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SEROPREVALENCE OF *TOXOPLASMA GONDII IN* NEWLYDIAGNOSED HIVSEROPOSITIVEPATIENTS

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Abstract

Toxoplasmosis, caused by *Toxoplasma gondii*, is a major opportunistic infection among HIV-positive individuals, particularly those who are immunocompromised. This study aimed to determine the seroprevalence of anti-*Toxoplasma* IgG antibodies in newly diagnosed ART-naïve HIV-positive patients and to assess its association with CD4 counts and selected demographic factors. A cross-sectional study was conducted among 400 adult HIV-positive patients attending a tertiary care hospital. Serum samples were tested for *Toxoplasma* IgG using ELISA. The overall seroprevalence was found to be **73%**. No statistically significant association was observed between IgG positivity and gender, age, education level, or place of residence. A significant correlation was detected between seropositivity and high-risk occupations such as farming and labor work ($p = 0.0005$). An inverse relationship was observed between IgG titers and CD4 counts ($r = –0.0305$), suggesting higher levels of chronic infection among severely immunocompromised individuals. Given the absence of routine screening and specific prophylaxis for toxoplasmosis under national guidelines, the study emphasizes the importance of screening all HIV-positive patients at diagnosis to prevent reactivation and development of toxoplasmic encephalitis.

Keywords: Toxoplasma *gondii, hive seropositive, seroprevalence, igg antibodies, cd4 count, opportunistic infections, toxoplasmosis, high-risk occupation, elisa, immunocompromised patients.*

Introduction

Toxoplasmosis, a zoonotic parasitic infection caused by *Toxoplasma gondii*, is estimated to have affected a large proportion (around one third) of the world population. (1, 2) Immunocompromised people mainly HIV-infected patients are at the greatest risk.

Even before the advent of AIDS, toxoplasmosis had been recognized as a cause of incapacitating disease and death among immunosuppressed patients. The incidence of toxoplasmosis among HIV infected individuals directly correlates with the prevalence of anti *T. gondii* antibodies, the degree of immunosuppression

(best measured by CD4 count), and (3) the immunologic response to antiretroviral drugs, and the use of effective prophylactic treatment regimens against the development of toxoplasmosis. AIDS associated toxoplasmosis is usually due to reactivation of a chronic (latent) infection that results from the progressive immune dysfunction that develops in these patients (5). It is estimated that 20-47% of AIDS patients who are infected with *T. gondii*, but are not taking anti-*Toxoplasma* prophylaxis or ART will ultimately develop toxoplasmosis. (3, 5) Case fatality may reach 100% if the infection is not treated or is treated very late in its course. Since such a high prevalence is usually observed and reactivation of chronic infection is the most common cause of toxoplasmosis in patients with AIDS, initial assessment of these patients needs an assay for anti *T. gondii* IgG antibodies. Those with positive result are at a high risk of reactivation of infection. Although fourfold decrease in both deaths and incidence have been reported after the wide availability of HAART regimens, (8-10) toxoplasmosis remains one of the most common CNS disorders in HIV infected patients, accounting for around 26% with a 1 year survival of 77%. (11) Thus there is a probable need of immediate initiation of primary prophylaxis in HIV positive patients with positive *T. gondii* IgG antibodies in addition to HAART. Under the National AIDS Control Programme, Ministry of Health and Family Welfare, Government of India, routine screening of anti-*Toxoplasma* IgG antibodies in not recommended and HIV positive patients are not put on prophylaxis for the same.(12)Presently, cotrimoxazole is used as prophylaxis for *Pneumocystis carinii* pneumonia, which has been proved to offer some protection against toxoplasmosis, however it is not protective in all cases and moreover some patients may not be able to tolerate the drug combination (6).

Materials and methods

Type of Study: Cross–sectional
Study Setting: Microbiology Department of a tertiary level teaching hospital
Sample size: 400 newly diagnosed ART naïve HIV seropositive patients
Study period: March 2019- January 2020
Methodology

After obtaining Institutional Review board permission the present study on **Seroprevalence of *Toxoplasma gondii* in newly diagnosed HIV Seropositive Patients** was initiated in the Microbiology Department of a tertiary level teaching hospital for the period of March 2020 to February 2021. 400 newly diagnosed ART naïve adult HIV positive patients coming for CD4 count estimation were enrolled for participation in the study. For the study, 4 ml of blood sample was collected aseptically from the patient. The sample was collected along with the blood sample for CD4 count estimation. There was no extra prick and the patient was not called a second time for giving the sample for *Toxoplasma* IgG estimation. The serum from the sample was separated by centrifugation at 3000rpm for 5mins, and the aliquot was stored in a sterile storage vial at -20^0 C. **Test Details:** Name of the test- E-TXG-K18 Toxoplasma IgG ELISA **Principle:** The test for the assay of Toxoplasma IgG is based on the capture of this immunoglobulin and the subsequent identification of those which are specific, making use of their ability to bind to an anti-human immunoglobulin conjugated with peroxidase. The capture is performed using purified and inactivated T. gondii antigens bound to the solid phase (microtitre wells).

Procedure:

All specimens and reagents were brought to room temperature (25^0 C) before use. There were 5 calibrators- 0-4. All calibrators were assayed in duplicate, except calibrator 1 which was assayed in triplicate. The micro titration strips to be used were marked. The serum samples were diluted to 1:101 by distributing 10µl sample to 1ml sample diluent.

1. 100µl of each diluted serum sample and calibrators were pipetted to the appropriate wells.
2. The plate was incubated for 45mins at 37^0 C.
3. Each well was aspirated and washed four times for 30seconds using washing solution using an automatic micro plate washer, they were then blotted dry by inverting plate onto absorbent material.
4. 100µl of Enzyme labelled 2nd Antibody was added into each well and then further incubated for 45mins at 37^0 C.
5. Each well was again aspirated and washed four times for 30seconds with washing solution using an automatic micro plate washer, and then blotted dry by inverting plate on absorbent material.
6. 100µl of TMB Chromogen solution was added to each well using dispenser and then incubated for 15mins at room temperature avoiding exposure to direct sunlight.
7. 100µl of stopping solution was added to each well after incubation.
8. The absorbance of the solution in the wells was read within 30mins, using a micro plate reader set at of dual wavelength of 450nm and 620nm.

Interpretation of results:

The mean absorbance for each calibrator and unknown was calculated.

Qualitative Results:

The Cut-off control corresponds to Calibrator 1. If the absorbance of the sample is higher than the Cut-off, the sample is positive for the presence of specific IgG. The ratio between the average OD value of the sample and that of the cut-off value was calculated.

The results were interpreted as follows:

Positive: if the ratio is >1.1 Doubtful: if +/- 10% of the cut off Negative: if the ratio is <0.9 If the result was doubtful, then the test was repeated. If it was still doubtful, then a new sample was collected.

Quantitative results:

A graph was constructed by plotting the U/ml against the average OD of the controls, when the OD of the sample was reported on the graph, the U/ml contained in the serum sample was calculated. Positive and negative results were expressed as follows: Positive: sample concentration >10IU/ml Negative: sample concentration < 9 IU/ml.

Statistical Analysis

Statistical analysis was done by doing Chi-square test and Karl Pearson correlation test. A P value of <0.05 was taken as significant.

Observation and Results

A total of 400 ART naive HIV positive patients fulfilling the inclusion criteria constituted the study population.

Observation 1

Number of patients positive for *Toxoplasma* IgG = 292 (73%).

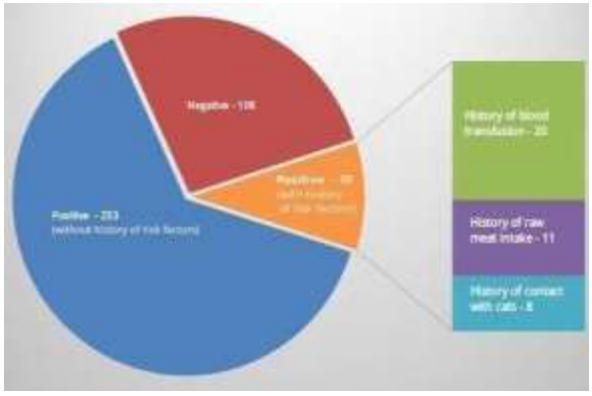

Figure 1: Seroprevalence of *toxoplasma* IgG with and without risk factors (n = 400)

Only 39 out of 292 Toxoplasma IgG positive patients provided us with definite history of risk factor exposure. In the remaining 253 patients, exposure to risk factor remained obscure even on meticulous questioning.

Table 2: Comparison of *toxoplasma* IgG positivity with gender (n = 400)

	***Toxoplasma* IgG status**			
	Positive	**Negative**	**Total**	**% Prevalence**
Female	151	60	211	71.56
Male	141	48	189	74.60
Total	292	108	400	73.00

Observation 2

The prevalence of *Toxoplasma* IgG in males and females were 74.6% and 71.56% respectively. P-value = 0.494.

There was no significant difference in the prevalence of anti-*Toxoplasma* IgG antibodies between females and males.

Table 3: Comparison of *toxoplasma* IgG positivity with age (n = 400)

	***Toxoplasma* IgG status**			
Age (in years)	**Positive**	**Negative**	**Total**	**% Prevalence**
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

Observation 3

Patients were divided into 3 age groups. The prevalence of *Toxoplasma* IgG in the different age groups ranged from 68.35% (≥45 yrs) to 76.58 %(18-34 yrs). P-value = 0.2474. There was no significant difference in the prevalence of anti-Toxoplasma IgG antibodies among the different age groups.

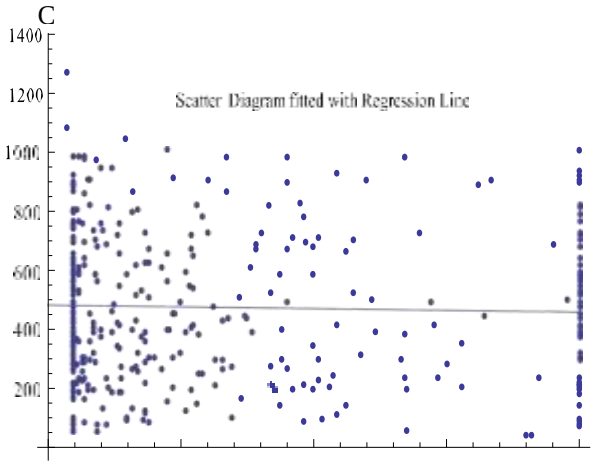

Figure 4: Correlation of *toxoplasma* IgG level (IU/ml) with $CD4^+$ count (cells/ul) (n = 400)

Observation 4

X and Y represent the sample data of Toxoplasma IgG levels and CD4 counts respectively.

- Karl-Pearson correlation coefficient (r) was -0.0305077 indicating that
- *Toxoplasma* IgG levels and CD4+ counts are inversely related.
- The regression line's equation was found to be y=482.401- 0.116313x.
- This also indicated that *Toxoplasma* IgG levels and $CD4^+$ counts are inversely related.
- The data points on the scatter plot shows 11 patients having CD4<200cells/mm^3 to have a *Toxoplasma* IgG levels >150IU/ml.

Table 5: Comparison of *toxoplasma* IgG positivity with employment status (n=400)

	Positive	Total	% Prevalence
Group A	**77**	**88**	**87.50**
Group B	**96**	**139**	**69.06**
Group C	**119**	**173**	**68.78**

Observation 5

Based on occupation, patients were classified into three groups and it was observed that, P value = 0.0005. There is a highly significant relationship between prevalence of anti-*Toxoplasma* IgG antibodies and Group A (High risk occupation for toxoplasmosis, like farmers, labourers).

Table 6: Comparison of *Toxoplasma* IgG positivity with education (n= 400)

Educational Status	Positive	Total	% Prevalence
Illiterate	58	77	75.32
Primary	127	175	72.57
Secondary	87	119	73.10
Tertiary	20	29	68.96

Observation 6

On classifying the educational level into four groups, the percentage prevalence of anti-*Toxoplasma* IgG antibodies ranged from 68.90-75.32. P value = 0.6094. There was no significant difference in the prevalence of anti-*Toxoplasma* IgG antibodies among the different educational levels.

Table 7: Comparison of *Toxoplasma* IgG positivity with place of residence (n=400)

	Total	Positive	% Prevalence
URBAN	383	279	72.84
RURAL	17	13	76.47

Observation 7

Since the number of patients hailing from rural areas were extremely small against those hailing from urban areas, comparison between the two is not statistically possible.

Discussion

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 on 400 ART naïve HIV positive patients to determine the seroprevalence of toxoplasmosis and to correlate the positivity with their CD4 counts., as immunocompromised patients are considered to be one of the high risk populations for toxoplasmosis. In the present study, the seroprevalence was found to be 73%, and the positivity was inversely proportional to the CD4 counts. The anti-Toxoplasma IgG levels had significant association with those employed in high risk occupations for toxoplasmosis; however it had no correlation with gender, age, education and place of residence.

Summary

The current study was conducted to know the seroprevalence of Toxoplasma gondii in newly diagnosed HIV positive patients and to correlate the positivity with their CD4 counts in a tertiary level hospital. 400 newly diagnosed ART naïve HIV positive patients were included in the study after written informed consent. 4 ml of blood was collected and was tested for the presence of Toxoplasma IgG by ELISA.

1. The seroprevalence was found to be 73%.
2. The prevalence of anti- *Toxoplasma* IgG antibodies between females and males was not statistically significant.
3. Maximum positive cases were in the age group of 18-34years (76.58%), but the difference was not statistically significant.
4. The positivity was inversely proportional to the CD4 counts. (Karl-Pearson correlation coefficient (r) = - 0.0305077)
5. Only 13.3% of the positive cases gave a history of exposure to risk factors.
6. Significantly higher seropositivity (87.5%) was found amongst those employed in high-risk occupations for toxoplasmosis (p= 0.0005)
7. No significant relation was found with the level of education.
8. Only 17 out of the total 400 were from a rural background, so a statistical comparison was not possible.

Conclusion

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, and sensitize them about the ways and means to prevent either acquisition or activation of infection, in order to prevent the development of Toxoplasmic encephalitis (TE).

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