ORIGINAL ARTICLE

Magnitude of Cryptococcal Antigenemia among HIV Infected Patients at a Referral Hospital, Northwest Ethiopia

Awoke Derbie[1,2]*, Workneh Ayalew[3], Daniel Mekonnen[1,4], Megbaru Alemu[1], Yihun Mulugeta[5]

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Affiliation and Correspondence:
[1]Department of Medical Microbiology, Immunology and Parasitology, College of Medicine and Health Sciences, Bahir Dar University, Bahir Dar, Ethiopia
[2]CDT-Africa, Addis Ababa University, Ethiopia
[3]Felege Hiowot Referral Hospital, Bahir Dar, Ethiopia
[4]Biotechnology Research Institute, Bahir Dar University, Ethiopia
[5]Department of Epidemiology and Biostatstics, College of Medicine and Health Sciences, Bahir Dar University, Bahir Dar, Ethiopia
*Email: awe.love2000@gmail.com

ABSTRACT

BACKGROUND: *Cryptococcosis is one of the common opportunistic fungal infections among HIV infected patients living in Sub-Saharan Africa, including Ethiopia. The magnitude of the disease at Felege Hiwot Referral Hospital (FHRH) in particular and in Ethiopia at large is not well explored.*
METHODS: *A retrospective document review and analysis was done on records of 137 HIV infected patients who visited FHRH ART clinic from 1 Sep to 30 Dec 2016 and had registered data on their sex, age, CD4 count and cryptococcal antigen screening result. The cryptoccocal antigen (CrAg) detection was done by the IMMY CrAg® LFA (Cryptococcal Antigen Lateral Flow Assay) kit from patient serum as per the manufacturer's instruction. All data were entered, cleared, and analyzed using SPSS v20. Descriptive data analysis and cross tabulation were done to assess factors associated with cryptococcal antigenemia. Statistical significance was set at p-value less than or equal to 0.05.*
RESULTS: *More than half of the participants, 54.7% (75/137), included in the study were females. The median age of the participants was 32.0 years (ranged: 8-52 years). The mean CD4 count was 51.8 with SD of 26.3 (range 3-98). All the patients were HIV stage IV. The proportion of positive cryptococal antigen from serum test was at 11.7% (95% CI: 7.3-18.1%). The IMMY CrAg® LFA result was found statically associated with patient sex (p= 0.045). However, it was not associated with patient age group and the CD4 count (P>0.05)*
CONCLUSIONS: *This study provided baseline data on the magnitude of cryptococcal antigenemia among HIV positive patients that is not touched before in the studied area. The results of the study showed that this opportunistic fungal infection is an important health concern among HIV patients. Further studies with sound design employing adequate sample size should be considered.*
KEYWORDS; *Cryptococcal antigenemia, IMMY CrAg® LFA, Bahir Dar, Ethiopia*

INTRODUCTION

Increasing access to antiretroviral therapy (ART) has transformed the prognosis of HIV infected patients in resource-limited settings. However, treatment coverage remains relatively low, and HIV diagnosis occurs at a late stage. As a result, many patients continue to die of HIV-related opportunistic infections (OIs) in the weeks prior to, and months following initiation of ART (1, 2). *Cryptococcus neoformans,* a causative agent of cryptococcosis remain a common cause of infectious morbidity and mortality, especially among HIV-positive patients living in Sub-Saharan Africa (3). *Cryptococcal* disease is one of the most important OIs, and a major contributor to this early mortality, accounting for between 13% and 44% of deaths among HIV infected people living in developing nations. In sub-Saharan Africa alone, there are more than 500,000 deaths each year due to cryptococcal meningitis (CM), which may exceed those attributed to tuberculosis (1).

Cryptococcus is a type of fungus that lives in soil, especially soil that is contaminated with large amounts of bird droppings. Some people inhale the spores from the environment and never get sick, but in people with weak immune systems, the fungus can cause an infection. The only way a person can get sick from this fungus is by directly inhaling the agent from the environment, making it no person to person transmission (1,4).

Cryptococcal infection is associated with a range of illness. In some people, it causes a lung infection similar to tuberculosis, or it can cause no symptoms at all. The incubation period is not known, but it is thought that the infection can remain dormant in the body for many years. In immunosuppressed people, particularly HIV-infected once with CD4 counts less than 100, the infection can reactivate and spread throughout the body. When this happens, the infection usually presents as meningitis which is a common cause of death among HIV/AIDS patients (5).

The case fatality rate in patients with cryptococcal meningitis, the commonest presentation of HIV-related cryptococcal disease in adults, remains unacceptably high, particularly in sub-Saharan Africa, between 35%-65%. This compares with 10%-20% in most developed countries. The main reason for this is a delay in presentation with diagnosis only when meningitis is advanced and treatment is less effective, mainly as a result of limited access to lumbar puncture (LP) and rapid diagnostic assays (1).

There are three categories of methods that can be used to diagnose cryptococcal meningitis: India Ink microscopy, which can be used on cerebrospinal fluid (CSF); culture, which can be used on CSF or blood; and antigen detection. There are several methods to detect cryptococcal antigen in CSF or serum: latex agglutination (LA), enzyme immunoassay (EIA), and lateral flow assay (LFA) (6-8). A patient who tests positive for cryptococcal antigen can take oral fluconazole to help the body fight the early stage of the infection. This could prevent the infection from developing into meningitis (9).

In Ethiopia, where there is poor surveillance system, there are few studies conducted on the level of cryptococcal infection and its associated risk factors (2,10-11). However, the data is missing in our study site where there are a number of HIV patients getting ART service in accordance with the national ART program. Some HIV patients in the study area get tested for cryptococcal infection during follow-up when there is suggestive clinical presentations. Thus, the aim of this study was to assess the prevalence of cryptococcal antigenemia and its associated factors among HIV infected patients at Felege Hiwot Referral Hospital (FHRH) setting.

MATERIALS AND METHODS

Study design, setting and data collection: A retrospective record review was done on records of 137 HIV infected patients who were attending ART monitoring at FHRH ART clinic. The hospital is located in Bahir Dar town, which is the capital of Amhara National Regional State located 565 km away from the capital Addis Ababa. The FHRH is a tertiary health care level hospital serving the population of Bahir Dar and remote areas of Northwest Ethiopia. The total population served by the hospital is about 12 million. In the

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hospital, the ART clinic is operating under the National HIV Program of Ethiopia, under which patients are diagnosed for HIV and get ART treatment and follow-up services (CD4 count, liver and kidney function tests and hematologic evaluation) for free.

HIV positive patients, regardless of their ART status, whose CD4 count was less than 100 and had headache with signs suggestive of meningitis were screened for CrAg in the hospital (1). Taking this background, those HIV patients who visited the hospital's ART clinic from 1 Sep to 30 Dec 2016 and had registered data on their sex, age, CD4 count and cryptococcal antigen screening result were included for analysis. Patient records that missed one of these variables were excluded from the study. Data were retrieved directly from laboratory registration logbook using data extraction sheet on 1to 10 January 2017.

Cryptococcal antigen test: Although culture is the standard method for definitive diagnosis, detection of cryptococcal antigen in serum or cerebrospinal fluid is used for presumptive diagnosis. Cryptococcal antigen screening in peripheral blood is also recommended for HIV-infected persons with CD4 cell counts <100/µl to reduce early deaths while receiving ART (6, 12).

In the studied area, the cryptoccocal antigen (CrAg) detection was done by the IMMY CrAg® LFA (Cryptococcal Antigen Lateral Flow Assay) kit as per the manufacturer's instruction. The IMMY CrAg® LFA is an immunochromatographic dipstick assay for the qualitative and semi-quantitative detection of cryptococcal antigen in serum, plasma, whole blood and cerebrospinal fluid (CSF). The CrAg® LFA is a prescription use laboratory assay which can aid in the diagnosis of cryptococcosis (6,12). In this study, from those patients who came with evidence of cryptococcal meningitis, 40µl serum was used to detect the CrAg. The serum and its one drop dilute were added into a test tube and the lateral flow device that is pre-coated with anti-CrAg monoclonal antibodies and gold conjugated control antibodies was submerged with its white end. After ten minutes of incubation, the result was read and recorded. The antigen antibody complex forms a test line causing a visible line to form. The IMMY CrAg® LFA has sensitivity and specificity of 100% using serum (12-13). Patients found positive for CrAg were managed using fluconzole based antifungal treatment based on the recommended approach.

Statistical analysis: All data were entered, cleared, and analyzed using SPSS statistical software package, Version 22.0. Descriptive data analysis was used to visualize differences within data, and cross tabulation was done to assess factors associated with cryptococcal antigen. Differences were considered significant when *p*-value was less than or equal to 0.05.

Ethical issues: Permission and ethical clearance were obtained from Amhara Regional Health Bureau Institutional Review Board (IRB) to utilize the data. As the data was collected retrospectively, no patient details were linked to the patient's identity and confidentiality was maintained.

RESULTS

In this study, we analyzed the records of 137 HIV infected patients to assess their cryptococcal antigenemia. More than half of the participants, 75(54.7%), were females. The median age of the participants was 32.0 years (ranged: 8-52 years). The mean CD4 count was at 51.8 with standard deviation of 26.3 (range 3-98). The proportion of positive cryptococal antigenemia from serum test was at 11.7% (95% CI: 7.3-18.1%) (Table1). Further, based on the WHO classification system, all of the HIV positive participants in this study were stage IV.

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Table 1: Demographic and related clinical data of HIV patients screened for cryptococcal antigenemia at FHRH, Bahir Dar, 2016.

Variable		Frequency	Percent
Sex	Male	62	45.3
	Female	75	54.7
	Total	137	100
Age group in years	0-14	3	2.2
	15-24	11	8.0
	25-34	69	50.4
	35-44	38	27.7
	>44	16	11.7
CD4 count/ µl	0-25	25	18.2
	26-50	46	33.6
	51-75	36	26.3
	76-100	30	21.9
CrAg LFA result	Positive	16	11.7
	Negative	121	88.3

We found that the IMMY CrAg® LFA result was statically associated with patient sex (p= 0.045). However, it was not found associated with the patient age group and the CD4 count (P>0.05) (Table).

Table 2: CrAg LFA test result of study subjects at FHRH, Bahir Dar, 2016.

Variable		No. Tested	*CrAg LFA result		*P value*
			Positive n (%)	Negative n (%)	
Patient Sex	Male	62	11 (17.7)	51 (82.3)	
	Female	75	5 (6.7)	70 (93.3)	0.045
Age groups in years	0-14	3	1 (33.3)	2 (66.7)	
	15-24	11	0 (0)	11 (100)	
	25-34	69	7 (10.1)	62 (89.9)	0.276
	35-44	38	7 (18.4)	31 (81.6)	
	>44	16	1 (6.3)	15 (93.7)	
CD4 count	0-25	25	3 (12)	22 (88)	
	26-50	46	7 (15.2)	39 (84.8)	0.793
	51-75	36	3 (8.3)	33 (91.7)	
	76-100	30	3 (10)	27 (90)	

*CrAg LFA: Cryptococcal antigen lateral flow assay test

DISCUSSION

Although the widespread availability of antiretroviral therapy (ART) in developed countries has helped reduce cryptococcal infections in these areas, it is still a major problem in developing countries, like Ethiopia, where access to healthcare is limited (9). Most of the patients with cryptococcal meningitis have serious disease and high fatality rate, and its clinical symptoms are not typical, misdiagnosis is common in early stage (4).

Each year, *Cryptococcus* is believed to cause more deaths than tuberculosis in Sub-Sahran Africa (14-15). Therefore, screening individuals with AIDS for serum cryptococcal antigen (CrAg), followed by treatment of CrAg positives

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with antifungals, may prevent cryptococcal meningitis (2, 6, 15). In this study, most of the participants living with HIV were females (54.7%). Similarly, in terims of their age category, the majority of HIV patients, 50.4% were in the 25-36 years group which implies that HIV still affects those individuals who are sexually active. Moreover, there is a similar report by Kharsany *et al's* study in which adolescent girls and young women aged 15-24 years had up to eight fold higher rates of HIV infection compared to their male peers. There remains a gap in women initiated HIV prevention technologies especially for women who are unable to negotiate the current HIV prevention options of abstinence, behavior change, condoms and medical male circumcision or early treatment initiation in their relationships (16).

In this study, we found that the overall prevalence of cryptococcal antigenemia was 11.7% among HIV infected patients with mean CD4 count at 51.8 cells/µl. According to Shiferaw *et al*'s study, in Addis Ababa-Ethiopia, the magnitude of cryptococcal antigenemia was 11% among HIV infected patients with CD4 count lessthan100 cells/µl (10). Similarly, according to Bitew *et al's.* study in Addis Ababa, the prevalence of cryptococcal antigenemia among HIV positive patients was 8.5% (11). An overall prevalence of 9.9% of *C. neoformans* infection among HIV patients was also documented by Egbe *et al.* in Nigeria (17) which makes our finding almost comparable with previous studies done in Ethiopia and elsewhere in the world.

It is indicated that among immunosuppressed persons, particularly HIV-infected people with CD4 counts under 100, cryptococcal infection is important (16) and is one of the AIDS defining infections (3). Cryptococcal antigenemia was not found associated with patient age group and their CD4 count (P>0.05) (Table 2). On a study done in Nigeria, authors demonstrated that CD4 count less than 200/µl was significantly associated with cryptococcal antigenemia but age group and gender did not show association (17). The statistical difference on CD4 might be because of the small sample size we have employed in the present study.

In this study, we found that the mean CD4 count of the study participants was 51.8 per 1µl of blood (range 3-98). At the same time, all of them were stage IV. Considering the fact that in such patients with CD4 counts < 100, screening them for CrAg and early ART initiation is the most important and cost-effective preventive strategy to reduce the incidence and high mortality associated with cryptococcal meningitis in HIV-infected patients (1). Since Amphotericin B is not available, the management protocol of CrAg positive HIV patients in FHRH was using fluconzole based antifungal treatment based on the WHO recommended approach (1) and an expert's opinion. The fluconzole based treatment was given in three phases: 1) induction phase (for two weeks, high dose-600mg BID), 2) consolidation phase (for up to eight weeks, 400mg BID) and 3) maintenance phase (200mg daily for 3-6 months until the patients attains CD4 count of >200). Lumber puncture was also given every 24 hours for these patients until they got relief from the headache.

The results of our study should be applied with caution. Our findings are subject to at least two limitations: selection bias and quite limited sample size. At the same time, due to the retrospective nature of the study, it was not possible to show detailed clinical picture of HIV patients which might have a significant role to indicate the overall profile of the study participants. No data was found on the ART status and antifungal treatment outcome of patients with positive CrAg test. However, our study is the first of its kind in the studied area that provide baseline information about the magnitude of cryptococcosis among HIV positive patients for further large scale study. The results of this study also showed that this opportunistic fungal infection is an important health problem among HIV patients that needs the attention of physicians who are in charge of attending them.

In conclusion, althrough we have employed quite limited study participants' data, the reported prevalence of cryptococcal antigenemia calls stakeholders to expand CrAg screening service for individuals with HIV/AIDS, especially for those with CD4 count <100/µl. Additional prospective

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study with adequate sample size is needed to determine the exact magnitude of the disease and to explore its determinants.

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