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Original Research Article

Seroprevalence of *Toxoplasma gondii* in HIV infected cases and its association with CD_4 count in Silchar medical college and hospital

Barnamoy Bhattacharjee*, Debadatta Dhar Chanda

Department of Microbiology, Silchar Medical College and Hospital, Silchar, Assam, India

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***Correspondence:**
Dr. Barnamoy Bhattacharjee,
E-mail: bornob90@gmail.com

ABSTRACT

Background: Toxoplasmosis caused by the intracellular coccidian parasite *Toxoplasma gondii* is one the most common AIDS defining condition in HIV infection. It may produce fatal consequences if gains upper hand in immunocompromised states like HIV infection or due to reactivation of latent disease or new infection. *Toxoplasma* encephalitis, affecting 15-40% of world's PLHIV, is the most common opportunistic parasitic co-morbidity conventionally when CD_4 count falls below 200/µl. But there has also been reports of asymptomatic toxoplasma IgG seropositivity in PLHIV with CD_4 count in the range of 200-500/µl. This study was carried out to find the prevalence of *Toxoplasma* IgG seropositivity and to determine the association of *Toxoplasma gondii* seropositivity with CD_4 count in HIV infected cases in south Assam.
Methods: The 200 HIV infected cases with CD_4 count <500/µl attending ICTC, ART centre and various clinical departments were screened by *Toxoplasma* IgG ELISA over 1 year.
Result: It was found that 94 HIV infected cases were seropositive for *Toxoplasma* IgG and in which CD_4 count was significantly less than 200/µl in 54% cases and in the range of 200-500/µl in remaining 46% cases.
Conclusions: This sero-surveillance revealed anti-*Toxoplasma gondii* IgG in 47% cases. IgG seropositivity was significantly higher in cases on ART and in females with history of first trimester abortion. The finding of this study calls for initiation of prophylaxis against *Toxoplasma* routinely even in HIV cases of this region with CD_4count in 200-500/µl range as quite good proportion of *Toxoplasma* seropositive HIV cases have CD_4 count in this range apart from the conventionally vulnerable group with CD_4 count below 200/µl.

Keywords: *Toxoplasma* IgG, CD_4 count, HIV, Assam

INTRODUCTION

Toxoplasmosis caused by the intracellular coccidian parasite *Toxoplasma gondii* is one the most common AIDS defining condition in HIV infected cases.[1] Itmay produce fatal consequences if gains upper hand in Immunocompromised states like HIV infection to produce life threatening complications due to reactivation of latent diseaseor new infection. *Toxoplasma* encephalitis, affecting 15-40% of world's PLHIV, is the world's most common opportunistic parasitic co-morbidity conventionally when CD_4 count falls below 200/µl. But there has also been reports of both symptomatic and asymptomatic *Toxoplasma* IgG seropositivity in PLHIV with CD_4 count in the range of 200-500/µl. Statistically significant *Toxoplasma* seropositivity has also been found in HIV cases with CD_4 count below 500/µl as reported by Anuradha et al.[2] Incidence of cerebral toxoplasmosis in adults is still found high despite the advent of HAART.[3] Though there are many studies on toxoplasmosis in HIV infected cases in northern, western and southern India providing a picture of seroprevalence of *Toxoplasma gondii* in the HIV cases but there is hardly any study in the eastern and north-eastern part of India on this topic. So, we do not find any concrete statistical information in North eastern

India and Assam for the same and there is paucity of knowledge about *Toxoplasma gondii* infection among HIV infected cases and its associated risk factors in this geographical region, especially when the burden of PLHA in this part of India is on a rising trend. Since this health care institute of Southern Assam caters to quite large proportion of the HIV patients of varied spectrum of clinical presentation from5 neighboring north-eastern states, conducting a study in this part of India would definitely help to bridge the statistical data gap with rest of India and help to estimate the seroprevalence of *Toxoplasma gondii* IgG in HIV infected cases and find the association of CD_4 count with already diagnosed *Toxoplasma* seropositivity which will in turn help provide data to formulate decision regarding initiation of prophylaxis for Toxoplasmosis routinely in PLHIV with CD_4 count less than 500/µl.

Aim

Aim of the study was to estimate the seroprevalence of *Toxoplasma gondii* IgG in HIV infected cases in the only referral tertiary care hospital of Southern Assam, to determine the association of *Toxoplasma gondii* IgG seropositivity with CD_4 count in HIV infected individuals reporting to ICTC, ART Plus center and various clinical departments and to study the association of demographic factors, various other risk factors and anti-retroviral therapy with *Toxoplasma gondii* seropositivity in HIV infected cases.

METHOD

This study was carried out with proper Ethical clearance as a prospective hospital based cross-sectional study in Silchar Medical College and Hospital from June 2018 to May 2019 in 200 HIV infected cases (Inclusion criteria-HIV seropositive case of age >12 years and HIV seropositive cases who had latest CD_4 count less than 500/µl.

Exclusion criteria were-1. HIV seropositive cases below 12 years of age, HIV seropositive antenatal cases, HIV seropositive cases with CD_4 count >500/µl and eligible cases who did not give voluntary consent for participation in the study were excluded) attending ICTC, ART Plus center and various clinical departments with formal consent. Conventional qualitative ELISA was done for anti-*Toxoplasma* IgG antibodies in the HIV positive serum samples using Diapro diagnostic Toxo IgG kit (with sensitivity and specificity > 98%) having 96 wells coated with purified gamma irradiation inactivated *Toxoplasma gondii* proteins. Positive cases were calculated based on the cut off as per the SOP of the ELISA kit.

CD_4 counting of the HIV cases were done in the state reference laboratory of department of microbiology of the said tertiary care hospital from 2 ml venous blood using flow-cytometryprinciple by Cyflow counter machine, a volumetric software controlled absolute count system.

Ethical clearance was taken from institutional ethical committee and Assam state AIDS control society.

Consent collection

In accordance with the direction from Assam State Aids Control Society, the entire procedure starting from sample collection, to testing and consequence of result was first explained to the patient himself/herself or to guardians of minor cases in their native or intelligible language. Thereafter, a formal written voluntary consent was taken in English or Bengali from patients themselves or parents/ guardian (in case of minor) in the form of signature or left thumb impression (in case of illiterate and extremely debilitated persons). Following this, an open-ended questionnaire was filled up which had patient's personal and habitual profile and their relevant clinical details, keeping in mind at each step that patients right to privacy and confidentiality was not infringed upon.

Statistical calculation

Was done by using online p value calculator from www.socscistatistics.com.

RESULTS

It has been calculated from result of qualitative anti-*Toxoplasma* IgG ELISA that out of 200 PLHA cases, 94 cases are reactive for anti-*Toxoplasma gondii* IgG with all the reactive cases having antibody titre more than 50 IU/mL since the cut off for the ELISA kit is set at 50 IU/mL. Thus, the seroprevalence of *Toxoplasma gondii* in HIV infected cases attending this tertiary care referral Hospital stands out to be 47% (Figure 1).

The 46% of *Toxoplasma* IgG seropositive HIV cases were found to have CD_4 count in the range of 200-500/µl and a little higher proportion of 54% PLHIV Toxoplasmosis cases had CD_4 count less than 200/µl (Figure 2). This comparative association of CD_4 count and *Toxoplasma* seropositivity in HIV has been found to be statistically significant with p value 0.000566. Highest *Toxoplasma* IgG seropositivity was noted in the age group of 31-40 years (Figure 3). The higher no. of male *Toxoplasma* seropositive cases as compared to females does not bear any statistical significance as p=0.83. With regards to influence of ART, statistically significant higher seroprevalence was noted in patients on ART (p=0.000635) (Figure 4). Thus, this study shows that lower number of *Toxoplasma* seropositive cases are found to have history of cat handling or contact with cats (Figure 6), which has been found to be statistically significant (p=0.02). Blood transfusion was not found to be a statistically significant risk factor for *Toxoplasma* seropositivity (p=0.508) in this study. *Toxoplasma*

seropositivity had statistically significant association with history of first trimester abortion or fetal death. This has been found to be a statistically significant association between the two factors (p=0.029). None of the participants had habit of or gave history of consumption of raw or undercooked meat, so the association of this factor with *Toxoplasma* seropositivity if any at all could not be found out.

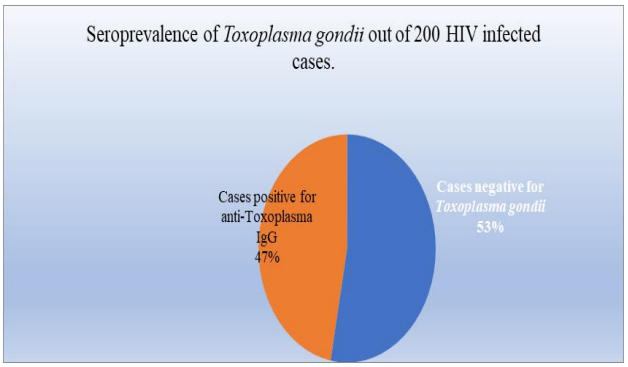

Figure 1: Seroprevalence of *Toxoplasma gondii*.

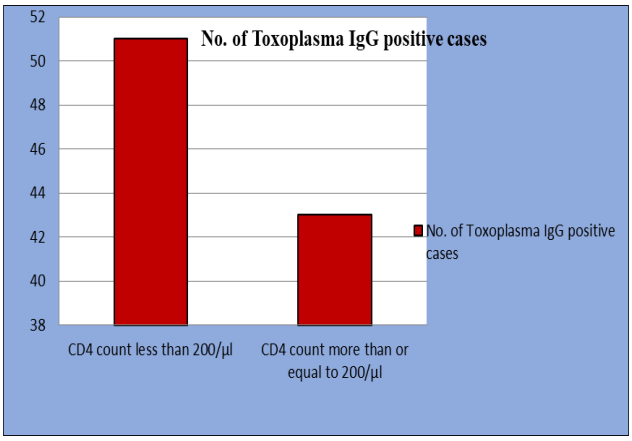

Figure 2: Variation of *Toxoplasma* seropositivity with CD4 count.

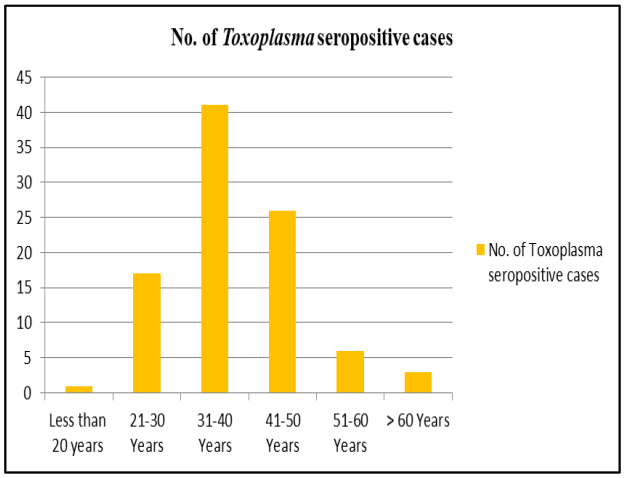

Figure 3: Variation of *Toxoplasma* seropositivity with age distribution.

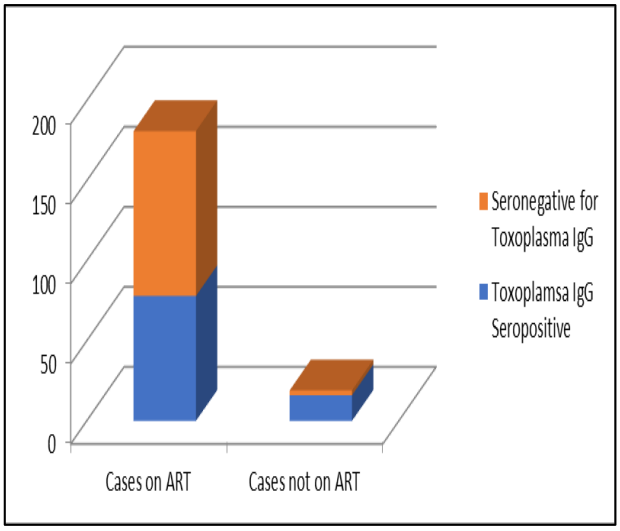

Figure 4: Influence of ART on *Toxoplasma* seropositivity.

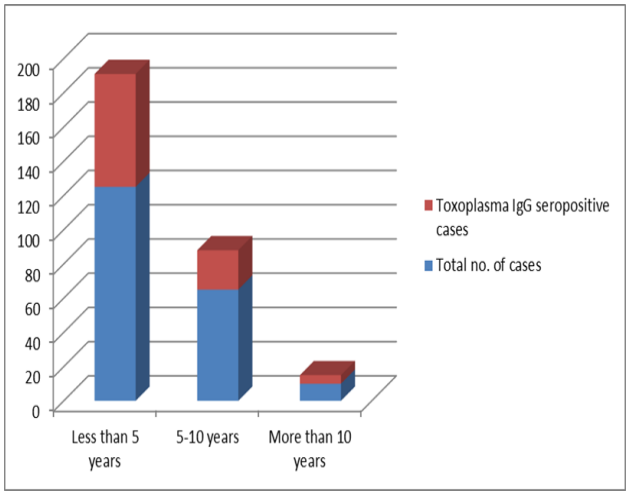

Figure 5: Variation in *Toxoplasma* seropositivity with duration of HIV infection.

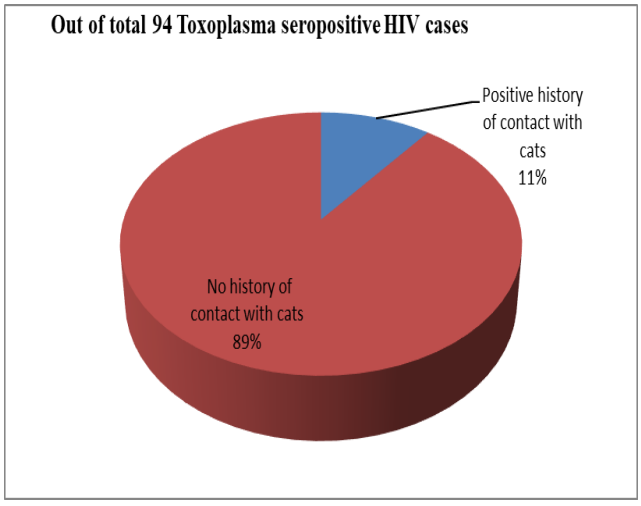

Figure 6: Influence of contact with cats and felines on *Toxoplasma* IgG seropositivity.

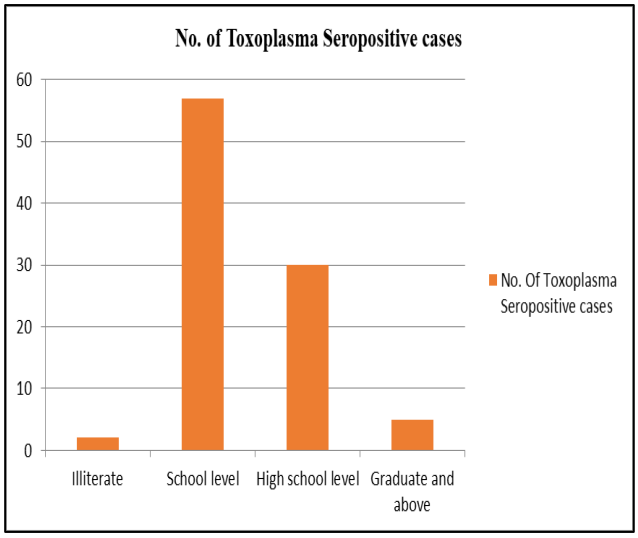

Figure 7: Level of education against *Toxoplasma* seropositivity.

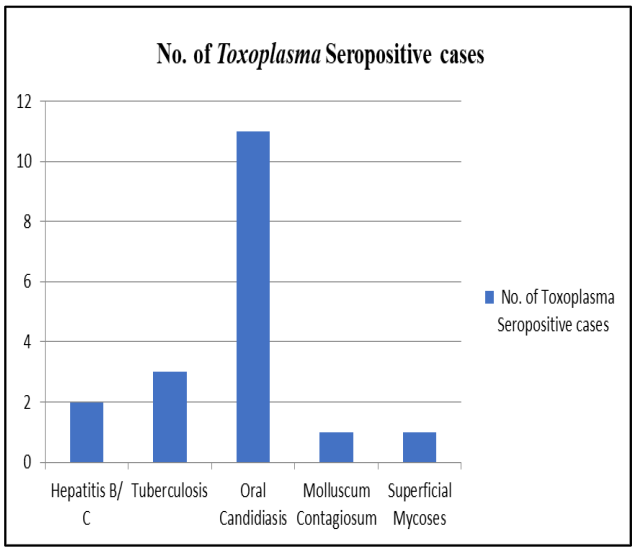

Figure 8: Seroprevalence of *Toxoplasma* IgG and opportunistic infections.

DISCUSSION

In this study the seroprevalence of anti-*Toxoplasma gondii* IgG in HIV infected patients from south-eastern part of Assam and neighboring North-Eastern states of Mizoram, Tripura, Manipur and Nagaland has been found to be 47% (94 seropositive cases out of 200). Similar seroprevalence has been found in a study carried out in 2012 by Bhattacharyya dept of microbiology KGMU Lucknow and Khurana et al from PGIMER Chandigarh on 100 HIV infected individuals from northern India.[4]

Mathew et al in a cross-sectional study on HIV patients attending the ART centre of Kozhikode medical college Kerala also found the seroprevalence of *Toxoplasma gondii* to be 40% which is almost in concordance with finding of this study.[5]

Basavraju et al found the seroprevalence of Toxoplasmosis to be 34.78% in a cross-sectional study carried out on 92 HIV infected patients attending the ICTC of Khammam Telangana in 2013.[2]

Nissapatorn et al in their study *Toxoplasmosis* in HIV/AIDS patient in Malaysia found the prevalence of seropositivity for *Toxoplasma* to be 41.2%.[6]

In a retrospective cohort study on 1130 HIV positive cases attending the AIDS centre of Prague from 1988-2012 by Machala et al, the seroprevalence was found to be 42%.[4,7] Similar seroprevalence (49.75%) was reported in a study carried out on 201 HIV infected cases by Alinaghi et al from Tehran, Iran Okwuzu et al from Lagos Nigeria also found 40.5% seroprevalence of *Toxoplasma gondii* IgG in 242 HIV infected cases.[8,9]

In contrast, low seroprevalence of 10.8%, 12%, 15% and 21% were found by Amuta et al, Malla et al, Sucilathangam et al and Perween et al respectively.[10-13] Walle et al found seroprevalence of Toxoplasmosis to be as high as 87.4%.[14] Cademartori et al in 2009-2010 also found 80% prevalence of *Toxoplasma* seropositivity in Brazil.[15] Very high seroprevalence of 94% was found in HIV infected women of reproductive age group in Southwest Ethiopia by Zelek et al.[16] Highest seroprevalence of 96.3% was recorded in a study by Madhavi et al in HIV infected cases in North of Iran.[17]

Association of Toxoplasma gondii seropositivity and CD_4 count

Amongst the *Toxoplasma gondii* IgG positive cases found in HIV infected cases with CD_4 count below 500/µl and attending Silchar medical college and hospital, higher number of *Toxoplasma* seropositive cases (51 out of 94) were found in patients with CD_4 count below 200/µl. This association has been found to be statistically significant (p=0.0005). Similar association has also been found by Basavraju et al in Khammam Telengana.[2]

Mukherjee et al in their study on HIV infected patients of a tertiary care hospital of Pune Maharashtra also found that *Toxoplasma* seropositivity was significantly associated with low CD4 counts.[18] Decreasing CD4 counts was significantly associated with increasing seroprevalence of anti-*Toxoplasma* IgG which is in concordance with our study. Bhattacharyya et al on 100 HIV infected patients from northern India found that the mean CD_4 cell count was significantly lower ($p<0.05$) in patients with seropositivity for IgG.[4] Similar negative correlation was also found by Malla et al which is at par with our study.[11] Osunkalu et al in their study on 380 HIV infected patients at Lagos university Teaching hospital found that there was an association between CD_4 cell count <100 cells/µL and Toxo-IgG antibody seropositivity in HIV-positive subjects without neurological symptoms which finding has also been seconded by Vasmehjani et al in their study in Jahrom of

Southern Iran found that CD_4 cell count<100 cells/µl was significantly associated with higher seropositivity for *Toxoplasma* in HIV infected cases.[9,19]

But findings of studies by Amuta et al from Benue state of Nigeria, Ogoina et al from Northern Nigeria, Nazari et al from West Iran, Nissapatorn et al from Malaysia are in discordance with our study as all of them have found that *Toxoplasma* seropositivity in HIV cases has no significant association with CD_4 count.[6,16,19-21]

Association of Toxoplasma seropositivity and anti-retroviral therapy

In this cross-sectional study on HIV seropositive subjects, we have found that 82% of the *Toxoplasma* seropositive cases are on ART which is only in concordance with study of Muluye et al from Gondar Northwest Ethiopia.[22]

On the contrary, most other studies viz. Mukherjee et al in their study in Maharashtra, Alinaghi et al from Tehran Iran, Cademartori et al in South Brazil, Vasmehejani et al found that seroprevalence of *Toxoplasma gondii* was significantly lower in HIV cases on ART.[8,15,18,19]

Association of Toxoplasma seropositivity and age and gender

In present study, though statistically insignificant it is seen that *Toxoplasma* seropositivity is highest in the age group of 31-40 years followed by age group of 41-50 years, which is at par with the findings of Anuradha et al, Khamman Telangana, Meisheri et al Bombay amongst the Indian publications and Nazari et al, West Iran, Vasmehjani et al from Jahrom of Southern Iran, Nissapatorn et al from Kuala Lumpur Malaysia amongst the international publications where it has been found that these two age groups are not statistically significant risk factor associated with *Toxoplasma* seropositivity.[2,6,19,23]

Chemoh et al from Southern Thailand and Osunkalu et al from their study in Lagos university teaching hospital found that male gender was significantly associated with *Toxoplasma* seropositivity.[9,25] Female gender and *Toxoplasma* seropositivity was found to be associated significantly by Tegegne et al.[26]

But both the findings are in discordance with the present study where we find that gender is not significantly associated with higher seroprevalence of *Toxoplasma gondii* and this finding of present study is in concordance with finding of Perween et al Northern India, Mathew from Southern India.[5,13] Various international studies in concordance with present finding are Mahdavi et al, Cademartori et al, Ogoina et al in their study in Northern Nigeria, Muluye et al in their cross-sectional study in Gondar university hospital Northwest Ethiopia, Aliaghi et al in their study on HIV infected subjects in Tehran, Iran, Vasmehjani et al from Jahrom of Southern Iran.[8,15,17,19,20,22]

Association of Toxoplasma seropositivity and contact with cats

In this study conducted here on the HIV patients attending Silchar medical college and hospital, handling of felines or living with cats has not been found to be a significant risk factor as lower seroprevalence of *Toxoplasma gondii* has been found in HIV cases with exposure to cats. This finding has echoed globally in the following studies-Chemoh et al from Southern Thailand, Cademartori et al from South of Brazil, Tegegne et al from Jimma Ethiopia, Nissapatorn et al in their study in Malaysian HIV infected population, Muluye et al from Northwest Ethiopia and Madhavi et al from Northern Iran.[6,15,17,22,25,26]

Association of occupation and level of education with Toxoplasma seropositivity

The present study could not find any significant association of either level of education or occupation of the participating HIV infected subjects with seropositivity of *Toxoplasma gondii* though majority of the *Toxoplasma* seropositive cases were either driver or mason and had school level education. This is in concordance with study of Muluye et al, Vasmehjani et al, Nissapatorn et al from Malaysia, Chemoh et al from Southern Thailand.[6,15,19,22] But Cademartori et al from South of Brazil reported that in their study of evaluation of seroepidemiological *Toxoplasmosis* in HIV/AIDS patients, the seroprevalence of Toxoplasmosis was significantly more in study population with school level education than in cases with high school level education unlike this study where the same finding is statistically insignificant.[15]

Duration of HIV infection and Toxoplasma seropositivity

This study shows that duration of HIV infection of the cases has no significant association with seropositivity of anti-*Toxoplasma* IgG. This is in concordance with studies of Madahvi et al from North of Vasmehjani et al in their study on HIV infected cases in Jahrom, Southern Iran.[17,19]

Association of Toxoplasma seropositivity and blood transfusion

Blood transfusion has not been found as a significant risk factor for *Toxoplasma* seropositivity in the present study. This has also been stated in studies by Chemoh et al from Southern Thailand and Cademartori et al from South of Brazil.[15,25] Apart from above mentioned risk factors and study of their associations with seroprevalence of *Toxoplasmosis* in HIV patients, it has been found that only the publication of Mukherjee et al reported that in their study on HIV patients in a tertiary care hospital of Pune, Maharashtra, they did not find any significant association of co-infections and seropositivity of *Toxoplasma gondii*.[18] This is in discordance with finding of present study where various co-infections like hepatitis

B, hepatitis C, tuberculosis, oral candidiasis, superficial mycoses etc. have been found to have significant association with anti-*Toxoplasma* IgG seropositivity in HIV infected individuals with CD_4 count below 500/µl (Figure 8).

Limitations

The present study could not go for estimation of anti-*Toxoplasma gondii* IgM in the antenatal HIV infected cases as the study has been conducted at authors own expense and no financial aid was received for this. So, conducting a large-scale ELISA based (both IgM and IgG) study including all sorts of HIV infected cases and covering different geographical territories of North-Eastern India will help us determine the precise magnitude of the burden of *Toxoplasma* seropositivity in HIV infected cases in this region and thus deal with its sequelae accordingly.

CONCLUSION

The seroprevalence of *Toxoplasma gondii* in HIV infected cases attending the tertiary care referral hospital of Southern Assam stands out to be 47%. This study asserts that CD_4 count <200/µl is significantly associated with higher prevalence of *Toxoplasma gondii* seropositivity. Demographic factors have not been found to be significant attributes towards anti-*Toxoplasma* IgG seropositivity in HIV cases in this study. Seroprevalence of *Toxoplasma gondii* has been found to be significantly higher in HIV infected cases on ART and in female study subjects with history of first trimester abortion or fetal death. The statistically significant observation found in this study reiterates in support of the need for initiating prophylaxis for Toxoplasmosis routinely in the HIV cases whenever CD_4 count goes below 500/µl as good proportion of Toxoplasma seropositive cases have also been found in the CD_4 count range of 200-500/µl in addition to finding higher number of Toxoplasmosis cases in PLHIV with CD_4 count less than 200/µl at par with the conventional knowledge.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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