61.53) of study participants were seropositive, whereas 48% of patients were non-reactors. 9 study participants were positive for both IgG and IgM. IgG positivity was seen in 24 patients, thus indicating past infection whereas IgM was positive in 19 patients, which indicates recent infection. The mean CD4 cell count was highest in the non-reactors. Among the reactors, those having IgG seropositivity had a higher CD4 count, followed by those with IgM seropositivity while the lowest mean CD4 count was seen in patients with both IgG and IgM seropositivity. It was thus seen that nonreactors have a significantly higher mean CD4 count ($t = 3.95$; $P < 0.01$).

Table 3 shows the distribution of seropositive and seronegative patients by the CD4 cell count levels. Decreasing CD4 counts was significantly associated with increasing seroprevalence (Chi-square for trends = 46.3 and $P < 0.0001$).

Table 4 shows the distribution of HIV-positive patients based on seropositivity, anti-retroviral therapy treatment, and co opportunistic infections (OIs).

Patients with toxoplasma seropositivity were suffering from concurrent OIs as follows: 3/6 patients with pulmonary tuberculosis had toxoplasma seropositivity. Toxoplasma

Table 1: Distribution of patients by age and sex

Age group (years)	Males	Females	Total (%)
10-20	0	0	0
20-30	10	3	13 (13)
30-40	46	1	47 (47)
40-50	33	0	33 (33)
>50	7	0	7 (7)
Total	96	4	100

Table 2: Seroprevalence of Toxoplasma infection and mean CD4 counts

Serology	Frequency and CI	Mean CD4 counts (SD)
IgG	24 (16.02-33.57)	226
IgM	19 (11.8-28.06)	160
IgG and IgM	09 (4.19-16.39)	98
Non reactors	48 (37.9-58.22)	351

IgG: Immunoglobulin G, IgM: Immunoglobulin M, CI: Confidence interval, SD: Standard deviation

Table 3: Distribution of patients by CD4 counts and serology

CD4 counts	IgG (%)	IgM (%)	IgG and IgM (%)	Total reactors (%)	Non-reactors (%)	Total (%)
<200	12 (38.70)	11 (35.48)	06 (19.35)	29 (93.53)	02 (6.47)	31 (100)
200-350	08 (34.78)	06 (26.08)	02 (8.69)	16 (69.55)	07 (30.45)	23 (100)
350-500	02 (9.09)	01 (4.54)	0	3 (13.33)	19 (86.37)	22 (100)
>500	02 (8.35)	01 (4.16)	01 (4.16)	4 (16.67)	20 (83.33)	24 (100)
Total	24	19	9		48	100

IgG: Immunoglobulin G, IgM: Immunoglobulin M. Chi-square for trends=46.3 and $P<0.000001$

Table 4: ART and coinfections in reactors and non-reactors

Factors	Reactors (%)	Non-reactors (%)	Total (%)	*P* value
ART				
Yes	45 (83.3)	9 (16.7)	54 (100)	$P<0.0001$
No	7 (15.21)	39 (84.78)	46 (100)	
Co-infections				
Yes	29 (58)	21 (42)	50 (100)	$P=0.23$
No	16 (44.44)	20 (55.56)	36 (100)	

ART: Anti-retroviral therapy

seropositivity was also found in 12 patients with disseminated tuberculosis, 2 patients with herpes zoster infection, 11 patients with oral candidiasis, 1 patient with pneumocystis jeroveci pneumonia, 1 patient with cryptococcal meningitis, and 7 patients of TE.

DISCUSSION

Our study showed a Toxo-IgG antibody seroprevalence rate of 19% and a Toxo-IgM seroprevalence of 24% in HIV-infected persons. Overall, 52% of the study participants were seropositive with anti-IgG antibodies or anti-IgM antibodies or both. Most of our patients were males, and out of the 4 females who were a part of the study, none were seropositive. In view of the fewer number of females in our study group, no correlations regarding seropositivity and gender can be made. The IgM antibody response to Toxoplasma infection is usually short lived and suppressed to undetectable levels in the setting of severe immunosuppression.[1,2] In consonance with this fact, our study also indicates lower rates of IgM seropositivity compared to IgG seropositivity. In our study, low CD4 cell count was significantly associated with seropositivity, wherein patients with lower CD4 cell counts were more likely to be seropositive. This can be explained by the reactivation of latent infection due to poor immune status, reflected by a low CD4 cell count. In our study, a significantly lower percentage of those on retroviral therapy was found to be seropositive, and the presence of co-OIs was not found to be significantly associated with seropositivity. The presence of OIs indicates lower immune status and thus may be associated with reactivation of Toxoplasma infection; however, this correlation was not apparent in our study.

Several other similar studies conducted in various parts of the world have shown toxoplasma seroprevalence varying from 15% to 68% depending on the geographical location of the conduct of the study. The seroprevalence figures among HIV-positive patients ranged from 40% in the USA,[6] 44% in Malaysia,[7] 53% in Thailand,[8] 67.8% in Mumbai, India,[9] 34.7% in Telangana, India,[10] 60% in Mexico,[11] 54% in Africa,[12] and 93% in Iran.[13] The variation in prevalence rates could be due to difference within the geographical locations as infection is more common in warm climates and at lower altitudes than in cold and mountainous regions, and because of differences in the sanitary conditions, personal hygiene, and other such related factors of the various populations being considered.[14] In our study, due to few number of females included in the study, no comments could be made on the association of male sex with seroprevalence. However, in a similar study carried out in Thailand, male gender was found to be the only variable significantly associated with the development of toxoplasma seropositivity. The same findings were also reported by other authors.[15-17] Male sex steroid hormones increase the susceptibility of men to acquiring several infections, including parasitic infections, by both influencing the disease resistance genes, decreasing immune responses, and by influencing their behavior in the form of increased risk taking attitude.[18,19] Our study found lower rates of IgM seropositivity (19%) as compared to IgG seropositivity (24%). Similar findings of lower rates of IgM seropositivity compared to IgG seropositivity in HIV-positive patients have also been documented in studies carried out in Mexico[11] and South Africa[20] and also by Meisheri et al.[9]

Low CD4 cell count was associated with seropositivity in our study. A similar finding was reported from studies carried out in France and Brazil, wherein patients with CD4 count of <200 cells/ul were more likely to be seropositive as compared to those with CD4 counts higher than 200 cells/ul,[21,22] similar correlation between low CD4 counts and toxoplama seropositivity has been reported by Anuradha and Preeti, in their study carried out in Telangana, India, and also in a similar study carried out in Tamil Nadu, India where CD4 counts <100 were found to be significantly associated with seropositivity.[10,23] However, studies carried out in Mexico, Malaysia, and Thailand[7,11,15] have shown no correlation between CD4 counts and seropositivity.

In our study, a significantly lower percentage of those on antiretroviral therapy was found to be seropositive.

However, in studies carried out in Thailand and Brazil, antiretroviral therapy was not significantly associated with seropositivity.[15,22] In our study, the presence of co-OIs was not found to be significantly associated with the development of seropositivity. In a study carried out in Nairobi among HIV-positive patients, no correlation between seropositivity and clinical stage of the disease was elicited.[24]

Limitation of the Study

This study was carried out in a large tertiary care hospital over a period of almost 2 years. However, due to patient referral procedures being followed, as the hospital was a tertiary care hospital, hence the results cannot be generalized to the primary care settings. Further, as there were only 4 females included in the sample, this is a limitation of the study.

CONCLUSION

Seropositivity for toxoplasma in HIV/AIDS patients in our study was 52%. Seropositivity was significantly associated with low CD4 count and not being on antiretroviral therapy. The presence of co OIs did not increase the risk of being seropositive.

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