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MAJOR ARTICLE

Prevalence and Factors Associated With Cryptoccocal Antigenemia Among Patients With Advanced Human Immunodeficiency Virus in Eastern Uganda: A Facility-Based Cross-sectional Study

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Background. Cryptoccocal infection remains an important cause of morbidity and mortality among people with advanced human immunodeficiency virus disease (AHD). In resource-limited settings, there is a paucity of data on cryptoccocal infections. We described the prevalence and factors associated with cryptoccocal antigenemia among people with AHD in Mbale Regional Referral Hospital in Eastern Uganda.

Methods. In this cross-sectional study, data on sociodemographic, clinical, and laboratory characteristics of adults with AHD were collected, and factors associated with cryptoccocal antigenemia were determined using multivariate logistic regression models.

Results. We enrolled 228 participants with a median CD4 cell count of 194/μL (interquartile range, 129–370/μL). The prevalence of cryptoccocal antigen was 10 in 228 (4.4% [95% confidence interval, 2.4%–80%]). CD4 cell counts <100/μL (adjusted odds ratio, 3.70) and poultry keeping were risk factors. The main predictors were headaches (adjusted odds ratio, 1), neck pains (8.817), confusion (6.323), and neck stiffness (676.217). No notable significant associations were found in the multivariate analysis

Conclusions. The prevalence of cryptoccocal antigen was 4.4%, and antiretroviral therapy was protective.

Keywords. antigenemia; cryptococcal; meningitis; HIV.

Cryptococcus neoformans is a ubiquitous fungus in the environment worldwide that causes cryptococcosis. It is often an opportunistic infection in people with severe immune suppression [1–3]. Globally in 2017, the prevalence of cryptococcosis varied between high- and low-income countries, but on average it was estimated to be 6.0% among people with advanced human immunodeficiency virus (HIV) disease (AHD). About 278 000 people are estimated to develop cryptococcal disease annually, resulting in 181 100 deaths [2]. A majority of people with cryptococcal disease have AHD, defined as CD4 cell count ≤200/μL or World Health Organization (WHO) clinical stage 3 or 4 [2, 4]. Cryptococcal meningitis is the most common and most severe form of cryptoccocal disease, accounting for about 80% of all cryptococcal infections globally [2].

Data indicate that early HIV diagnosis and early initiation of antiretroviral therapy (ART) results in a significant decrease in the number of associated cryptococcal morbidity, complications, and deaths [2, 5], and this has paralleled the efforts of universal ART access since 2010. Despite marked improvements due to the rollout of ART, cryptococcal disease remains the second most common cause of AIDS-related deaths, after tuberculosis [6, 7]. Cryptococcal antigenemia is not highly specific to the disease stage as it represents both mild and severe forms of infection.

A 2015 meta-analysis carried out in Uganda estimated a cryptococcal antigenemia prevalence of about 7.1%, with a mortality rate of up to 60% among people with AHD [8]. Even with ART initiation and administration of recommended cryptoccocal treatment, 14-day mortality rates of cryptococcal meningitis among AHD range between 20% and 42% in Ugandan cohorts [5, 9, 10]. These rates were attributed to delayed diagnosis and intervention [11]. Early detection of cryptococcal antigenemia not only provides an opportunity for early diagnosis and early treatment but also minimizes the risk of immune reconstitution inflammatory syndrome when ART is initiated [6, 12]. The WHO and the Uganda Ministry of Health recommend screening for cryptococcal antigenemia in all patients with AHD [4, 13]. However, delaying screening until the onset of AHD is likely to cause a delay in the disease

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treatment. Where early screening for cryptococcal disease has been successfully done, it is cost-effective [14]. Prioritizing routine screening for cryptococcal antigenemia is therefore important.

There are few data, if any, on cryptococcosis in Eastern Uganda. In the current study, we screened HIV-infected adult patients with AHD at Mbale Regional Referral Hospital (MRRH), Uganda, to determine the prevalence and factors associated with cryptococcal antigenemia.

METHODS

Study Setting and Context

This cross-sectional study was performed at MRRH and its collaborating HIV clinics in Eastern Uganda between May to June 2019. Quantitative data were collected using a pretested and customized questionnaire administered by trained research assistants. MRRH is the largest health facility in Eastern Uganda, located at the heart of Mbale City, about 222 km east of Kampala, the country's capital city. It is a public health facility with a capacity of about 455 beds, in collaboration with the Infectious Diseases Research Collaboration and The AIDS Support Organisation in HIV care in Eastern Uganda, making these the largest HIV care facility–based services in the region.

For laboratory quality assurance, we used the MRRH Laboratory to perform laboratory investigations. It offers services in hematology, biochemistry, immunology, serology, microbiology, and blood transfusion services. It is accredited to International Organisation for standardisation 15189 competence by the South African National Accreditation System. These are standard internal and external quality assurance mechanisms.

Study Population

The study targeted HIV-positive consenting adult patients (aged ≥18 years) with CD4 cell counts < 200/μL and/or WHO HIV clinical stage 3 or 4 who were receiving services at MRRH and its associated HIV clinics. We excluded patients receiving fluconazole prophylaxis, preemptive treatment for cryptococcal infection, and past or current treatment for cryptococcal meningitis.

Sample Size Calculation and Sampling Technique

For objective 1 (prevalence), using the prevalence of 7.1% from a previous study carried out in Uganda [8] and a 95% confidence interval (CI) and precision of 0.05, the sample was calculated to be 102 patients, adjusting for a nonresponse rate of 10% and design effect of 2. The estimated sample size was 227 patients. The nonresponse of 10% is based on our experience from similar studies based on MRRH, in which we have observed nonresponse ranges from 2.5% to 10%. We chose the upper end to maximize the statistical impact of the sample size. Design effect was adjusted for because of the potential correlation of information among persons served in a single locality thus creating "clusters." A design effect of 2.0 was conventionally selected because we could not find any previous literature on the same subject regarding this effect, and we had no pilot data to estimate it.

For objective 2, we used the prevalence of factors associated with cryptococcal antigenemia (CD4 cell count, body mass index [BMI], headaches, and convulsions) in a similar study by Micol et al [15], basing on a 95% CI and precision of 0.05. The sample size was calculated as 185 and then adjusted for 10% nonresponders to give us a final sample size of 206. In the current study, we used the bigger sample size of 227, as noted above for objective 1.

Patient Recruitment

Participants were recruited by consecutive sampling techniques from the MRRH and its associated HIV clinics. Each of these clinics formed an entry point for the study where a trained research assistant worked with the principal investigator to identify and recruit eligible study participants and obtain their consent. The research assistants were trained before study commencement and had daily orientation on the study for purposes of quality. Patients who met the inclusion criteria were recruited. On average, 7 patients were recruited consecutively each day until the sample size was attained, and we used patient study serial numbers to harmonize recruitment at the different sites.

Data Collection and Quality Assurance

The study site was assessed before data collection, which was achieved initially by a carefully designed, prerecorded, and pretested questionnaire including sociodemographic, clinical, and laboratory questions and factors associated with serum cryptococcal antigen (CrAg) positivity. The questionnaires were administered by trained research assistants, and the principal investigator ensured data completeness for every questionnaire.

Participants' presenting symptoms, coexisting diagnoses, and current treatment were extracted by administering the questionnaire and also from participants' records. A physical clinical examination was performed to determine signs, the presence of other opportunistic infections, and anthropometric measurements. Participants' weight and height were measured to determine body mass index (BMI).

Laboratory Procedures

Sample Processing

For each enrolled patient, 3–4 mL of blood was collected into 2 vacutainer tubes by the laboratory technician and processed as follows: blood was allowed to clot at room temperature for 30 minutes and centrifuged at 3000 rpm for 5 minutes. The resulting serum in a red-top vacutainer was used to test for CrAg. A purple-top vacutainer was used to collect samples to determine

the CD4 cell count for participants without a record of a recent CD4 cell count.

Cerebrospinal Fluid Sampling

For all participants testing positive for serum CrAg, a lumbar puncture was performed by the principal investigator under aseptic conditions. Cerebrospinal fluid (CSF) opening pressures were not measured owing to the unavailability of manometers at the study setting.

Serum and CSF CrAg Testing

CrAg testing was performed by a trained laboratory technician who used daily positive controls to ensure data quality. To minimize bias, the laboratory technician was blinded to all patient data. CrAg testing was performed using a commercially available point-of-care test kit (diluent and test strips) from Immuno-Mycologics (IMMY), a lateral flow assay, which has been shown to have higher performance than latex particle agglutination tests [16].

CD4 Cell Count and Viral Load

For the participants already receiving long-term care at the HIV clinics, data on CD4 cell counts and HIV viral load in the past 6 months were recorded from their files. CD4 cell counts, and viral load tests were performed if no recent test results (within <6 months) were recorded in their files. HIV viral loads were recorded as suppressed if the load was <1000 copies/mL blood; viral loads ≥1000 copies/mL were considered nonsuppressed and were categorized as either 1000–100 000 or >100 000 copies/mL. For the participants with newly diagnosed HIV infection (within the past 6 months), viral loads were recorded as not applicable because the national policy does not provide for viral load testing of people living with HIV/AIDS (PLWHA) who have been on ART for <6 months.

Data Analysis

Electronic data were exported from EPIDATA to Stata software (version 13; StataCorp) for statistical analyses. Exploratory data analyses were conducted to check the cleanliness of data and assembled into analytic data sets. Frequency statistics were used for descriptive data presentation, and logistic regression models were used to describe factors associated with CrAg positivity at a 95% CI.

Ethical Approval and Patient Consent Statement

The study conformed to the provisions of ethical standards in Uganda and internationally. Ethical approval was sought from the MRRH Research and Ethics Committee on behalf of MRRH and its collaborators, The AIDS Support Organisation Uganda Limited Research and Ethics Review Committee. The project was approved by the Mbale Regional Referral Hospital Research and Ethics Committee and registered with the Uganda National Council for Science and Technology, while local permission to conduct the project was obtained from administrative leaders of the MRRH and The AIDS Support Organisation. The Uganda National Health Research Organization and the Mbale Clinical Research Institute approved the publication of this manuscript.

In addition, the Busitema University Higher Degrees Committee approved the study because it was a master's program dissertation from that university. Informed consent was sought from each of the participants. If that was not possible because of the participant's unstable condition, consent was sought from the next of kin. No patient identification data were recorded on the data collection tool, but study documents were coded using study numbers that were linked only to patient clinical notes via patient identification numbers. This was to maintain the utmost confidentiality. Study procedures were explained in detail to all participants.

RESULTS

Baseline Sociodemographic Characteristics

We approached 234 individuals who met the inclusion criteria and recruited 228 participants; 6 declined to consent (Figure 1). The median CD4 cell count was 194/μL (interquartile range, 129–370/μL). Most participants (66.7%) were female, 47.8% had at least a primary school education, and 60.1% were not married (Table 1).

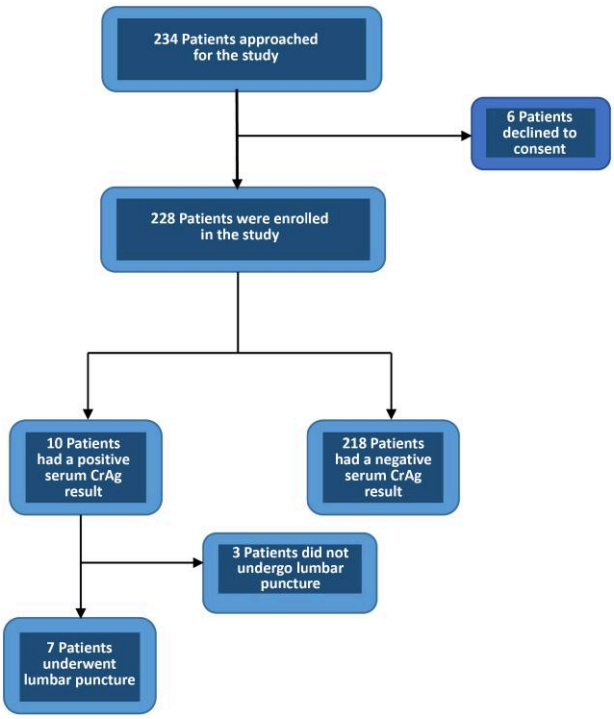

Figure 1. Study profile. Abbreviation: CrAg, cryptococcal antigen.

Table 1. Baseline Sociodemographic and Clinical Characteristics of Study Participants

Characteristic	Participants, No. (%)[a] (N = 228)
Age, mean (SD)	42 (12.4)
Age ≥40 y	121 (53.1)
Female sex	152 (66.7)
Marital status	
Married	91 (39.9)
Other	137 (60.1)
Highest level of education	
Primary school	109 (47.8)
Other	119 (52.2)
Occupation	
Farm-worker	136 (59.6)
Other	92 (40.4)
Criteria for recruitment	
HIV clinical stage 3/4	99 (43.4)
CD4 cell count <200/μL and/or HIV stage 3/4	129 (56.6)
Time since HIV diagnosis	
≥20 y	11 (4.8)
10–20 y	75 (32.9)
1–10 y	94 (41.2)
6 mo to 1 y	22 (9.6)
≤6 mo	26 (11.4)
CD4 cell count, cells/μL	
Median (IQR)	194 (129–370)
<100	41 (18)
101–200	85 (37.3)
>200	99 (43.4)
ART status	
Yes	201 (88.2)
No	27 (11.8)
ART duration	
>6 mo	175 (76.8)
<6 mo	26 (11.4)
HIV viral load	
Suppressed	131 (57.5)
1000–10 000 copies/mL	51 (22.4)
>10 000 copies/mL	27 (11.8)
Not applicable	19 (8.3)

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range; SD, standard deviation.

[a]Data represent no. (%) of participants unless otherwise specified.

Prevalence of Cryptococcal Antigenemia and Factors Associated With Cryptococcal Antigenemia

The overall prevalence of cryptococcal antigenemia was 4.4% (95% CI, 2.4%–80%). Bivariate analysis found the following significant associations: cell count <100/μL, poultry keeping at home, and clinical features suggestive of meningeal irritation, including headaches, neck and back pain, altered vision, recent confusion, and neck stiffness. Taking ART was protective (Table 2). In bivariate analysis, of the 10 study participants with a positive serum CrAg result, 9 (90%; odds ratio [OR] (CI) 52.313 [6.682–2304.004]) had a CD4 cell count ≤100/μL, all 10 (100%; $P < .001$) kept poultry at home. All 10 participants with a positive serum CrAg result were fully conscious, with a Glasgow coma scale score of 15 (maximum possible score) after the study, 9 of 10 were started on treatment for cryptococcal meningitis based on CSF CrAg positivity or the presence of meningeal signs, and 1 was treated with fluconazole preemptive treatment for cryptococcal antigenemia without evidence of neurological disease (asymptomatic). All 7 lumbar punctures yielded CrAg-positive results.

DISCUSSION

Compared with the global prevalence estimates of 6.0% in 2017 [2] and Uganda's national estimated prevalence of 7.1% in 2015 [8], our findings of a prevalence of 4.4% suggest that prior data need updating in different settings. We postulate that, in the era of ART, the prevalence of cryptococcal antigenemia is reduced over time despite low immunity in AHD, but longitudinal studies are needed to generate further evidence. The lower prevalence of cryptococcal antigenemia in our study is probably because we recruited a heterogeneous population of study participants with either CD4 cell counts <200/μL or WHO clinical stage 3 or 4 events. The latter may include patients with CD4 cell counts >200/μL at the time of the study, contrary to the findings of previous studies reporting higher prevalence among participants with more severe immunosuppression (CD4 cell counts ≤100/μL).

We found that the prevalence of cryptococcal antigenemia was 22% among those who were severely immunosuppressed (with CD4 cell count <100/μL), which is similar to the 19% reported in another cross-sectional study earlier performed in 2012 in Kampala by Oyella and colleagues [17]. In their study, 367 participants with AHD had median CD4 cell counts of 23/μL (interquartile range, 9–51/μL). Unlike earlier studies, our study was conducted with ongoing Ugandan systems and advocacy for improved early screening and presumptive treatment for positive cases to reduce the burden of cryptococcal antigenemia. Nonetheless, our study has generated important data for public health actions locally and in other similar settings globally.

We found that poultry keeping was associated with serum CrAg positivity. In rural subsistence communities and economic empowerment programs for PLWHA, avoidance of exposure to poultry would be preventive for the disease in AHD. In a study carried out in 49 cities across the world where 122 pigeon excreta samples were tested, Takahara and colleagues [18] found high positivity rates for *C. neoformans*. Using these data, well-targeted programs can be designed for PLWHA, especially since their CD4 counts are regularly monitored.

The clinical characteristics of patients with serum CrAg positivity were indicative of cryptococcal meningitis, as described by Harrison et al [3] and consistent with data reporting that

Table 2. Factors Associated With Cryptococcal Antigenemia Among People With Advanced HIV at Mbale Regional Referral Hospital: Bivariate and Multivariate Analysis

	Study Participants, No. (%)		Bivariate Analysis		Multivariate Analysis	
Characteristic	CrAg Positive (n = 10)	CrAg Negative (n = 218)	Crude OR (95% CI)[a]	*P* Value	Adjusted OR (95% CI)[b]	*P* Value
Age group						
18–30 y	1 (10)	41 (18.8)	0.48 (.01–3.64)	.482	...	...
>30 y	9 (90)	177 (81.2)	2.08 (.27–93.56)	.482	...	...
Sex						
Male	6 (60)	70 (32.1)	3.17 (.72–15.70)	.067	...	...
Female	4 (40)	148 (67.8)	0.32 (.06–1.39)	.067	...	...
Poultry keeping						
Yes	10 (100)	94 (43.1)	…	…	…	…
No	0 (0.0)	124 (56.9)	…	…	…	…
ART status						
On ART	6 (60.0)	195 (89.4)	0.18 (.03–.93)	.02	0.230 (.008–7.00)	.399
Not on ART	4 (40.0)	23 (10.5)	5.65 (1.077– 25.602)	.02	…	…
CD4 cell count						
<100/μL	9 (90.0)	32 (14.7)	52.31 (6.68– 2304.00)	<.001	1	…
100–199/μL	1 (10.0)	84 (38.5)	0.020 (.00–.15)	<.001	0.060 (.002–2.408)	.135
≥200/μL	0 (0.0)	99 (45.4)	…	<.001	0.080 (.001–5.599)	.244
Viral load						
Suppressed	5 (62.5)	126 (62.7)	0.99 (.19–6.57)	.99	1	.764
Nonsuppressed	3 (37.5)	75 (37.3)	1.01 (.15–5.35)	.99	1.623 (.068–38.597)	…
Persistent headaches						
Yes	9 (90.0)	83 (38.1)	14.640 (1.95–646.13)	<.001	0.3717 (.015–9.295)	.547
No	1 (10.0)	135 (61.9)	0.068 (.002–.51)	<.001	1	…
Altered vision						
Yes	7 (70.0)	50 (22.9)	7.84 (1.70–480.150)	.003	0.970 (.017–55.190)	.988
No	3 (30.0)	168 (77.5)	0.18 (.02–.59)	.003	1	…
Recent neck or back pain						
Yes	9 (90.0)	42 (19.3)	37.71 (4.901661.67)	<.001	11.9 (.45– 312.29)	.138
No	1 (10.0)	176 (80.7)	0.03 (.001–.20)	<.001	1	…
Reported confusion						
Yes	7 (70.0)	15 (6.9)	31.578 (6.20– 200.50)	<.001	7.03 (.09–563.75)	.383
No	3 (30.0)	203 (93.1)	0.023 (.004–.12)	<.001	1	…
Seizures or loss of consciousness						
Yes	7 (70.0)	11 (5.1)	43.91 (8.23– 284.25)	<.001	0.034 (.0–11.75)	.290
No	3 (30.0)	207 (94.9)	0.02 (.004–.12)	<.001	1	…
Neck stiffness						
Yes	6 (60.0)	1 (0.5)	325.50 (25.97– 15007.9	<.001	24.24 (.066– 858.7)	.290
No	4 (40.0)	217 (99.5)	0.003 (.00–.040)	<.001	1	…
Kernig sign						
Positive	7 (70.0)	0 (0.0)	…	<.001	35.22 (.088–139.9)	.243
Negative	3 (30.0)	218 (100.0)	…	<.001	1	…

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; CrAg, cryptococcal antigen; OR, odds ratio.

[a]Crude ORs were obtained from tab Odds which is a statistical software.

[b]Adjusted ORs were obtained with multiple logistic regressions.

70%–90% of participants who were CrAg positive also presented with these clinical features [11, 12, 15, 17, 19]. We found that the proportions of CrAg-positive individuals were similar between those on ART and those who were ART naive (6 vs 4 patients, respectively); the 6 individuals in the first group had taken ART for >6 months, similar to findings in a study in Kampala by Rheins et al [20], which found CrAg positivity in 46% on ART versus 54% of the ART naive, an indicator that ART does not play a role but immunity does. Our further finding of ART being a protective factor in our study (adjusted OR, 0.240), similar to findings in other studies [21], underscores the role of ART in building immunity in PLWHA. Therefore, in the early stages of ART when immunity is still low, the risk of cryptococcus is similar between ART-naive and nonnaive individuals.

Pathophysiologically, it would be expected that patients with HIV viral load suppression (<1000 copies/mL) have better immunity and are at low risk of positive serum CrAg. Conversely, our

finding showed that half of the participants with positive serum CrAg results had a suppressed HIV viral load, while the other half had a high viral load of >100 000 copies/mL. These findings are critical in informing guidelines that currently exclude PLWHA with suppressed viral load from serum CrAg screening. People who are new to ART or not yet on ART have extremely high HIV viral loads because the HIV is actively multiplying without hindrance by ART [3] and should also be a priority target for serum CrAg screening, irrespective of WHO clinical stage status. In patients with both high and low viral load counts we found that the common feature was low CD4 cell counts, indicating that this is a critical biomarker for the risk of cryptococcal infections. In our study, 5 (62.5%) of the CrAg-positive patients with a CD4 cell count <200/μL also had a suppressed viral load, indicating possible immunological failure.

A meta-analysis performed by Parkes and colleagues [8] showed a prevalence of 7.1% among the estimated 56 000 PLWHA with CD4 cell counts <100/μL and high viral loads; serum CrAg positivity was associated with higher HIV viral load. This is probably because higher HIV viral loads lead to a steady depletion of the CD4 cell count, as observed in patients with viral nonsuppression, including the ones in the current study [3]. While half of the individuals with a positive serum CrAg result in our study were found to have normal BMI and nutrition status, this was a sharp contrast to other studies showing that a low BMI and malnutrition were independently associated with cryptococcal antigenemia [17]. This suggests that using guidelines in which BMI is a criterion for priority screening would miss patients like those we included in our study.

In conclusion, the prevalence of cryptococcal antigenemia among patients with AHD under care in MRRH was 4.4%—lower than the national and global estimates, possibly because of increased screening and presumptive treatment. Across the disease spectrum, there was a high prevalence of 22% among patients with severe immune suppression (CD4 cell count <100/μL). CrAg positivity was associated with CD4 cell count <100/μL and poultry keeping. Being on ART was protective against cryptococcal antigenemia.

We recommend that fluconazole prophylaxis be considered for people with AHD who are CrAg negative but also keep poultry at home. Further research in this area should be considered, given that our study was not adequately powered to answer this question. Screening protocols should be strengthened to emphasize routine serum CrAg screening for patients with suppressed viral loads but with CD4 cell counts <100/μL.

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Data availability. The study data are available on request to the corresponding author.

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