ORIGINAL REPORT

Cryptococcal Antigenemia in Anti-Retroviral Naïve AIDS Patients: Prevalence and Its Association with CD4 Cell Count

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Abstract- *Cryptococcus neoformans* is the most incriminated fungal pathogen causing meningitis in acquired immune deficiency syndrome (AIDS) patients, and is known to constitute a major cause of deaths in AIDS patients. This study aimed to determine the sero-prevalence and effect of CD4 count on seropositivity for *Cryptococcus neoformans* antigen (crag) in antiretroviral- naïve (ART-naïve) AIDS patients. This study included 150 (61 males and 89 females) ART-naïve AIDS patients attending the Human Immunodeficiency Virus (HIV) clinic of the University of Benin teaching hospital, Benin City, Nigeria within the period February 2011- July 2011. 40 (18 males and 22 females) HIV positive outpatients with CD4 counts >200 cells/µl who are ART-naive were recruited and used as controls. The prevalence of crag in the patients and control group was determined using the cryptococcal antigen latex agglutination system (CALAS) (Meridian Bioscience, Europe) and CD4 counts were measured using flow cytometry (Partec flow cytometer, Germany). Of 150 ART-naïve AIDS patients with CD4 counts ≤ 200 cells/µL, 19 (12.7%) were positive for serum Cryptococcal antigen. ART-naïve AIDS patients with CD4 count ≤50 cells/µl had the highest prevalence of serum crag. Lower CD4 counts were significantly associated with positivity for serum crag (P<0.001). Age and Sex had no significant effect on the sero-positivity for serum crag. 1 (2.5%) of the control was sero-positive for crag. Serum crag was significantly associated with AIDS but not with HIV (P<0.001). This study uncovers a high prevalence of crag in ART-naïve AIDS patients in Benin City. There is an urgent need to introduce early and routine screening for crag in ART-naïve AIDS patients for prompt intervention.

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Keywords: Cryptococcal antigen; ART-naïve AIDS patients; Sero-prevalence; CD4 counts

Introduction

Cryptococcus neoformans is the most incriminated fungal pathogen causing meningitis in patients infected with Acquired immunodeficiency virus (AIDS) (1,2). The type of cryptococcoccis encountered in human immunodeficiency virus (HIV) is quite different when it has progressed to AIDS. Meningoencephalithis and cryptococcal pneumonia are the common cryptococcal infections found in HIV and AIDS respectively (3). Obligatory and effective administration of anti-retroviral agents in AIDS has been proven to reduce the incidence of cryptococcosis (4). Development of cryptococcosis as with other opportunistic infections during AIDS is associated with a decline in CD4 T (Cluster of differentiation 4) T cell counts in patients (5). Clinical manifestation of infection with *Cryptococcus neoformans* in AIDS patients is generally more evident at CD4 cells ≤ 50 cells/µl (6).

Cryptococcoccis in AIDS is usually asymptomatic and its defining illness is commonly not found in the early course of infection (7). Onset of clinical cryptococcoccis in AIDS is found with unspecific clinical symptoms as it is found in most pulmonary and meningeal diseases. Coughing, sweating, fevers, malaise, shortness of breath are common presenting symptoms. The finding of cryptococcal antigen in the blood represents a condition of systemic invasion with the fungus (8). At this stage there is the capacity of the fungus to disseminate to major parts of the body. The

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central nervous system is the commonest site of its dissemination, though cutaneous and adrenal dissemination which are rare are also found in some cases (9).

The clinical course an antiretroviral-naïve (ART-naïve) AIDS patient may follow will be rightly tracked and planned if diagnosis of cryptococcal antigenaemia is done before initiation of ART. This study thus aimed to determine the incidence of serum cryptococcal antigenaemia in ART-naïve AIDS patients with varying CD4 counts in University of Benin teaching hospital (UBTH), Benin City, Nigeria.

Materials and Methods

This study was carried out in the period February 2011-July 2011 at the HIV clinic of the University of Benin teaching hospital, Benin City, Nigeria a referral hospital for AIDS patients in Southern Nigeria. It also houses the South-south regional headquarters of the Institute of human virology, Nigeria and the Action project molecular research laboratory for HIV diagnosis. Ethical approval for the study was granted by the ethical committee of the University of Benin teaching hospital (UBTH), Benin City, Nigeria. A total of 150 ART-naïve AIDS patients (61 Males and 89 females) within the age groups < 20 to > 50 who were counseled, consented for the study and were included in this study. They were patients confirmed to have progressively developed AIDS with CD4 T cell count < 200 cells/ µl but had not been on antiretroviral medications in the period of the study. 40 (18 males and 22 females) HIV patients with CD4 counts >200 cells/µl who are ART-naive were recruited from the special treatment clinic of UBTH and used as controls. History of cryptococcal meningitis, prior positivity for *Cryptococcus neoformans* and anti-retroviral use were used as exclusion criteria.

Blood samples were collected by venipuncture and centrifuged to obtain serum. Laboratory analysis of samples was done at the PEPFAR (President's Emergency Plan for AIDS Relief) laboratory of UBTH, Benin City. Cryptococcal antigen testing was done using cryptococcal antigen latex agglutination system (CALAS®) (Meridian Bioscience Inc, Europe). This detection kit is simple, sensitive, qualitative and semi-quantitative latex test which detects capsular polysaccharide antigens of *Cryptococcus neoformans* in serum and cerebrospinal fluid. Samples were initially pre-treated by incubation at 50°c for 15 mins with CALAS® pronase (Meridian Bioscience Inc, Europe) to reduce non-specific interference with cryptococcal antigen latex test. All tests were carried out following the manufacturer's instruction. Determination of CD4 T cell counts in the patients was done using flow cytometry (Partec flow cytometer, Germany). In brief, equal volumes (20µl) of CD4 PE antibody and Ethylene diamine tetra acetic acid blood was mixed and incubated for 15mins.800 µl of CD4 buffer was added before reading in the cell counter.

Data was analyzed for significance using chi-square with Statistical packages for social sciences (SPSS) V. 15.

Results

150 patients with CD4 cells< 200 cells/µl were studied. 19 (12.7%) were positive for cryptococcal antigen. Prevalence of cryptococcal antigen was quite varied with CD4 cells levels; CD4 count ≤ 50 cells/µl had a higher prevalence of cryptococcal antigen (Table 1). This was followed by CD4 cell counts >50-≤100 cells/µl and >100-≤200 cells/µl. Lower CD4 counts were significantly associated with positivity for serum cryptococcal antigen (P<0.001).1 (2.5%) of the control was sero-positive for crag. Serum crag was significantly associated with AIDS but not with HIV (P<0.001). Females were more positive for cryptococcal antigen when compared to males (13.5% and 11.5% respectively). Also, age group 31-40 recorded higher cryptococcal antigenaemia when compared to other age groups (Table 2). Age and sex had no effect on sero-positivity for crag.

Table 1. Prevalence of serum crag in antiretroviral-naïve AIDS patients in Benin City.

Variables	No. tested (%)	No. positive (%)	*P*-value
CD4 count (cells/µl) of patients			
≤50 cells/µl	39	11 (28.2)	
>50-≤100 cells/µl	42	6 (14.3)	
>100- ≤200 cells/µl	69	2 (2.9)	<0.001
ART- status			
ART- naïve AIDS patients	150	19 (12.7)	
Control	40	2 (2.5)	<0.001

Table 2. Age and sex based prevalence of cryptococcal antigenemia in the studied patients.

Characteristics	No. tested (%)	No. positive (%)
Age (years)		
<20	4	0
21-30	33	2 (6.1)
31-40	55	9(16.4)
41-50	45	7(15.6)
>51	13	1(7.6)
Male	61	7 (11.5)
Female	89	12 (12.7)

Discussion

This study demonstrates a high prevalence of serum cryptococcal antigen in ART-naïve AIDS patients in Benin City, Nigeria. 19 (12.7%) of the studied patients were positive for serum cryptococcal antigen. The finding of this study concurs with the 12.2% in Congo, 12.9% in Bangkok and 13.5% in Kampala, Uganda (10,11,12). The prevalence report of this study is quite higher than prevalence rates of other countries, 7% was reported in a retrospective study on ART-naïve AIDS patients in South Africa (13) and 9.2% in Thailand (14). Higher prevalence of cryptococcal antigenaemia has been reported in Cambodia (21%) (15).This high rate recorded in these patients represents the burden of cryptococcal infection in AIDS patients in Benin City. An earlier study evaluating the prevalence of fungal opportunistic infections in AIDS patients largely on ART-therapy in this hospital using culture and microscopy recovered a 9.7% prevalence of *Cryptococcus neoformans* (16). This study further goes to confirm the burden of cryptococococcis in AIDS patients of this hospital. Screening for cryptococococcis in AIDS patients should be made a routine.

The distribution of cryptococcal antigenemia was highly varied with CD4 cell levels. Patients with CD4 cell count ≤50 cell/µl had the highest prevalence of serum crag; this was closely followed with patients with CD4 cells >50-≤100 cell/µl and >100-≤200 cells/µl. Several studies evaluating the prevalence of serum cryptococcal antigenemia in AIDS patients (17,18), has reported a consistently higher prevalence of serum crag in patients with lower CD4 cell counts. HIV is characteristically associated with T lymphocyte depletion and is highly marked in ART-naïve patients (13,19). Females had more seropositivity for crag when compared to their male counterparts. More men compared to women have been reported to carry a higher burden in the United States (20), but there were no significant difference in the prevalence in men and women in ART-naïve AIDS patients in Benin City. Age did not also play a significant role in serum crag positivity in the patients.

In conclusion, 12.7% ART-naïve AIDS patients in UBTH, Benin City, were crag positive. Crag prevalence was significantly associated with lower CD4 counts. ART- naïve AIDS patients with CD4 counts ≤50 cells/µl had the highest prevalence. The sero-prevalence was high; screening for *Cryptococcus neoformans* antigen should be made a routine in ART- naïve AIDS patients to reduce the quick mortality from Cryptococcal meningitis.

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