

# Screening for Cryptococcal Antigenemia in Anti-Retroviral Naïve AIDS Patients in Benin City, Nigeria

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## Abstract

**Objectives:** *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 thus aimed to determine the baseline sero-prevalence of *Cryptococcus neoformans* infection in anti-retroviral naïve (ART-naïve) AIDS patients using the serum Cryptococcal antigen (crag) detection method. Baseline effect of variation in CD4 counts, as well as sex and age with sero-positivity for crag were also determined.

**Methods:** This descriptive cross-sectional study included 150 (61 males and 89 females) ART-naïve AIDS patients attending the Human Immunodeficiency Virus clinic (HIV) at the University of Benin Teaching hospital, Benin City, Nigeria, within the period from February 2011- July 2011. Forty (18 males and 22 females) HIV positive outpatients with CD4 counts >200 cells/ $\mu$ L who were ART-naïve were recruited and used as controls. The sero-prevalence of crag in the patients and the control group was measured using the cryptococcal antigen latex agglutination system (CALAS) (Meridian Bioscience, Europe) and CD4 counts were measured using flow cytometry (Partec flow cytometer, Germany).

**Results:** Of the 150 ART-naïve AIDS patients with CD4 counts  $\leq$ 200 cells/ $\mu$ L; 19 (12.7%) were positive for serum Cryptococcal antigen. ART-naïve AIDS patients with CD4 count  $\leq$ 50 cells/ $\mu$ L 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. One (2.5%) of the controls was sero-positive for crag. Thus, serum crag was significantly associated with AIDS but not with HIV ( $p < 0.001$ ).

**Conclusion:** This study uncovers a high prevalence of crag in ART-naïve AIDS patients in Benin City. The prevalence of crag was higher in ART-naïve AIDS patients with lower CD4 counts. There is an urgent need to introduce routine screening for crag in ART-naïve AIDS patients in our locality to reduce the rapid mortality from *Cryptococcal meningitis* which accounts for a majority of the morbidity factor if undiagnosed during ART therapy.

**Keywords:** Cryptococcal antigen; ART-naïve AIDS patients; Sero-prevalence; CD4 T cell counts.

## Introduction

*Cryptococcus neoformans* is the most incriminated fungal pathogen causing meningitis in patients with acquired immunodeficiency syndrome (AIDS).<sup>1,2</sup> The type of cryptocococcus encountered in human immunodeficiency virus (HIV) is quite different when it has progressed to AIDS. Meningoencephalitis and cryptococcal pneumonia are the common cryptococcal infections found in HIV and AIDS respectively.<sup>3</sup> Obligatory and effective administration of anti-retroviral agents in AIDS have been proven to reduce the incidence of cryptococcosis.<sup>4</sup> The development of cryptococcosis as with other opportunistic infections during AIDS is associated with a decline in CD4 (Cluster of differentiation) T cell counts in patients.<sup>5</sup> Clinical manifestation of infection with *Cryptococcus neoformans* in AIDS patients is generally more evident at CD4 cells  $\leq$ 50 cells/ $\mu$ L.<sup>6</sup>

Cryptococcosis in AIDS is usually asymptomatic and its defining illness is commonly not found in the early course of infection.<sup>7</sup> Onset of clinical cryptococcosis 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.<sup>8</sup> At this stage there is the capacity of the fungus to disseminate to major parts of the body. The central nervous system is the commonest site of its dissemination, though cutaneous and adrenal dissemination which are rare are also found in some cases.<sup>9</sup>

The clinical course an ART-naïve AIDS patient that may follow will be rightly tracked and planned if diagnosis of cryptococcal antigenemia is done before initiation of ART. This study thus aimed to determine the prevalence of serum Cryptococcal

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antigenemia in antiretroviral-naïve AIDS patients with varying CD4 counts at the University of Benin teaching hospital, UBTH, Benin City, Nigeria.

## Methods

This descriptive cross-sectional study was carried out in the period of 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 anti-retroviral 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/ $\mu$ l but had not been on anti-retroviral medications in the period of the study. Forty (18 males and 22 females) HIV patients with CD4 counts >200 cells/ $\mu$ l who were ART-naïve 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 veni-puncture 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 (CALASR) (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 (R) pronase (Meridian Bioscience; Inc, Europe) to reduce non-specific interference with cryptococcal antigen latex test. All tests were carried out according to the manufacturer's instructions. Determination of CD4 T cell counts in the patients was done using flow cytometry (Partec flow cytometer, Germany). In brief, equal volumes (20  $\mu$ l) of CD4 PE antibody and Ethylene diamine tetra acetic acid blood was mixed and incubated for 15 mins, and 800  $\mu$ l of CD4 buffer was added before reading in the cell counter.

Data was analyzed for significance using the chi-square test with Statistical packages for social sciences (SPSS) V. 15 and  $p < 0.05$  was taken as significant.

## Results

One hundred and fifty patients with immunologic AIDS (CD4 cell count <200 cells/ $\mu$ l) who were not on anti-retroviral therapy were studied. Nineteen (12.7%) were positive for cryptococcal antigen. Prevalence of Cryptococcal antigen was quite varied with CD4 cells levels; CD4 count  $\leq 50$  cells/ $\mu$ l had a higher prevalence

of cryptococcal antigen (Table 1). This was followed by CD4 cell counts >50- $\leq 100$  cells/ $\mu$ l and >100- $\leq 200$  cells/ $\mu$ l. Lower CD4 counts were significantly associated with positivity for serum cryptococcal antigen ( $p < 0.001$ ). One (2.5%) of the control was sero-positive for crag. Serum crag was significantly associated with AIDS but not with HIV ( $p < 0.001$ ). Sex was not associated with crag positivity. Also, higher cryptococcal antigenemia were recorded among the age group 31-40 when compared to other age groups (Table 2). However, 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
<b>CD4 count (cells/<math>\mu</math>l) of patients</b>			
$\leq 50$ cells/ $\mu$ l	39	11(28.2)	<0.001
>50- $\leq 100$ cells/ $\mu$ l	42	6(14.3)	
>100- $\leq 200$ cells/ $\mu$ l	69	2(2.9)	
<b>ART- status</b>			
ART- naïve AIDS patients	150	19 (12.7)	<0.001
Control	40	1(2.5)	

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

Characteristics	No. tested (%)	No. positive (%)
<b>Age (years)</b>		
<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)
<b>Male</b>	61	7(11.5)
<b>Female</b>	89	12(12.70)

## Discussion

This study demonstrates a high prevalence of serum cryptococcal antigen in ART-naïve AIDS patients in Benin City, Nigeria. Nineteen (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.<sup>10-12</sup> The prevalence report of this study is relatively higher than the prevalence rates of other countries; 7% was reported in a retrospective study on ART-naïve AIDS patients in South Africa,<sup>13</sup> and 9.2% in Thailand.<sup>14</sup> Higher prevalence of cryptococcal antigenemia has been reported in Cambodia (21%).<sup>15</sup> The 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 a hospital using culture and microscopy recovered a 9.7% prevalence of *Cryptococcus neoformans*.<sup>16</sup> The study further goes to confirm the burden of cryptocococcus in AIDS patients at that hospital. Thus, screening for cryptocococcus in AIDS patients should be made a routine.

The distribution of cryptococcal antigenemia was highly varying with CD4 cell levels. Patients with CD4 cell count  $\leq 50$  cell/ $\mu$ l had the highest prevalence of serum crag; this was closely followed with patients with CD4 cells  $>50$ - $\leq 100$  cell/ $\mu$ l and  $>100$ - $\leq 200$  cells/ $\mu$ l. 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.<sup>17,18</sup> HIV is characteristically associated with T lymphocyte depletion and is highly marked in ART-naïve patients.<sup>13,19</sup> 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,<sup>20</sup> but there were no significant difference in the prevalence in men and women in ART-naïve AIDS patients in Benin City. Age also did not play a significant role in serum crag positivity in the patients.

The present study was aimed to determine the baseline serum cryptococcal antigen prevalence in ART-naïve AIDS patients in Benin City, Nigeria. A one year follow-up evaluation of the prevalence of serum crag, mortality and morbidity ratio in ART-naïve AIDS patients who were seropositive or sero-negative for crag in the preliminary survey is being studied and will be reported later. The key to reducing the mortality from *Cryptococcal meningitis*, which is a consequent of crag positivity, is early diagnosis of crag in ART-naïve AIDS patients prior to initiation of ART therapy and the integration of both ART therapy and primary prophylaxis with Fluconazole; this has been shown to reduce mortality from *Cryptococcal meningitis* consequent of crag positivity even at CD4 cell count within the range of  $\leq 100$  cells/ $\mu$ l to 25 cells/ $\mu$ l.<sup>12,21-23</sup>

## Conclusion

Overall, 12.7% ART-naïve AIDS patients at UBTH, Benin City, were crag positive. Crag prevalence was significantly associated with lower CD4 counts. ART-naïve AIDS patients with CD4 counts  $\leq 50$  cells/ $\mu$ l had the highest prevalence. The sero-prevalence of crag was high; thus screening for *Cryptococcus neoformans* antigen should be made a routine in ART-naïve AIDS patients before initiation of ART therapy to reduce the rapid mortality from *Cryptococcal meningitis*.

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