



Cryptococcal antigenemia among HIV seropositive patients accessing care in antiretroviral therapy (ART) clinics in Calabar, South Southern Nigeria

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Abstract

Background: *Cryptococcus neoformans* infection is a life threatening disease especially when associated with immunosuppression like HIV/AIDS. The greatest burden of disease occurs in sub-Saharan Africa, where mortality is estimated to be 17%. The finding of cryptococcal antigen in the blood represents a condition of systemic invasion with the fungus. Clinical manifestation of infection with *Cryptococcus neoformans* in AIDS patients is generally more evident at CD4 cells ≤ 100 cells/ μ l. This study was carried out to determine cryptococcal antigenemia among HIV seropositive patients accessing care in ART clinics as patients are not screened routinely despite reports of relatively high co-infection rates with HIV.

Methods: This prospective cross-sectional study was carried out on ART-treated and ART-naïve HIV positive adult patients attending ART clinics in two tertiary hospitals in Calabar, Nigeria. The seroprevalence of cryptococcal antigen among the patients was determined using the cryptococcal antigen latex agglutination system (CALAS) (Wampole Laboratories, USA), according to the manufacturer's instruction. The CD4 count levels of each patient were determined by flow cytometry using the fluorescent activated cell sorter BD FACS Count system (Becton Dickinson).

Results: Out of the 272 HIV positive subjects enrolled in the study, 116(42.6%) were ARV-naïve and 156(57.4%) were ARV-treated patients. A 5.1% cryptococcal antigenemia prevalence was established in the study. Infection rates were higher among subjects receiving ART 11/156 (7.1%) than in ART-naïve subjects 3/116 (2.6%). Infection rates 5(35.7%) peaked at age 25-34 years. The mean CD4 counts of subjects with cryptococcal infection were 100.7 ± 67.8 cells/ μ l, with a minimum CD4 count of 13.0. All the infections occurred among subjects with CD4 counts ≤ 200 cells/ μ l of blood. There was a statistically significant effect of cryptococcal antigenemia on the CD4 counts of the subjects ($t=3.7$, $p=0.002$).

Conclusion: This study reveals that cryptococcal antigenemia is a health problem among HIV/AIDS patients in our locality. Cryptococcal antigenaemia seem to be more common among HIV patients on ART. The CD4 count levels among the ART treated subjects could have been boosted by administration of ART

Keywords: Cryptococcal antigen, HIV/AIDS patients, CD4 counts, antiretroviral therapy (ART)

Introduction

Cryptococcosis is one of leading pulmonary and meningeal mycoses of worldwide occurrence. It causes an estimated one million cases of cryptococcal meningitis per year among HIV/

AIDS patients resulting in nearly 625,000 deaths [1]. The greatest burden occurs in Sub-Saharan Africa, where mortality is estimated to be 17% [2,3].

The disease predominantly occurs in immunocompromised

patients with underlying predisposing factors, such as advanced HIV disease, organ transplantation, haematological malignancies and aggressive cancer therapy [4,5]. It is a disease that has become the focus of the attention in Europe, America, Africa, and Southeast Asian countries [6,7]. Prior to the availability of highly active antiretroviral treatment (HAART), the disease was considered the fourth major cause of mortality in individuals with AIDS [1]. Extrapulmonary cryptococcosis is an AIDS-defining illness. The most frequent clinical presentation is disseminated meningoencephalitis which is rapidly fatal in the absence of antifungal treatment [8,9].

Cryptococcus neoformans is an encapsulated fungal pathogen [10]. On routine laboratory media, colonies of *C. neoformans* develop within 36 to 72 hours. Colonies appear as white to cream coloured, with mucoid consistency and may be several millimeters in diameter. Colonies may also develop sectors that differ in pigmentation [11]. The amount of capsule can be judged from the degree of colonial mucosity. Highly encapsulated colonies may coalesce and slowly trickle down a slant to puddle in the bottom of the tube or drip off the medium of inverted plates [12].

Cryptococcus neoformans is a spherical, budding, encapsulated yeast cell in both tissue and culture microscopic examinations. The yeast cells measure between 5 to 10 μm in diameter and exist as single and multiple buds [13], but majority of cells in tissue and culture lack buds because they readily detached from their parent cells. On rare occasions, filamentous and short hyphal variants have been isolated [4].

The mode of transmission to humans is by inhalation of basidiospores or dehydrated haploid yeast. These small propagules lodge in the lung alveoli from where they are spread to the central nervous system to cause meningoencephalitis [14-16].

On entry into the lungs, the yeast cells become rehydrated and acquire the characteristic polysaccharide capsule. In the case of basidiospores, these would convert to encapsulate blastoconidia [17,18].

Methods

Specimen collection

Five ml of blood was collected from all subjects by trained Medical Laboratory scientists and Nurses by venopuncture allowed to clot and centrifuged to obtain serum. This was used to confirm the HIV status of the subjects before enrolling them for the studies using the Determine rapid test kit (Abbott Laboratories, Tokyo, Japan) and STAT-PAK HIV 1/2 assay (ChemBio, Medford, NY, USA). A part of the patient's serum was used to determine CD4 counts by flow cytometry using the BD fluorescent-activated cell sorter system (BD Biosciences, San Jose, CA, USA) as per the manufacturer's instructions. The sero-prevalence of cryptococcal antigen in the patients and the control group was measured using the cryptococcal antigen latex agglutination system (CALAS) (Wampole Laboratories, Cranbury, NJ, USA) according to manufacturer's instructions.

Ethical approval

Ethical approval was obtained from the ethical research committee, UCTH, Calabar, Nigeria. Informed consent was also obtained from all the subjects.

Serology and assessment of immune status

Serological tests were performed on the serum collected from the patients for cryptococcal antigen detection, using the Crypto-LA Test (Wampole Laboratories, New Jersey, USA). The kit is a simple, sensitive, qualitative and semi-quantitative latex test which detects capsular polysaccharide antigens of *Cryptococcus neoformans* in Serum and cerebrospinal fluid. Samples were initially heat inactivated in a water bath at 56°C for 30 minutes to reduce non-specific interference with cryptococcal antigen latex test [19-21]. All tests were carried out according to the manufacturer's instructions. The CD4 counts were determined by flow cytometry using the fluorescent activated cell sorter BD FACS Count system (Becton Dickinson) as per the manufacturer's instruction [19].

Data analysis

The data obtained in this study were analyzed with Epi-Info CDC, 2000 and Microsoft excel data analysis packages. Descriptive statistics were carried out. Frequencies were calculated for categorical variables. Interactions between specific categorical clinical variables were tested for significance using the χ^2 test. Student's *t* test was used to compare the means between two variables. A p-value of 0.05 was considered statistically significant.

Results

Out of the 272 HIV positive subjects enrolled in the study, 116(42.6%) were ARV-naïve while 156(57.4%) were ARV-treated patients (Table 1). A total of 14(5.1%) subjects were positive for Cryptococcal antigenaemia (CRAg). The infection rates were higher among subjects receiving ART 11/156 (7.1%) than in ART-naïve subjects 3/116 (2.6%). Infection rates 5(35.7%) peaked at age 25-34 years (Table 1).

Females were more infected than males and had a lower mean CD4 counts (97.7 \pm 64.9 cells/ μl) than males (112.0 \pm 92.4 cells/ μl) (Table 2).

The mean CD4 count of subjects with cryptococcal infection was 100.7 \pm 67.8 while those without cryptococcal infections had a mean CD4 count of 355.8 \pm 253.1. All the infections occurred among subjects with CD4 counts \leq 200 cells/ μl of blood. There was a statistically significant association between *Cryptococcus* infection and the CD4 counts of the subjects ($t=3.7$, $p=0.002$) (Table 3).

Table 4 shows the comparative mean CD4 counts of ART-naïve and ART-treated subjects. The ART-naïve subjects had a lower mean CD4 count (71.3 \pm 47.5 cells/ μl) than the ART-treated subjects (108.8 \pm 72.1 cells/ μl). There was a statistically significant difference in mean CD4 counts between the two groups ($t=3.25$, $p=0.001$).

Table 1. Age distribution of cryptococcal antigenemia among subjects.

Age (years)	ART Treated		ART Naïve		Total No. tested	Total No. (%) positive
	No. tested	No. (%) positive	No. tested	No. (%) positive		
15-24	22	2(9.1)	26	0(0.0)	48(17.6)	2(14.3)
25-34	60	3(5.0)	44	2(4.5)	104(38.2)	5(35.7)
35-44	40	3(7.5)	23	1(4.3)	63(23.2)	4(28.6)
45-54	24	3(12.5)	15	0(0.0)	39(14.3)	3(21.4)
55-64	8	0(0.0)	5	0(0.0)	13(4.8)	0(0.0)
65-74	2	0(0.0)	3	0(0.0)	5(1.8)	0(0.0)
Total	156(57.4)	11(7.1)	116(42.6)	3(2.6)	272	14(5.1)

Table 2. Comparative mean CD4 counts of subjects positive for cryptococcal antigenaemia by gender.

Gender	No. tested	No. (%) positive	Mean CD4 counts	Statistics
Female	167	11(6.6)	97.7±64.9	t=0.31
Male	105	3(2.9)	112.0±92.4	p=0.7

Table 3. Comparative mean CD4 counts of subjects with and without cryptococcal infections.

Cryptococcal antigenaemia	No. (%) of subjects (n=272)	Mean CD4 counts	Statistics
Positive	14(5.1)	100.7±67.8	t=3.75
Negative	258(94.9)	355.8±253.1	p=0.002

Table 4. Comparative mean CD4 counts of ART treated and ART-naïve (HIV/AIDS) subjects with cryptococcal infections.

Cryptococcal antigenaemia	ART-treated	ART-naïve	Statistics
No. (%) of subjects	11(7.1)	3(2.6)	t=1.96, p=0.05
Mean CD4 counts (cells/μl)	108.8±72.1	71.3±47.5	t=3.25, p=0.001

Discussion

Cryptococcal antigen (CRAG) can be detected weeks before the onset of symptoms, and those who are asymptomatic but positive for cryptococcal antigen have a high risk of subsequent cryptococcal meningitis and mortality [22]. In this study fourteen (5.1%) of the study subjects were positive for serum cryptococcal antigen. Mamoojee et al., [23] reported 2% CRAG prevalence among HIV patients enrolled for antiretroviral therapy in Ghana while Chukwuanukwu et al., [24] reported a 13.1% cryptococcal antigenemia (CRAG) among HIV positive women attending prevention of mother to child transmission (PMTCT) clinics in South Eastern Nigeria. The higher (5.1%) prevalence in our study may be due to the fact that Mamoojee and colleagues' [23] enrolled fewer number of subjects in their study. On the other hand, Chukwuanuku and colleagues' [24]

higher prevalence (13.1%) may be due to further depression of immune status by pregnancy among their subjects.

The (7.1%) prevalence of serum cryptococcal antigen recorded among ART treated subjects in this study is lower than the 12.2% reported in Congo, 13.1% in Thailand and 13.5% in Kampala, Uganda [25-27]. Also a (2.6%) serum cryptococcal antigenemia was recorded among ART naive patients. This finding is also lower than the (12.7%) prevalence reported in Benin city, Nigeria [28]. The low cryptococcal antigenaemia among our subjects could be due to the significant reduction in HIV burden in Cross River State from 12% to 6% between 1994 to 2005 [29]. The massive HIV intervention strategy carried out by Global HIV Initiatives (GHAIN), Presidents Emergency Program for AIDS Relief (PEPFAR) and the World Bank, in the last 15 years in Nigeria and Cross River State in particular has led to this reduction in HIV burden in the State.

Females had more seropositivity 11/167 (6.6%) for CRAG when compared to their male counterparts 3/105 (2.9%). The reason could be due to the lower CD4 count levels among females than males. Age did not also play a significant role on serum CRAG positivity among patients ($\chi^2=2.42$, $p=0.49$).

Several studies evaluating the prevalence of serum cryptococcal antigenemia in AIDS patients have reported a consistently higher prevalence of serum CRAG in patients with lower CD4 cell counts [28]. Subjects with cryptococcal antigenaemia had a lower mean CD4 counts 100.7±67.8 cells/μl than the CRAG negative subjects with CD4 count levels of 355.8±253.1 cells/μl of blood. This could reflect further depletion of CD4 cells by cryptococcal infection among the patients. Cryptococcal antigen (CRAG) screening directed at all newly diagnosed HIV cases with CD4 counts ≤200 cells/μl is likely to identify patients at risk of developing cryptococcal meningitis.

Cryptococcal antigenaemia seem to be more common among HIV patients on ART. The higher *Cryptococcus neoformans* infection rates among ART-treated patients than ART-naïve patients could be explained with the fact that some of those subjects just commenced the ART therapy. The mean CD4 counts 108.8±72.1 cells/μl among ART-treated patients were also higher than the ART naive subjects 71.3±47.5 cells/μl which depicts that the administration of ART could have boosted the

CD4 count levels among these subjects.

Conclusion

This study reveals that cryptococcal antigenemia is a health problem among HIV/AIDS patients in our locality. Although cryptococcal antigenaemia was more common among HIV patients on ART, the higher CD4 count levels among them depicts that the administration of ART could have boosted the CD4 count levels among these subjects. Thus, Cryptococcal antigen screening should be made a routine for all HIV positive patients accessing care in ART clinics in Calabar. This will improve the lives of the patients, reduce morbidity and reduce preventable deaths which arise from cryptococcal meningitis.

Recommendation

Cryptococcal antigen (CRAG) screening is lacking in our institutions, it is necessary to incorporate this as a routine screening test for HIV programme in Calabar and Nigeria in general.

Competing interests

The authors declare that they have no competing interests.

Authors contributions

O. Ogba and L. Abia Bassey were involved in the writing of manuscript and study designing. O. Ogba carried out the laboratory and data analysis and conceived the study.

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