

Prevalence of cryptococcal antigenuria at initial HIV diagnosis in KwaZulu-Natal

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Objectives

The World Health Organization (WHO) recommends screening HIV-infected people for cryptococcal antigens to identify cryptococcosis, a major cause of AIDS-related deaths. As the burden of cryptococcosis is unknown in South Africa's KwaZulu-Natal province, we assessed the cryptococcal antigenuria prevalence among newly diagnosed HIV-infected adults there.

Methods

We conducted a cross-sectional study of newly diagnosed HIV-infected adults who received voluntary HIV testing in an out-patient clinic. Participants provided a urine specimen in a sterile container, and we performed testing with a WHO-endorsed rapid cryptococcal antigen lateral flow assay (Immy Inc., Norman, OK, USA) per the manufacturer's specifications. We assessed cryptococcal antigenuria prevalence among participants with CD4 counts < 200 cells/ μ L, and stratified results by CD4 count categories.

Results

Among 432 participants, the mean (\pm standard deviation) age was 36.1 ± 9.9 years and 172 (40%) were female. The overall estimated prevalence of cryptococcal antigenuria was 9.0% [95% confidence interval (CI) 6.5–12.1%]. CD4 counts were available for 319 participants (74%); the median CD4 count was 75 cells/ μ L [interquartile range (IQR) 34–129 cells/ μ L]. Participants with a negative cryptococcal antigenuria screening test had a median CD4 count of 79 cells/ μ L (IQR 36–129 cells/ μ L), while participants with a positive cryptococcal test had a median CD4 count of 41 cells/ μ L (IQR 10–112 cells/ μ L). The estimated prevalence of cryptococcal antigenuria among participants with CD4 counts < 50 cells/ μ L was 12.5% (95% CI 7.0–20.1%), which was significantly higher than that among participants with CD4 counts of 50–200 cells/ μ L (4.8%; 95% CI 2.3–8.7%).

Conclusions

Nearly 1 in 10 newly diagnosed HIV-infected adults with CD4 counts < 200 cells/ μ L in KwaZulu-Natal had evidence of cryptococcal antigenuria. Point-of-care CD4 count testing and cryptococcal antigen screening may rapidly identify cryptococcosis at the time of HIV diagnosis.

Keywords: CD4 count, cryptococcal urine antigen, cryptococcosis, cryptococcus, diagnosis, HIV/AIDS, South Africa

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Introduction

Cryptococcosis, a fungal infection that leads to cryptococcal meningitis, is a leading cause of AIDS-related mortality world-wide [1]. The World Health Organization (WHO) recommends screening all HIV-infected people with CD4 counts \leq 100 cells/ μ L for cryptococcal antigens [2], as levels of circulating cryptococcal antigens predict the development of cryptococcal meningitis and mortality [3–5]. Two

studies have shown a new, rapid lateral flow assay to have excellent sensitivity (combined sensitivity of 98%) when performed on urine specimens and compared with latex agglutination testing of serum specimens [3,6].

In South Africa, cryptococcosis accounts for approximately 63% of meningitis cases, which is attributable in part to the high burden of HIV/AIDS [7]. The KwaZulu-Natal province has the highest HIV prevalence in South Africa [8], yet there have been no studies on cryptococcosis prevalence in this region. We assessed the prevalence of cryptococcal antigenuria, which is strongly correlated with circulating serum and plasma cryptococcal antigens [3], among newly diagnosed HIV-infected adults attending several out-patient clinics in KwaZulu-Natal. We chose to assess urine specimens as urine has been reported to contain cryptococcal antigen and has additional appeal as a noninvasive biological specimen. The acceptability and validation of an inexpensive rapid test could lead to widespread adoption of cryptococcosis screening in HIV care and treatment programmes.

Methods

Study sites and participants

We conducted a cross-sectional study on adults presenting for voluntary HIV counselling and testing from October 2011 to January 2013. The parent study has been described elsewhere [9]. In brief, participants were recruited from four out-patient clinical areas offering HIV testing services: McCord Hospital, St Mary's Hospital, and two municipal health clinics in urban and peri-urban areas of Durban. All patients were approached sequentially as they presented for HIV testing. Throughout the study, all four clinical sites offered free HIV counselling and rapid testing on week days. Eligible participants were ≥ 18 years old, not receiving antiretroviral therapy, and seeking out-patient care for various reasons. We excluded those known to be HIV-infected, pregnant, or unwilling to disclose HIV test results to the research team. All participants were informed of the study's objectives, enrolled in a private space, and provided written informed consent to participate in the study prior to HIV and cryptococcal antigen testing. Participant data were maintained in a secure database and participant confidentiality was preserved throughout the study. The ethics committees of McCord Hospital (IRB00005803) and St Mary's Hospital in Durban, and Partners HealthCare in Boston (Protocol #: 2006-P-001379/40) approved the study.

Study procedures

In this study, a urine sample was requested from those who were HIV-infected. Urine samples were collected in a sterile container. Urine specimens were refrigerated within 6 h of collection before transfer to a -20°C frost-free freezer (actual temperature ranged from -18° to -22°C) for testing at the completion of cohort enrolment. All participants were offered CD4 count testing as part of their routine care. All HIV testing, care, and treatment were provided in accordance with current South African Department of Health guidelines.

We performed cryptococcal antigen testing using a rapid lateral flow assay (Immy Inc., Norman, OK, USA) on urine samples of participants with CD4 counts < 200 cells/ μL . The rapid cryptococcal antigen assay has been approved by the United States Food and Drug Administration and endorsed by the WHO [2]. The rapid lateral flow assay has an estimated sensitivity of 98% when used on urine specimens [3,6]. According to the manufacturer, the rapid lateral flow assay performs as well on thawed urine samples. Test kits were maintained in a sealed pouch at room temperature (15 – 26°C) before usage. All urine samples were allowed to thaw completely before testing. For each test, one drop of diluent buffer solution, provided by the test manufacturer, was added to a small vial. We pipetted 40 μL of urine into the vial and placed a rapid test into the vial. After 10 min, two researchers (PKD and JMK), blinded to participants' demographics and CD4 count, independently interpreted the test results using a reference card provided by the manufacturer. Participants were considered urine cryptococcal antigen positive by the appearance of a colour band of any intensity, as defined by the manufacturer. If the 'control' band was not present or the two readers were not in agreement, the test process was repeated using the same urine sample until the two readers reached agreement.

Statistical analyses

In primary analyses, we measured the overall prevalence of cryptococcal antigenuria among the cohort. In secondary analyses, we stratified the prevalence of cryptococcal antigenuria by CD4 count stratum and compared the prevalence in participants with CD4 counts < 50 cells/ μL with that in participants with CD4 counts of 50 – 200 cells/ μL . As this is a descriptive study, we calculated exact 95% confidence intervals (CIs) for the overall and stratified estimates of prevalence. The *P*-values were generated to determine the significance of differences between people who were cryptococcal antigenuria positive and those who were cryptococcal antigenuria negative, and to compare estimated cryptococcal antigenuria prevalences among

Table 1 Baseline characteristics

	Total (<i>n</i> = 432)	CrAg negative (<i>n</i> = 393)	CrAg positive (<i>n</i> = 39)	<i>P</i> -value
Age (years) (mean ± SD)	36.1 ± 9.9	36.2 ± 9.9	35.3 ± 10.1	0.59
Gender [<i>n</i> (%)]				
Female	172 (39.8)	157 (39.9)	15 (38.5)	1.00
Male	260 (60.2)	236 (60.1)	24 (61.5)	1.00
CD4 cell count				
Result available [<i>n</i> (%)]	319 (73.8)	295 (75.1)	24 (61.5)	0.08
CD4 count (cells/μL) [median (IQR)]	75 (34, 129)	79 (36, 129)	41 (10, 112)	0.28

We used Fisher's exact test to compare percentages, *t*-tests to compare means, and the Mann–Whitney test to compare medians. CrAg, cryptococcal antigenuria; IQR, interquartile range; SD, standard deviation.

categories of CD4 cell count. We used Fisher's exact test to compare categorical data, *t*-tests to compare continuous data, and the Mann–Whitney test to compare medians. The sample size was generated to provide an exact 95% binomial CI of ± 3%, assuming 10% CrAg prevalence in this cohort. All reported *P*-values were two-tailed, and a *P*-value < 0.05 was considered statistically significant. We collected data using Microsoft Excel and conducted statistical analyses using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Results

We tested urine specimens from 432 newly diagnosed HIV-infected adults. Mean age was 36.1 years (standard deviation ± 9.9 years) and 40% were female (Table 1). CD4 counts were available for 319 participants (74%). Among the entire cohort, the median CD4 count was 75 cells/μL [interquartile range (IQR) 34–129 cells/μL]. There was no significant difference in cryptococcal antigenuria at initial HIV diagnosis by age or gender.

Median CD4 count was 41 cells/μL (IQR 10–112 cells/μL) among participants with a positive cryptococcal antigenuria result, and 79 cells/μL (IQR 36–129 cells/μL) among participants with a negative cryptococcal antigenuria result. Fewer participants with cryptococcal antigenuria obtained a CD4 count result, but this was not statistically significant. Participants who had evidence of cryptococcal antigenuria had lower CD4 counts compared with participants who tested cryptococcal antigenuria negative, but this difference was not statistically significant.

Thirty-nine participants were urine cryptococcal antigen positive, giving an overall estimated cryptococcal antigenuria prevalence in the cohort of 9.0% (95% CI 6.5–12.1%) (Table 2). When stratified by CD4 count, the estimated cryptococcal antigenuria prevalence was 7.5% (95% CI 4.9–11.0%) among participants with CD4 counts < 200 cells/μL, and 8.8% (95% CI 5.2–18.8%) among participants with CD4 counts < 100 cells/μL. Among participants with CD4 counts < 50 cells/μL, the estimated cryptococcal

Table 2 Cryptococcal antigenuria prevalence among HIV-infected adults stratified by CD4 count category

	Number positive/ number tested	Prevalence (%) (95% CI)
Total	39/432	9.0 (6.5–12.1)
Stratified by CD4 count category		
<200 cells/μL	24/319	7.5 (4.9–11.0)
<100 cells/μL	17/192	8.8 (5.2–18.8)
<50 cells/μL	14/112	12.5 (7.0–20.1)
Missing CD4 cell count	15/113	13.3 (7.6–21.0)

CI, confidence interval.

antigenuria prevalence was 12.5% (95% CI 7.0–20.1%). Among the 113 participants who did not obtain a CD4 count, the estimated prevalence of cryptococcal antigenuria was 13.3% (95% CI 7.6–21.0%).

Overall, cryptococcal antigenuria prevalence was greater among participants with lower CD4 counts. There was no difference in estimated cryptococcal antigenuria prevalence between those with CD4 counts of 100–200 cells/μL (5.5%; 95% CI 2.2–11.0%) and those with CD4 counts < 100 cells/μL (*P* = 0.29). However, the estimated prevalence of cryptococcal antigenuria among participants with CD4 counts < 50 cells/μL was significantly higher than that in participants with CD4 counts of 50–200 cells/μL (4.8%; 95% CI 2.3–8.7%) (*P* = 0.01).

Discussion

In a cohort of newly diagnosed HIV-infected adults with CD4 counts < 200 cells/μL in KwaZulu-Natal, the estimated prevalence of cryptococcal antigenuria at HIV diagnosis was nearly 1 in 10 patients. As expected, we found the prevalence of cryptococcal antigenuria to be higher among participants with lower CD4 counts. We also found a high rate of cryptococcal antigenuria prevalence among participants who did not obtain a CD4 count result, which is a group at risk for being lost to follow-up. As death from

cryptococcosis has been observed in one African study with a CD4 count as high as 365 cells/ μ L [10], current WHO screening recommendations (CD4 count \leq 100 cells/ μ L) may miss some HIV-infected people with potentially life-threatening cryptococcosis.

To our knowledge, this is the first prevalence study of cryptococcal antigenuria in KwaZulu-Natal province, which has a very high burden of both HIV infection and tuberculosis (TB). Our prevalence estimates are consistent with those of most other studies of HIV-infected adults in sub-Saharan Africa [11–14], which have been as high as 21% among adults with CD4 counts $<$ 150 cells/ μ L in Ethiopia [15]. A study in Cape Town, South Africa reported an estimated overall cryptococcal antigenaemia prevalence of 7% at the time of HIV diagnosis [4]. Few studies have examined the prevalence of cryptococcal antigens among HIV-infected adults with CD4 counts $>$ 200 cells/ μ L. A recent study in Ethiopia reported an estimated serum cryptococcal antigenaemia prevalence of 5.8% (three of 52) among HIV-infected adults with CD4 counts of 200–350 cells/ μ L [15]. Our observed prevalence of cryptococcal antigenuria among newly diagnosed HIV-infected adults is probably attributable to a high burden of cryptococcosis in the study region, but others have suggested greater sensitivity of the rapid lateral flow assay as compared with standard latex agglutination assays [6].

Our study had several strengths and limitations. First, we performed testing on frozen, not fresh, urine samples. Cryptococcal antigens circulate at lower levels in urine than in plasma or serum [3], and may be less detectable in frozen samples. Thus, our methodology may have led to some false negative results, thereby underestimating the true burden of infection. Secondly, as this was a pilot study to assess the burden of disease, we did not conduct a laboratory reference standard test for cryptococcosis or assess patient outcomes. Two studies, however, have demonstrated that the rapid lateral flow assay has 98% sensitivity when performed on urine specimens and compared with latex agglutination testing of serum specimens [3,6], and there have been no studies of test specificity. Finally, we evaluated the rapid cryptococcal antigen test among HIV-infected adults in a TB-endemic region, so these results may not be generalizable to other populations.

In conclusion, we found a prevalence of cryptococcal antigenuria of around 10% among newly diagnosed HIV-infected adults with CD4 counts $<$ 200 cells/ μ L in KwaZulu-Natal. Because the rapid lateral flow assay is a simple, inexpensive and rapid test to detect cryptococcal antigenuria, screening HIV-infected people at the clinical point of care may be appropriate [12]. As the WHO recommends routine cryptococcosis screening for HIV-infected patients with CD4 counts \leq 100 cells/ μ L and the relationship

between cryptococcal antigenuria and patient outcomes is uncertain, test validation studies for urine should include HIV-infected people with CD4 counts $>$ 100 cells/ μ L and assess clinical outcomes.

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Conflicts of interest: We declare that we have no conflicts of interest.

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