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ORIGINAL ARTICLE

Cryptococcal antigen prevalence in HIV patients from a tertiary care centre in South India

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ABSTRACT

Background and Objectives: Cryptococcosis is an opportunistic mycosis, caused by *Cryptococcus neoformans*. Cryptococcal meningitis is one of the most fatal opportunistic infections associated with human immunodeficiency virus (HIV) infection. The aim of this study was to find the prevalence of cryptococcal antigenemia in people living with HIV (PLHA) and also to find the prevalence of opportunistic infections among these patients.

Materials and Methods: A total of 204 non duplicate samples were collected from people with HIV aged above 18 years. Samples with CD4 count less than 300 were included in the study. Cryptococcal antigen detection was done by CrAg Lateral flow assay.

Results: None of the patients in our study were positive for cryptococcal antigen. Opportunistic infections were observed in 82 (40.2%) HIV positive patients. Candidiasis, tuberculosis and *Pneumocystis jiroveci* pneumonia were the most common opportunistic infections.

Conclusion: This is the first study from the southern state of Kerala on the prevalence of Cryptococcal antigenemia among HIV positive individuals. The study showed that routine screening for cryptococcal antigen will not be cost effective in our population. Similar to other studies, eventhough candidiasis, tuberculosis and PCP were more commonly seen among people with CD4 count < 200 cells/mm3, there was no statistically significant association.

Keywords: Cryptococcosis; Toxoplasmosis; Opportunistic infections; Tuberculosis; Candidiasis

INTRODUCTION

Cryptococcosis is a serious opportunistic fungal infection among those with weakened immune systems, such as those with advanced HIV/AIDS. Cryptococcosis is an opportunistic mycosis, caused by an encapsulated yeast *Cryptococcus neoformans* (1). This yeast is usually found in soil contaminated with bird droppings particularly from pigeons and chickens, usually inhaled through lungs and remain dormant for many years (2). Reactivation which leads to infection is common among immunocompromised individuals like people living with human immunodeficiency virus/acquired immunodeficiency syndrome (PLHA) (3). Cryptococcal meningitis is one of the most fatal opportunistic infections associated with human immunodeficiency virus (HIV) infection (4).

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The common methods used in most laboratories for diagnosis of cryptococcal infection include the India ink stain of body fluids for encapsulated yeasts and culture of body fluids. India ink lacks sensitivity and is often negative in patients. Culture can take more days to a result, and require large specimen volumes. In 2009, a lateral flow immunoassay (LFA) for the detection of cryptococcal antigen (CrAg) was introduced by IMMY (Immuno-Mycologics, Inc., OK, USA) as a point of care test for diagnosis of cryptococcal infection. This test is stable at room temperature (20 ± 25ÊC), has a rapid turnaround time and does not require technical expertise. Its sensitivity is almost 100% with both serum, plasma and CSF samples (5). The CrAg test can detect the target antigen from peripheral blood on average of 22 days prior to the development of CM (Cryptococcal Meningitis) and about 11% of patients will have positive CrAg test more than 100 days prior to the onset of the disease, CM (6).

The use of preemptive therapy in asymptomatic cases with positive antigenemia is not well defined, but international recommendation has suggested its use based on expert opinion (7). World Health Organizations (WHO) in 2011 recommended CrAg screening for HIV positive persons with CD4 below 100 cells/μL, followed by preemptive fluconazole treatment in areas with high prevalence (8). However, in India no formal recommendations for CrAg screening have been issued. The management of CM requires prolonged hospitalization resulting in significant increase in health care costs. Treatment of asymptomatic cryptococcal infection with oral fluconazole is a much less expensive and easily available option compared to standard-of-care treatment for meningitis (9, 10). There are very few data from India on the prevalence of cryptococcal antigenemia.

Our suggestion is that routine testing may result in early detection of asymptomatic infected subjects. Therefore, the aim of this study was to find the prevalence of cryptococcal antigenemia in people living with HIV (PLHA), especially the advanced cases and also to find the prevalence of opportunistic infections among these patients.

MATERIALS AND METHODS

This prospective study was conducted in the Department of Microbiology, Government Medical College, Alleppeyfrom November 2018- February 2019.

Samples were collected from a total of 204 people with HIV aged above 18 were included in the study.

Ethical approval. The study was approved by the Institutional Review committee of Government Medical College, Alleppey and written consent was obtained from patients. After obtaining consent, serum samples were collected from study subjects which included the following.

Exclusion criteria. (i) HIV patients with cryptococcal disease diagnosed within the previous year, (ii) HIV patients on antifungals within last 14 days and (iii) subjects who are not willing to participate in the study.

Inclusion criteria. (i) HIV patients enrolled in ART clinic, (ii), HIV patients with advanced disease and (iii) Newly diagnosed HIV patients.

Specimen collection. Under aseptic precaution 2 ml blood will be collected from the cubital fossa by venipuncture by phlebotomist for CD4 count. An additional 2ml blood will be taken without additional puncturing for testing for cryptococcal antigen. The serum was separated and test was performed using CrAg LFA as per manufacturers instructions. The IMMY CrAg® LFA is an immunochromatographic dipstick assay for the qualitative and semi-quantitative detection of cryptococcal antigen in serum, plasma, wholeblood and cerebrospinal fluid (CSF). The results will be read in 10 minutes and reported.

The following variables were collected from medical records and/or patient interviews and recorded on proforma form and transcribed to an Excel® spreadsheet: age, gender, CD4 cell count, highly active antiretroviral therapy (HAART) status, WHO clinical stage, opportunistic infections.

Statistical analysis. Data was entered in excel and analyzed using JASP 18.0. Data was expressed in percentage and proportions. Difference in proportions were calculated using Chi-square test.

RESULTS

In this study, we collected data of 204 HIV infected patients and tested their serum for the presence of

cryptococcal antigen. Among the 204 patients, 122 (59.8%) were males and 82 (40.2%) were females. Mean age of the participants was 42.5 years and mean CD4 count was 175.15 with standard deviation of 78.26. HIV positive patients were classified according to the WHO classification system (Table 1).

Demographic and Clinical details of HIV patients screened for cryptococcal antigenemia during the study is shown in Table 2.

None of the patients in our study were positive for serum cryptococcal antigen. Opportunistic infections (OI) were observed in 82 (40.2%) HIV positive patients. Multiple OI were observed in 15 patients and a total of 97 OI were found. Distribution of OI are shown in Table 3. Opportunistic infections distributed according to their CD4 count is shown in Table 4.

Opportunistic infections like Tuberculosis, Candidiasis and *Pneumocystis jiroveci* pneumonia were commonly seen in HIV patients with CD4 count <200 but there was no statistical significance.

Table 1. WHO Classification of HIV positive patients (CD4 count< 300)

Stage	n (%)
1	78 (38.2)
2	40 (19.6)
3	59 (28.9)
4	27 (13.2)

Table 2. Details of HIV patients (CD4 count< 300) screened for cryptococcal antigenemia

Variable		n (%)
Sex		
Male		122 (59.8)
Female		82 (40.2)
Age group	0-14 years	3 (1.5)
	15-24 years	10 (4.9)
	25-34 years	30 (14.7)
	35-44 years	67 (32.8)
	45-59 years	82 (40.2)
>59 years		12 (2.9)
CD4 Count	0-30	6 (2.9)
	31-60	18 (8.8)
	61-90	15 (7.4)
	91-120	19 (9.3)
	121-150	21 (10.3)
	151-180	13 (6.4)
	181-210	31 (15.2)
	211-240	30 (14.7)
	241-270	29 (14.2)
	271-300	22 (10.8)
Marital Status	Single	32 (15.7)
	Married	153 (75)
	Divorced	2 (1)
	Widowed	17 (8.3)

DISCUSSION

The findings from our study indicated a 0% prevalence of cryptococcal antigenemia among HIV patients. This is concordant with the study conducted by Hajiabdolbaghi et al. in Iran which also showed a prevalence of 0% (10). Prevalence of Cryptococcal antigen among HIV patients in various studies is shown in Table 5 (11-17). One study conducted in Eastern India (Maharashtra) showed a prevalence in cryptococcal antigenemia of 8% (17). One of the main reasons for the zero prevalence of cryptococcal antigen was probably that only 43 (21.1%) patients in the study group had a CD4 count below 100.

Table 3. Distribution of Opportunistic infections among HIV patients (CD4 count< 300)

Opportunistic infections (n=97)	Frequency	Percent
Tuberculosis	27	27.8
Toxoplasmosis	1	1
Cytomegalovirus infection	1	1
Candidiasis	51	52.6
Non Hodgkins Lymphoma	1	1
Herpes Zoster infection	6	6.2
Pneumocystis jiroveci pneumonia	10	10.3

Table 4. Frequency of Opportunistic infections distributed according to their CD4 count

Opportunistic infections (n=97)	CD4 > 200 (n=31)	CD4< 200 (n=66)	P value
Tuberculosis	7	20	0.42
Toxoplasmosis	0	1	0.49
Cytomegalovirus infection	0	1	0.49
Candidiasis	19	32	0.24
Non Hodgkins Lymphoma	0	1	0.49
Herpes Zoster infection	3	3	0.33
Pneumocystis jiroveci pneumonia	2	8	0.38

Table 5. Prevalence of Cryptococcal Antigen in various studies

Author	Place	Year	Total patients	Prevalence of Cryptococcal Antigen	Reference
Smith et al.	Vietnam	2009-2012	226	4%	11
McKenney et al.	United states	1986-2012	1872	2.9%	12
Ezenabike et al.	Nigeria	2020	300	19.67%	13
Liechty et al.	Uganda	2003-2004	377	13.5%	14
Micol et al.	Cambodia	2004	327	21%	15
Jemal et al.	Ethiopia	2019	140	11.43%	16
Kadam et al.	India	2011-2012	208	8%	17

To the best of our knowledge, this is the first study from the southern state of Kerala showing the prevalence of cryptococcal antigen among HIV patients. HIV infection is one of the main risk factors for tuberculosis. Tuberculosis is one of the main causes of morbidity and mortality among HIV patients. In the present study tuberculosis was a common opportunistic infection with a prevalence of 27.8%. Fungal infections are important causes of morbidity among patients with HIV and are common OIs among HIVpositive individuals and can affect up to 94% of 5 infected individuals, depending on the stage of the infection and the population analyzed (18, 19). Candidiasis was the most common infection observed before the use of Anti retroviral therapy. Candidiasis was the most common opportunistic infection seen in our study with a prevalence of 52.6%.

The most common opportunistic infection among HIV patients in the western world is *Pneumocystis jiroveci* pneumonia or PCP (20). Studies in India on prevalence of PCP are scanty. A study conducted by Udwadia et al. showed a prevalence in PCP pneumonia of 13% among HIV patients (21). Candidiasis, tuberculosis and PCP were the three most common OIs in our study. This finding was concordant with the study conducted in Kerala by Vinod et al. (22). Study conducted by Vinod et al. showed a prevalence in PCP of 15% which was higher than the finding in our study (10.3%). Herpes zoster infection, Non Hodgkins lymphoma, toxoplasmosis, cytomegalo virus infection were the other OIs found in our study.

The Lateral flow assay (LFA) is an ideal point of care test, as very little technical expertise is required. The assay can be performed at room temperature. It does not not require refrigeration or heat inactivation. The assay can be performed on remanent blood samples used for routine testing, reducing the need of additional visits (23). Direct microscopy, culture and India ink staining are the methods used commonly in most laboratories to diagnose cryptococcal infections but sensitivity is limited for India ink and direct microscopy while culture takes several days of incubation (24). In December 2011, World health organization had suggested to use rapid CrAg assays for ART-naive patients initiating treatment in high burden cryptococcal populations for patientswith CD4 cells <100 cells/mm3 (25). Despite this, India is yet to adopt routine screening for asymptomatic cryptococcal infection and the burden of asymptomatic cryptococcaemia remains unknown in many parts of the country.

India has the world's third largest burden of HIV, and more than 35% of them enter HIV care with CD4 counts <200 cells/mm3 (26). A routine CrAg screening program in India might drastically help to avert significant morbidity and mortality but the cost efficiency of such a program will be a problem. A study conducted by Meya et al. showed that cryptococcal screening is cost-effective in populations where the prevalence of antigenemia is greater than 3% (27). Preemptive fluconazole therapy should be given in CrAg-positive patients to reduce progression to cryptococcal disease.

CONCLUSION

This is the first study from the southern state of Kerala on the prevalence of cryptococcal antigenemia among HIV positive individuals. The study showed that prevalence of cryptococcal antigen among HIV patients was 0% and hence routine screening for cryptococcal antigen will not be cost effective in our population. Similar to other studies, candidiasis, tuberculosis and PCP were the most common OIs in our study and even though they were more common-

ly seen among people with CD4 count < 200 cells/mm3, there was no statistically significant association. Our study had a few limitations. One of the main reasons for the absence of cryptococcal antigen was possibly due to the fact that only 21.1% patients in the study group had a CD4 count below 100 cells/mm3.

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