



Prevalence of Toxoplasmosis among HIV/AIDS Patients and Correlation of Radiological Investigations with Laboratory Findings

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Authors' contributions

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ABSTRACT

Background: *Toxoplasma gondii* an intracellular protozoan causes toxoplasmosis a worldwide neglected tropical disease, which also causes severe disease in immunocompromised patients. The Toxoplasmosis was known for cerebral involvement more commonly among patients with HIV/AIDS and serious than extra-cerebral toxoplasmosis. For cerebral toxoplasmosis patients the definitive diagnosis is crucial by demonstrating the presence of the tachyzoite form of *Toxoplasma gondii* directly in the cerebral tissues. This study was undertaken to assess the prevalence of toxoplasmosis among HIV patients and role of radiological investigation in the diagnosis.

Materials and Methods: A prospective cross-sectional study was conducted at Department of

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Microbiology, Government Medical College and Hospital, Nagpur, Maharashtra, India. A total of 362 HIV positive patients attending ART clinic and provides informed consent were included in this study. The relevant investigations of each patient with clinical history were noted. ELISA test was carried out from blood samples to detect anti-toxoplasma IgM and IgG antibodies as per the manufacturer's instructions. Data was analyzed using SPSS version 21.

Results: Out of 362 HIV positive patients, Majority of the male patients were positive for HIV positive than females, 62.43% and 37.57% respectively. About 99.17% of patients were married and most of them were from urban area (86.46%), Majority of the patients were labourers (39.78%) followed by house wives (20.72%). A total of 23.48% HIV positive patients were co-infected with toxoplasmosis of which 21.55% patients were married and from urban area. Most of the HIV positive patients and co-infected with toxoplasmosis were between the age groups of 25-34 and 35-44 years. The overall correlation of serological and radiological features was seen in 68.23%. Although radiological investigations were helpful in providing better localization of toxoplasmosis but less confirmatory than ELISA. So combination of modalities should be used in diagnosis of toxoplasmosis for appropriate management. Both the ELISA and the CT-scan were simultaneously positive in 75 (20.72%) and negative in 172 (47.51%) cases. Among all HIV-toxoplasma co-infected patients, hyper density was seen in 15.29% patients, hypo density was present in 43.52% patients. Ring enhancement was observed among 29.41% cases.

Conclusion: We conclude from this study that the prevalence of toxoplasmosis in HIV positive patients was high (23.48%) and more commonly found between the age groups of 25-34 and 35-44 years. Radiological investigations were helpful in providing better localization of toxoplasmosis but less confirmatory than ELISA.

Keywords: *Toxoplasmosis; prevalence; sero-positivity; HIV/AIDS; ART Centre.*

1. INTRODUCTION

In immunocompromised patients *Toxoplasma gondii* causes severe disease [1-2]. Among the immunocompromised patients who previously acquired latent infection can lead to develop reactivated toxoplasmosis with encephalitis. The disseminated toxoplasmosis and toxoplasmic encephalitis have been noted in patients with immunodeficiency because of various underlying causes, such as Hodgkin's disease or immunosuppressive therapy. Disseminated toxoplasmosis may also complicate the transplantation of organs resulting due to either from transplantation of an organ from a *Toxoplasma gondii* infected donor to a susceptible recipient or from reactivation of a latent *Toxoplasma gondii* infection in the recipient due to immunosuppressive therapy [3-5].

The prevalence of toxoplasmosis infection varied dependent on the geographical regions and socio-demographic characteristics. In European and other tropical regions, prevalence of toxoplasmosis is over 50% [6]. In US, majority of HIV-infected patients had antibodies against *T. gondii* and sero-prevalence data of HIV-infected patients was at the range of 3-22% [7,8]. It was 9.8% in Hong Kong [9], Nigeria 75.4% [10], 58.4% in Tunisia [11], 28.5% in Benin [12],

40.2% in Senegal [13], 74.5% in South Brazil [14], 63.7% in Paris [15], Kodym et al. [16] reported 30% in Chezech republic. A study from Telangana, India, reported the sero-prevalence of 34.78% among HIV-positive patients [17]. Another study by Sucilathangam et al. [18] observed 15% of *Toxoplasma* sero-positivity in HIV-positive people.

The initial presentation of toxoplasmic encephalitis among patients with HIV/AIDS may be subacute. The patients present with various clinical symptoms such as headaches, fever and altered mental status, associated with focal neurologic deficits. The progression of the infection could lead to further symptoms like confusion, drowsiness, seizures, hemiparesis, hemianopsia, aphasia, ataxia, and cranial nerve palsies. As disease prolongs the motor weakness and speech disturbance are seen. If the patients are not treated promptly, they may progress to coma within few days to weeks. Toxoplasmosis rarely present as a rapidly fatal form of diffuse or global encephalitis with profound changes in mental status, nausea and vomiting usually with elevated intracranial pressure [19-23].

The cerebral involvement is common among HIV/AIDS patients and more serious than extracerebral toxoplasmosis. For cerebral

toxoplasmosis patients the definitive diagnosis is crucial by demonstrating the presence of the tachyzoite form of *Toxoplasma gondii* directly in the cerebral tissues. For cerebral toxoplasmosis the presumptive diagnosis including the clinical symptoms, In clinical practice the radiological findings, serological and molecular diagnosis for *Toxoplasma* infection and good response to anti-toxoplasma therapy are widely accepted. The favorable outcome of cerebral toxoplasmosis is the improvement of clinical and radiological features after 2 to 3 weeks of therapy. The clinical diagnosis is a dilemma because of the cerebral toxoplasmosis mimics with other brain diseases. Which makes it difficult to diagnose. Differential diagnosis of HIV/AIDS-associated cerebral toxoplasmosis is extremely important. The local neuro-epidemiology and the degree of immunosuppression in the host are two key factors involved [24-26].

The radiological observations, either by computed tomography (CT) or magnetic resonance imaging (MRI) are useful modalities in the presumptive or empirical diagnosis of cerebral toxoplasmosis. These findings are however not pathognomonic of cerebral toxoplasmosis. Radiological diagnosis can be classified as typical findings of hypodense lesions with ring enhancing and perilesional edema are observed in nearly 80% of cerebral toxoplasmosis cases [27].

Cerebral toxoplasmosis poses a diagnostic problem that relies on classical serological methods to detect anti-toxoplasma immunoglobulins because clinical blood samples from patients with immunodeficiency can fail to produce sufficient titers of specific antibodies. Sero-evidence of toxoplasma infection, independent of antibody levels is generally seen in all patients before developing cerebral toxoplasmosis. Most cerebral toxoplasmosis patients have high titers of anti-toxoplasma IgG antibodies with high IgG avidity that provides serological evidence of infection and this also supports a conclusion that this is the result of a secondary reactivation of latent or chronic toxoplasma infection. Therefore, it is important to determine the toxoplasma sero-status in all HIV-infected patients in order to define the population at risk for cerebral toxoplasmosis. At the onset of cerebral toxoplasmosis, significant rises in anti-toxoplasma antibody titers are found in only a marginal number of these patients. The level of rising titers may occur before the onset of cerebral toxoplasmosis and it does not seem to predict the occurrence of cerebral toxoplasmosis.

Anti-Toxoplasma IgM antibody, as measured by the indirect fluorescent or ELISA tests, is rarely found in cerebral toxoplasmosis patients. In cases of cerebral toxoplasmosis, a negative or low titer of serological results or even the absence of anti-toxoplasma antibodies does not exclude positive diagnosis and the anti-toxoplasma therapy should be started without delay if clinical and radiological presentations are consistent with cerebral toxoplasmosis. A positive serology result seems to be even less useful in areas where there is a high prevalence of toxoplasmosis in the general population, while a negative result does have a high negative predictive value [28-29]. However, there is paucity of adequate information on the prevalence of *Toxoplasma gondii* infection among HIV/AIDS patients in India. Therefore, the present study was undertaken to assess the prevalence of toxoplasmosis among HIV patients and role of radiological investigation in the diagnosis.

2. MATERIALS AND METHODS

2.1 Study Design

The present prospective hospital based study was conducted in the Department of Microbiology, Government Medical College and Hospital, Nagpur, Maharashtra, India. A total of 362 HIV positive patients included through following criteria attending ART clinic during this study period. The convenient sampling method was adopted and the demographic data were recorded on data collection sheet such as age, sex, marital status, occupation and residency, risk factors for HIV transmission, clinical and laboratory data on toxoplasmosis.

2.2 Inclusion Criteria

All volunteered confirmed HIV/AIDS positive patients with CNS signs and symptoms suggestive of toxoplasmosis such as headache, fever, increased intracranial tension, seizure, altered sensorium, papilloedema, cerebellar signs were included in this study.

2.3 Exclusion Criteria

Patients with immunocompromised status due to other than HIV infection were excluded from this study.

To maintain the strict confidentiality and to conceal the identity of the patient, coding system for sample was followed which was known only

to investigator and password protected data was stored electronically. After taking written informed consent, detailed clinical history and all relevant investigations (including radiological for the diagnosis of toxoplasmosis) of each patient was done and findings were noted as per standard predesigned and pretested clinical proforma.

Blood samples to provide the sera for ELISA test were taken by venipuncture. At least 5 ml of blood was obtained to ensure that there will be enough serum for the test. Immediately blood was transferred from the syringe into dry stoppered sterile tube and allowed to clot. When the serum has separated, it was pipetted off into a sterile tube [30]. Serum samples were then subjected to ELISA test for detecting anti-toxoplasma IgM and IgG antibodies as per the manufacturer's instructions. Process was performed as per standard protocol [31].

Data was compiled in MS Excel and checked for its completeness and correctness. Then it was analysed using SPSS version 21, quantitative tests were applied with p value of < 0.05 was considered statistically significant for interpretation of the findings. Final diagnosis (based on ELISA) was compared with the

radiological findings to assess the accuracy of radiological investigations. The prior ethical approval was sought for this study by the institutional ethics committee.

3. RESULTS

Out of these 362 HIV positive patients, 226 (62.43%) were males and 136 (37.57%) were females. Most of the HIV positive patients, 359 (99.17%) were married and only 3 (0.83%) were unmarried and 313(86.46%) were from urban area whereas 49 (13.54%) were from rural area. Majority of the patients were laborers 144 (39.78%) followed by house wives, skilled employee, drivers, farmers and others were 75 (20.72%), 64 (17.68%), 52 (14.91%), 12 (3.31%) and 15 (4.14%) respectively. Total 85 (23.48%) HIV positive patients were co-infected with toxoplasmosis. 65 (17.96%) were males and 20 (5.52%) were females. All of the co-infected patients were married and 78 (21.55%) from urban area. Whereas, 7 (1.93%) were from rural area. Majority of the co-infected patients were laborers 35 (9.67%) followed by drivers, skilled employee, house wives, farmers and others were 17 (4.70%), 13 (3.60%), 12 (3.31%), 3 (0.82%) and 5 (1.38%) respectively (Table 1).

Table 1. Prevalence of toxoplasmosis co-infection among HIV positive patients according to socio-demography

Variables	HIV positive (%), N=362	IgG positive (%), N=71	P value	IgM positive (%), N=14	P value	IgG + IgM (%)
Age in years						
15-24	23 (6.32)	3 (0.82)	0.488	0 (0)	0.734	3 (0.82)
25-34	171(47.23)	32 (8.84)		6 (1.66)		38 (10.50)
35-44	133(36.74)	31 (8.56)		5 (1.38)		36 (9.94)
45-54	29(8.01)	5 (1.38)		2 (0.55)		7 (1.93)
55 and above	6 (1.66)	0 (0)		1 (0.28)		1 (0.28)
Total	362 (100)	71(19.61)		14 (3.86)		85 (23.48)
Sex						
Male	226 (62.43)	53 (14.72)	0.018*	12 (3.31)	0.093	65 (17.96)
Female	136 (37.57)	18 (4.97)		2 (0.55)		20 (5.52)
Marital status						
Married	359 (99.17)	71 (19.61)	0.390	14 (3.86)	0.737	85 (23.48)
Unmarried	3 (0.83)	0 (0)		0 (0)		0 (0)
Residence						
Rural	49 (13.54)	6 (1.66)	0.162	1 (0.28)	0.531	7 (1.93)
Urban	313 (86.46)	65 (17.96)		13 (3.60)		78 (21.55)
Occupation						
Driver	52 (14.91)	15 (4.14)	0.488	2 (0.55)	0.749	17 (4.70)
Farmer	12 (3.31)	3 (0.82)		0 (0)		3 (0.82)
Laborer	144 (39.78)	28 (7.73)		7 (1.93)		35 (9.67)
Housewife	75 (20.72)	11 (3.03)		1 (0.28)		12 (3.31)
Skilled employee	64 (17.68)	11 (3.03)		2 (0.55)		13 (3.60)
Others	15 (4.14)	3 (0.82)		2 (0.55)		5 (1.38)

As shown in Table 2, the toxoplasmosis co-infection among HIV positive patients according to signs, symptoms and other risk factors was assessed. The signs and symptoms associated with co-infection showed that majority of the patients had headache (22.10%) and fever (19.06%). Whereas, 8.01%, 3.04%, 2.49%, 1.93%, and 0.56% co-infected patients had symptoms of increased intracranial tension, seizure, papillo-edema, altered sensorium and cerebellar signs respectively. Almost half of the patients (43 (11.88%) had history of pet contact, 67 (18.50%) history of meat ingestion and 5 (1.38%) had past history of toxoplasmosis, which were statistically significant. Mode of transmission was through sexual contact among 76 (20.10%), whereas, among 9 (2.49%) patients the transmission was unknown. Decreased CD4 cell count was observed <100 among 22 (6.08%), 101-200, 201-500 and >501 CD4 cells among 13 (3.60%), 37 (10.22%) and 13 (3.60%) respectively. However, majority of the patients, 63 (17.40%) responded to treatment.

The correlation between the ELISA seropositivity and radiological feature is shown in Tables 3 and 4, both the ELISA and the CT-scan were simultaneously positive in 75 (20.72%) and negative in 172(47.51%) cases, the overall correlation seen in 247(68.23%). Strong association between ELISA and CT-scan by McNemars χ^2 test was found. 10 (2.76%) cases were positive by ELISA and negative by radiological examination for toxoplasma. The Odds ratio was found to be 12.29 with 95% CI = (5.84-26.53) which was found to be statistically significant.

As shown in Table 5 the radiological features suggestive of toxoplasmosis in HIV-toxoplasmosis co-infected patients in the CT-scan head (n=85). Out of these hyper density and hypo-density was present in 13 (15.29%) and 37 (43.52%) patients respectively. Whereas, 25 (29.41%) cases showed only ring enhancement as the radiological feature. Only 4 (4.70%) patients had hyper density and

Table 2. Prevalence of toxoplasmosis co-infection among HIV positive patients according to signs, symptoms and other risk factors

Variables	IgG positive (%)	P value	IgM positive (%)	P value	IgG + IgM
Signs and symptoms					
Headache	68 (18.78)	0.000*	12 (3.31)	0.019*	80 (22.10)
Fever	57 (15.75)	0.000*	12 (3.31)	0.013*	69 (19.06)
Increased intracranial tension	21 (5.80)	0.555	8 (2.20)	0.004*	29 (8.01)
Seizure	6 (1.66)	0.898	5 (1.38)	0.000*	11(3.04)
Altered sensorium	4 (1.10)	0.573	3 (0.82)	0.024*	7 (1.93)
Papilloedema	6 (1.66)	0.095	3 (0.82)	0.001*	9 (2.49)
Cerebellar sign	2 (0.55)	0.547	0 (0)	-	2 (0.56)
History of pet contact					
Yes	37 (10.22)	0.000*	6 (1.66)	0.002*	43 (11.88)
No	34 (9.40)		8 (2.20)		42 (11.60)
History of meat ingestion					
Yes	54 (14.91)	0.000*	13 (3.600)	0.000*	67 (18.50)
No	17 (4.70)		1 (0.28)		18 (4.98)
Past history of toxoplasmosis					
Yes	5 (1.38)	0.000*	0 (0)	-	5 (1.38)
No	66 (18.23)		14 (3.87)		80 (22.10)
Mode of transmission					
STD	64 (17.68)	0.487	12 (3.31)	0.516	76 (20.10)
Blood transfusion	0 (0)		0 (0)		0 (0)
Unknown	7 (1.93)		2 (0.55)		9 (2.49)
CD4 cell count					
<100	20 (5.52)	0.227	2 (0.55)	0.292	22 (6.08)
101-200	10 (2.76)		3 (0.82)		13 (3.60)
201-500	28 (7.73)		9 (2.49)		37 (10.22)
>501	13 (3.59)		0 (0)		13 (3.60)
Response to treatment					
Yes	51 (14.10)	0.000*	12 (3.31)	0.000*	63 (17.40)
No	20 (5.52)		2 (0.55)		22 (6.08)

Table 3. Correlation between radiological features in CT-scan head and ELISA for anti-toxoplasma antibodies

Radiology	ELISA		Total (%)
	Positive (%)	Negative (%)	
Positive	75 (20.72)	105 (29.00)	180 (49.72)
Negative	10 (2.76)	172 (47.51)	182 (50.28)
Total (%)	85 (23.48)	277 (76.52)	362 (100)

McNemars χ^2 test=78.48 (Software used: Stata ver.10.0), Odd's Ratio =12.29, 95% Confidence Interval = (5.84-26.53); p value <0.0001 considered significant

Table 4. Comparative evaluation of radiological test with ELISA

Statistic	Formula	Value	95% CI
Sensitivity	$\frac{a}{a+b}$	88.24%	79.43% to 94.21%
Specificity	$\frac{d}{c+d}$	62.09 %	56.10% to 67.83%
Positive Likelihood Ratio	$\frac{\text{Sensitivity}}{100 - \text{Specificity}}$	02.33	1.96 to 2.76
Negative Likelihood Ratio	$\frac{100 - \text{Sensitivity}}{\text{Specificity}}$	00.19	0.11 to 0.34
Positive Predictive Value	$\frac{a}{a+c}$	41.67% (*)	34.38% to 49.23%
Negative Predictive Value	$\frac{d}{b+d}$	94.51 % (*)	90.13% to 97.33%

Note: a-true positive, b-false positive c-false negative, d-true negative [32]

Table 5. Radiological features in HIV-toxoplasmosis co-infected patients (N=85)

Radiological features	No. of cases (%)	p-value
Only Hyperdensity	13(15.29)	0.408
Only Hypodensity	37(43.52)	0.026*
Only Ring-enhancement	25(29.41)	0.000 *
Hyperdensity + Ring-enhancement	04(4.70)	0.384
Hypodensity + Ring-enhancement	06(7.05)	0.280
Total (%)	85(100)	-

ring-enhancement features. Whereas, 6(7.05%) cases showed hypo density and ring-enhancement. Only hypo density and only ring-enhancement were the radiological features which were found to be statistically significant (p<0.05).

4. DISCUSSION

Toxoplasmosis is the most common opportunistic infection in HIV-infected patients. A high seroprevalence of anti-*Toxoplasma gondii* IgG antibody has been reported in HIV-infected subjects [32-35]. This study showed that the seroprevalence of *Toxoplasma gondii* was 23.48%. The co-infection occurred most among married male from urban population with the

history of pet contact, meat ingestion and unsafe sexual activity. This rate is almost comparable with the other studies reported elsewhere such as study by Holliman [36], in which the seropositivity of toxoplasmosis found to be 26.06%, Sykora et al. [37] found seropositivity of toxoplasmosis 29.8% in HIV positive patients. Brindle et al. [38] found seropositivity of toxoplasmosis to be 22%. Oksenhendler et al. [39] found that 25.4% was seropositivity rate. Similarly, Minkoff et al. [40] found that 20.2% was the seroprevalence of toxoplasmosis. Millogo et al. [41] found seropositivity in 25.4%. The seropositivity was found as 67.8%, 23.2%, 22.4%, & 21% by studies conducted by different authors like Sukthana et al, Nissapatorn et al and Nissapatorn et al. respectively [42-44]. Also, Hari

et al. Akanmu et al. and Oshinaike et al. carried out study to find out seroprevalence of toxoplasmosis and found out to be 8%, 54% and 85.5% respectively [45-47]. This study revealed that there is high prevalence of *Toxoplasma gondii* co-infection among HIV/ AIDS patients suggesting that HIV-infected populations should be protected from *Toxoplasma gondii* infection to reduce the morbidity and burden of the disease.

In our study, both the ELISA and the CT-scan were simultaneously positive in 75 (20.72%) and negative in 172 (47.51%) cases, the overall correlation seen in 247 (68.23%). In fact we found a strong association between ELISA and CT-scan by McNemars χ^2 test. Also, statistically was found to be highly significant ($p=0.000$). A retrospective study was done by Venugopal A et al. 2012, among AIDS patients in a tertiary care hospital. The diagnosis was based on clinical features, demonstration of elevated IgG by ELISA and associated CT-scan findings. 2826 HIV positives attended Infections Disease Cell from 2000 –2010, of which 33 (1.12%) had CNS toxoplasmosis. Mean level of IgG was 255.69. CT / MRI finding of ring enhancing lesion or cerebritis was seen in 79% of the cases with 18% of lesions in both basal ganglia and parietal lobes. Cerebritis was most common lesion in CT/MRI, seen in 16 cases while ring enhancing lesions were seen in 10 cases. 82% improved with treatment and 18% died of complications. The possibility of cerebral toxoplasmosis should be considered in every HIV-positive patient with neurological symptoms parietal lobe lesions were common in their study, contrary to other existing data which say toxoplasma lesions are usually midline lesions [48].

In the present study, out of these 85 HIV-toxoplasma co-infected patients, only hyperdensity was seen in 13(15.29%) patients, only hypodensity was present in 37 (43.52%) patients whereas 25 (29.41%) cases showed only ring enhancement as the radiological feature. The patients had both hyperdensity & ring-enhancement features were 4 (4.70%). Whereas, 6 (7.05%) cases showed hypodensity and ring-enhancement simultaneously. Similar radiological features were reported in a study by Vidal et al. [23] showed that typical findings of hypodense lesions with ring enhancing and perilesional edema were present in nearly 80% of cerebral toxoplasma patients. An another study reported that in patients with toxoplasma encephalitis various lesions found were hypodense lesion with ring-enhancement and

perilesional edema, nodular enhancement and perilesional edema with small, enhancing asymmetric nodule along wall of the lesions [28].

5. CONCLUSION

This study concludes that the majority of the HIV positive patients were males (62.43%) than females (37.57%). Majority of them were married (99.17%) and from urban area (86.46%), Majority of the patients were labourers (39.78%) followed by house wives (20.72%). A total of 23.48% HIV positive patients were co-infected with toxoplasmosis of which 21.55% patients were married and from urban area. Most of the HIV positive patients and co-infected with toxoplasmosis were between the age groups of 25-34 and 35-44 years. The overall correlation of serological and radiological features was seen in 68.23%. Although radiological investigations were helpful in providing better localization of toxoplasmosis but less confirmatory than ELISA. So combination of modalities should be used in diagnosis of toxoplasmosis for appropriate management.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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