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Geographical Variation in Prevalence of *Cryptococcal* Antigenemia among HIV-infected Treatment-Naïve Patients in Nigeria: A multicenter cross-sectional study

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Abstract

Objective—Worldwide, HIV-associated cryptococcal meningitis affects approximately 1 million persons and causes 600,000 deaths each year mostly in sub-Saharan Africa. Limited data exist on cryptococcal meningitis and antigenemia in Nigeria, and most studies are geographically restricted. We determined the prevalence of cryptococcal antigenemia (CrAg) among HIV-infected treatment-naïve individuals in Nigeria.

Design/Methods—This was a retrospective, cross-sectional study across four geographic regions in Nigeria. We performed CrAg testing using a lateral flow immunoassay on archived whole blood samples collected from HIV-infected participants at US PEPFAR-supported sites

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Conflicts of Interest: The University of Nevada, Reno, has licensed monoclonal antibodies used for construction of the CrAg LFA to IMMY. Consistent with University of Nevada, Reno policies, a portion of royalties are distributed to T. Kozel. All remaining authors declare no conflicts of interest.

selected to represent the major geographical and ethnic diversity in Nigeria. Eligible samples were (1) stored in an -80° freezer; (2) collected from consenting patients (>15 years) naïve to antiretroviral therapy with CD4+ count less than 200 cells/mm³.

Results—A total of 2,752 stored blood samples were retrospectively screened for CrAg. A majority of samples were from participants aged 30 - 44 (57.6%), and 1,570 (57.1%) were from women. The prevalence of CrAg positivity in specimens with CD4 < 200 cells/mm³ was 2.3% (95% CI = 1.8%-3.0%), and varied significantly across the four regions ($p < 0.001$). At 4.4% (3.2%-5.9%), the South East contained the highest prevalence.

Conclusion—The significant regional variation in CrAg prevalence found in Nigeria should be taken into consideration as plans are made to integrate routine screening into clinical care for HIV-infected patients.

Keywords

AIDS; cryptococcal antigen (CrAg); cryptococcal meningitis; retrospective cross-sectional study; sub-Saharan Africa; Nigeria

Introduction

Cryptococcal meningitis (CM) is caused by the encapsulated fungi *Cryptococcus gattii* and *C. neoformans*, which is commonly found in soil enriched with bird guano. Exposure occurs via inhalation of the fungal spores and likely occurs early in life. However the disease remains dormant due to the suppressive effects of the immune system.¹ In people with HIV/AIDS, *C. neoformans* may reactivate, causing primary pulmonary or disseminated infection, including CM.² The highest risk for the development of CM is among those with a CD4 count <100 cells/mm³.³

Worldwide, HIV-associated CM is estimated to affect approximately 1 million persons and cause 600,000 deaths each year, with the largest burden in sub-Saharan Africa.⁴ In-hospital mortality rates range from 20-50% in resource-limited settings (RLS),^{5,6} with higher mortality rates in settings of suboptimal treatment.⁷ Optimal treatment of CM requires potentially toxic drugs and regular lumbar punctures to manage elevated intracranial pressure. These treatments are expensive, require close monitoring and inpatient hospitalization, and are not widely available in most RLS.

One method to prevent the development of CM in the first place involves screening patients at high risk for developing CM for early cryptococcal infection.⁸ This is done via a blood test that detects cryptococcal antigen (CrAg). Research has shown that CrAg is detectable in patients' sera for a median of three weeks prior to the development of symptomatic CM, creating a window for intervention with antifungal treatment to prevent CM.⁹ In 2011, the World Health Organization (WHO) issued an integrated guideline recommendation for cryptococcal screening among persons with CD4<100 cells/mm³ who were initiating HIV care.¹⁰ Subsequent studies have shown that treatment of early cryptococcal disease can improve outcomes among persons with advanced HIV, and is cost effective down to a CrAg prevalence of 0.6%.¹¹⁻¹³

In Nigeria, the high number of new HIV infections combined with late presentations following infection and inadequate treatment coverage results in serious opportunistic infections like CM.^{4,14} Limited data exist on cryptococcal meningitis in Nigeria. Osazuwa et al. evaluated the prevalence of CrAg among ART naïve patients with CD4<200 cells/mm³ in Benin, a town in southern Nigeria, and found a prevalence of 12.7%.¹⁵ Due to Nigeria's size and previous reports which suggest that *C. neoformans* may have a differential environmental distribution in some areas, studies of CrAg-prevalence from multiple geographical areas are needed to guide effective and efficient implementation of cryptococcal screening in Nigeria.^{16,17}

Methods

Study setting and design

This was a retrospective, cross-sectional study to perform CrAg testing on archived whole blood samples collected from HIV-infected participants at PEPFAR-supported sites across Nigeria. Sites in four regions, the South East, South West, North West and North Central, were selected to represent the major geographical and ethnic diversity seen in Nigeria. Due to the low volume of obtainable samples in the North West and North Central regions, samples from these regions were consolidated and their region was considered “North”.

Written informed consent was obtained from all patients on enrollment into care with permission to use archived samples for future studies. The study protocol was approved by the IRB and Ethics committee of University of Nigeria teaching Hospital, Enugu, Institute of Human Virology Nigeria and the 68 Nigerian Army Reference Hospital, Yaba, Lagos.

Samples were collected between April 2004 and August 2014 and were tested for CrAg positivity between May 2014 and September 2014. All samples on initial collection and processing were separated into aliquots, placed in cryovials and stored at -80°C. During the study, samples meeting criteria were identified, retrieved and kept in a liquid nitrogen freezer until testing. CD4 testing had been performed on samples at the time of initial collection, and resulting CD4 counts were obtained from patients' medical records for use in the analyses of this study.

Study Participants

All stored samples from participants presenting to an out-patient ambulatory setting were selected if they met the inclusion criteria. Samples must have been (1) processed and stored at -80°C; (2) collected from patients with known CD4+ cell count less than 200 cells/mm³; (3) collected from patients naïve to antiretroviral therapy at the time of collection; (4) collected from patients with written consent to allow performance of further HIV-related testing on archived samples.

Statistical Analysis

All statistical analyses were conducted using STATA version 12. Overall similarities between the three regions of the study in terms of demographic and disease characteristics were examined. Demographic characteristics of the populations sampled were compared

using Pearson's chi-square tests. Median CD4 count and age amongst groups were compared using non-parametric equality-of-medians tests. Differences in proportions of CrAg-positive individuals within and across regions were compared using Fisher's exact test. CrAg prevalence by CD4 count stratum, sex and age group were only compared between the South East and South West regions; the small number of CrAg cases in the North precluded meaningful comparison.

Prevalence ratios for factors related to CrAg screening were calculated using log-binomial regression models.¹⁸ For each variable, the prevalence ratio and 95% confidence interval were calculated, adjusting for sample clustering by study site using a generalized linear model. A stratified analysis was also conducted to obtain the prevalence odds ratio for low CD4 count (<100 cells/mm³) by region.

Results

A total of 2,752 stored blood samples from three geographic regions were retrospectively screened for CrAg. The numbers of samples screened by region are as follows: 999 in the South East, 1,329 in the South West, and 424 in the North. The majority of samples were collected from individuals aged 30-44 ($n=1,585$; 57.6%), and 1,570 (57.1%) were collected from women. Median CD4 counts were 121 in the North, 84 in the South East and 95 in the South West and were significantly higher amongst individuals in the North compared with those in the South East and South West ($p < 0.001$). Mean CD4 counts among CrAg positive specimens was 77 in the North, 46 in the South East and 41 in the South West without significant differences ($p=0.88$).

The overall prevalence of CrAg positivity in specimens with $CD4 < 200$ cells/mm³ was 2.3% (95% CI = 1.8% - 3.0%), though this number varied significantly across the three regions ($p < 0.001$, Table 1). The South East had the highest prevalence at 4.4%. The prevalence of CrAg positivity in specimens with $CD4 < 100$ cells/mm³ was 3.6% and varied significantly across the three regions ($p < 0.001$, Table 1).

Of patients found to be CrAg-positive, the median CD4 count was 45 cells/mm³ (range: 7-178 cells/mm³). Patients with advanced immunosuppression ($CD4 < 100$ cells/mm³) were 3.89 times more likely to have been CrAg-positive (95% CI = 3.33 – 4.54) than those with CD4 counts between 100-200 cells/mm³ ($p < 0.001$, Table 2), adjusting for sex and age.

Median CD4 counts of CrAg-positive patients were not significantly different overall ($p=0.89$). Among patients from the South East and South West, respectively, 82% and 83% who tested positive for CrAg had $CD4 < 100$ cells/mm³. At a cutoff of $CD4 < 150$ cells/mm³, the number of CrAg-positive cases detected increased to 91% and 100% in the South East and South West, respectively.

Discussion

WHO's 2011 Rapid Advice on cryptococcal disease recommends consideration of routine screening for cryptococcal antigen (CrAg) in asymptomatic patients with CD4 count below 100 cells/mm³ in areas with a CrAg prevalence $\geq 3\%$.¹⁰ In light of these recommendations,

this study suggests two key findings. First, of the regions studied, cryptococcal disease was found to be most prevalent in Nigeria's South East. With a prevalence of 6.2% among immunocompromised patients (CD4 <100) screened in this region, cryptococcal disease prevalence in this group is comparable to countries where CrAg screening has been incorporated into national ART guidelines. Even in Nigeria's South West, where this study found prevalence to be low (1.4), screening still would likely be cost-effective, according to recent estimates by Jarvis et al citing cost-effectiveness of this intervention down to a prevalence as low as 0.6% in South Africa.¹²

It is worth noting that CrAg estimates from other settings were most often obtained using the WHO recommended screening threshold of CD4 < 100 cells/mm³. This CD4 cutoff used in screening for cryptococcal disease brings up the second key finding of this study: almost one-fifth of patients who tested positive for CrAg in the South West and South East would not have been captured by screening under the WHO guideline. Magambo and colleagues also noted a high prevalence of asymptomatic cryptococcal antigenemia in patients with CD4 count between 100 and 200.¹⁹ This calls into question the use of 100 CD4 cells/mm³ as a cutoff. However, very little data exists on the cost-effectiveness of screening patients with CD4 100-200 cells/mm³, and little is known about differences in development of CM and survival of patients in this range as compared with those with CD4<100 cells/mL.

We found differences in the prevalence of CrAg by region as well as a lower prevalence when compared to other studies.¹⁵ *C. neoformans* survives and multiplies in soil at high humidity and relatively low temperatures (4-24 C)²⁰. We saw higher prevalence of CrAg in the Southern regions of Nigeria compared to the North region. The Northern region of Nigeria has hotter temperatures and lower humidity compared to the Southern region which might decrease *C. neoformans* survival. Additionally, mean CD4 count was higher in the North. We also found lower prevalence of CrAg than has been reported in other studies.¹⁵ This might be due to different sample sizes and participant populations (e.g. participants recruited from hospitals vs from outpatient clinics).

Although there was not a significant difference between men and women in the total sample, there were differences in the North and Southeast regions. This may be due to a differential risk of exposure to fungal spores based on occupation or cultural norms.²¹

Estimates from this study provide insight into the complexity of Nigeria's cryptococcal disease burden and, on a larger scale, the country's evolving HIV epidemic, though several notable limitations exist. Few samples were analyzed in the North region of the country. Political instability in this region limited the amount of sample collection and testing that could be carried out.

Because of the limitations of the data collected in North, few sound conclusions were able to be drawn about the region. However, other studies do suggest that cryptococcal disease may still pose a public health problem in the northern regions. A hospital-based prevalence study in Shika, a town in the North West region, found that CrAg was prevalent in a slightly larger percentage (2.23%) of HIV patients attending a care center than was seen in this study.²²

To our knowledge, this is the largest cryptococcal prevalence study to date in the world. It provides baseline estimates for the prevalence of CrAg in the North, South West, and South East regions of Nigeria, highlighting the regional variation of cryptococcal disease. Additionally, the prevalence found in two regions exceeds current estimates for minimum prevalence at which cryptococcal screening is a cost-effective intervention; consideration should be given to implementing cryptococcal screening in these areas. Further research is needed to gain a more complete understanding of the regional complexities of this high-mortality AIDS-related problem facing a country with the second highest HIV burden worldwide.²³

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Table 1

CrAg Prevalence by CD4 Count, Age, and Sex in Three Regions

Region	Total (n = 2,752)			North (n = 424)			South East (n = 999)			South West (n = 1,329)		
	CrAg Positive (%)	CrAg Negative (%)	p-value*	CrAg Positive (%)	CrAg Negative (%)	p-value*	CrAg Positive (%)	CrAg Negative (%)	p-value*	CrAg Positive (%)	CrAg Negative (%)	p-value*
Total CrAg Cases	64 (2.3)	2,688 (97.7)		2 (0.5)	422 (99.5)		44 (4.4)	955 (95.6)		18 (1.4)	1,311 (98.7)	
CD4 Count (cells/mm ³)			<0.001			0.604			<0.001			0.008
100 - 199	12 (0.9)	1,289 (99.1)		1 (0.6)	156 (99.4)		8 (1.9)	407 (98.1)		3 (0.5)	616 (99.5)	
<100	52 (3.6)	1,399 (96.4)		1 (0.4)	266 (99.6)		36 (6.2)	548 (93.8)		15 (2.1)	695 (97.9)	
Sex			0.374			0.121			0.276			0.637
Male	31 (2.6)	1,151 (97.4)		2 (1.4)	146 (98.7)		22 (5.3)	395 (94.7)		7 (1.1)	610 (98.9)	
Female	33 (2.1)	1,537 (97.9)		0 (0.0)	276 (100.0)		22 (3.8)	560 (96.2)		11 (1.5)	701 (98.5)	
Age			0.854			0.582			0.431			0.264
15 - 29 years	10 (1.9)	503 (98.1)		0 (0.0)	169 (100.0)		10 (4.5)	212 (95.5)		0 (0.0)	122 (100.0)	
30 - 44 years	38 (2.4)	1,547 (93.6)		2 (0.9)	220 (99.1)		22 (3.8)	551 (96.2)		14 (1.8)	776 (98.2)	
45+ years	16 (2.4)	638 (97.6)		0 (0.0)	33 (100.0)		12 (5.9)	192 (94.1)		4 (1.0)	413 (99.0)	

* Calculated using Fisher's exact test

[†] Comparing South East and South West regions only due to low case number in North

Table 2
Prevalence risk ratio (PRR) of CrAg+ patients by various risk factors

Variable	Crude PRR	<i>p</i> -value	Adjusted PRR* (95% CI)	<i>p</i> -value
CD4 Count				
< 100 cells/mm ³	3.89 (2.09, 7.25)	<i>p</i> < 0.001	3.89 (3.33, 4.54)	<i>p</i> < 0.001
100-200 cells/mm ³	REF	-	REF	-
CD4 Count (<i>South East</i>)				
< 100 cells/mm ³	3.20 (1.50, 6.81)	<i>p</i> = 0.003	-	-
100-200 cells/mm ³	REF	-	-	-
CD4 Count (<i>South West</i>)				
< 100 cells/mm ³	4.36 (1.27, 14.99)	<i>p</i> = 0.019	-	-
100-200 cells/mm ³	REF	-	-	-
Age				
15 – 29 years	REF	-	REF	-
30 – 44 years	1.23 (0.62, 2.45)	<i>p</i> = 0.556	1.23 (0.51, 2.99)	<i>p</i> = 0.647
>45 years	1.26 (0.57, 2.74)	<i>p</i> = 0.569	1.26 (0.69, 2.29)	<i>p</i> = 0.460
Sex				
Female	1.25 (0.77, 2.03)	<i>p</i> = 0.370	1.36 (0.94, 1.97)	<i>p</i> = 0.099
Male	REF	-	REF	-

* Calculated using generalized linear model accounting for clustering by study site; clustering not adjusted for in regional stratified analysis because South East and South West regions each contained only one study site