



Prevalence of Disseminated Cryptococcosis among Human Immunodeficiency Virus Infected Patients in Benin City, Nigeria

Gabriel Joseph¹, Ephraim Ogbaini-Emovon^{2*}, Benson U. Okwara³, Abel Onunu³
and Emmanuel Kubeyinje³

¹Department of Dermatology and Infectious Diseases, Federal Medical Centre, Lokoja, Kogi State, Nigeria.

²Institute of Lassa Fever Research and Control, Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria.

³Department of Dermatology, University of Benin Teaching Hospital, Benin-city, Edo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author JG designed the study, wrote the protocol and wrote the first draft of the manuscript. Author EO managed the laboratory work and data, author BUO managed the literature search. Author AO contributed to physical examination of patients, data analysis and review of the manuscript. Author EK handled patients selection, management of clinical data and review of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2015/14576

Editor(s):

(1) Roberto Manfredi, Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy.

Reviewers:

(1) Anonymous, France.

(2) Anonymous, Uganda.

(3) Nwako Okechukwu Francis, Department of Medicine, FMC Owerri, Nigeria.

Complete Peer review History: <http://www.sciencedomain.org/review-history.php?iid=906&id=12&aid=7636>

Original Research Article

Received 7th October 2014
Accepted 12th November 2014
Published 5th January 2015

ABSTRACT

Aim: To determine the prevalence of disseminated cryptococcosis among symptomatic HIV-infected patients, attending the Antiretroviral Treatment Clinic at the University of Benin Teaching Hospital, Benin City, Nigeria.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Internal Medicine and Department of Medical Microbiology, University of Benin Teaching Hospital, Edo State, Nigeria, between September 2010

*Corresponding author: Email: epogbaini@yahoo.com;

and August 2011.

Methodology: Five hundred consecutive symptomatic HIV-infected patients, on ART were enrolled into this cross-sectional study (266 males, 234 females, age range 18-81 years, mean age, 40.08 years). A blood sample collected from each participant was screened for serum cryptococcal antigen (CRAG) using the cryptococcal Latex agglutination test. The viral load and CD4+ T-cell count were also determined in parallel. A structured questionnaire was used to gather information on socio-demographic characteristics, medical and treatment history of participants. Data collected and the results of laboratory tests were analyzed using the SPSS software, version 22.0.

Results: The prevalence of serum cryptococcal antigen was 9.8%. Majority (66.8%) of the participants had a CD4+ T-cell count of less than 100 cells/ μ l. The association between serum CRAG and CD4+ T-cell was found to be significant ($P < .001$). Viral load done for only 90 of the participants was high in 51.1%. The association between serum CRAG and viral load was found to be significant ($P < .001$).

Conclusion: The prevalence of serum CRAG was high among symptomatic HIV-infected patients on ART, in Benin city, Nigeria, despite ART implementation. There is need therefore for a routine cryptococcal antigen test for all symptomatic HIV-infected patients on ART, while further microbiological investigations for those with positive result are recommended for appropriate medical intervention.

Keywords: *Cryptococcus antigen; HIV-patients; CD4+ cell count; viral Load.*

1. INTRODUCTION

Cryptococcosis is an infection caused by the yeast-like fungus, *Cryptococcus neoformans* [1]. This infection occurs in people with immune-suppression, but rare in immune-competent individuals [2,3]. In the last 20 years, the incidence of cryptococcosis has increased with the occurrence of life-threatening infection among HIV-infected individuals with profound immune-suppression [4,5]. Cryptococcosis is acquired by inhalation of the fungus into the lungs. Pulmonary infection has the tendency to spontaneously resolve and is frequently asymptomatic [1]. While localized pulmonary infection may manifest as pneumonia [2], it may disseminate to other systems of the body, with a wide clinical spectrum, which may include cryptococcaemia [6], cutaneous and oral cryptococcosis [7], diarrhea [8], and most commonly, central nervous system (CNS) manifestations [1,3,4].

Globally, cryptococcosis remains a significant cause of mortality among HIV-infected persons, despite the advent of highly active antiretroviral therapy (HAART). In developed countries, the 10-week mortality in HIV patients remains high, ranging from 10%-25% [9,10], while in resource-poor countries such as Uganda, a 14-day mortality rate of 20%-42% has been reported among patients with cryptococcal meningitis, despite treatment with amphotericin B [11]. In Zambia, the 6-month mortality was 100%, with a

median survival of only 19 days, among AID patients without access to antiretroviral therapy, who were treated with fluconazole [12].

Serum cryptococcal antigen (CRAG) is a marker for invasive or disseminated cryptococcal infection and the most sensitive and specific indicator for systemic cryptococcosis [13]. A positive test therefore warrants a search for disseminated disease. In resource-poor settings, knowledge of the prevalence of cryptococcal antigenaemia, is an important criterion for introducing routine screening among HIV-infected population and in deciding pre-emptive treatment for disseminated cryptococcosis before commencing ART. Nigeria ranks among the top ten countries in sub-Saharan Africa with the highest prevalence of HIV [14]. The prevalence of serum cryptococcal antigen among ART-naive HIV-infected patients in Mid-Western Nigeria was reported as 12.7% [15]. Also, among HIV-infected pregnant women in Nigeria, Chukwuanuku et al. [16] reported a serum CRAG prevalence of 13.1%, while Gomerep et al. [17] reported a prevalence of 36% in a cohort of 100 HIV-infected patients, with a mean CD4 T-cell count of 87 ± 60 cells/ mm^3 , suspected of having meningitis or meningoencephalitis. The problem however, is that these data are disjointed and insufficient for an objective assessment of the burden of disseminated cryptococcosis among HIV patients in Nigeria. This study was undertaken to determine the prevalence of disseminated cryptococcosis among

symptomatic HIV-infected patients on ART, using serum CRAG as a marker; and to assess the effect of CD 4 T-cell count and viral load on prevalence.

2. MATERIALS AND METHODS

2.1 Study Design and Patients

This was a cross-sectional study carried out at the University of Benin Teaching Hospital (UBTH), a 700-bed tertiary hospital, located in southern part of Nigeria. UBTH has about 3000 registered HIV-infected patients and a dedicated laboratory for HIV investigations which is supported by PEPFAR (Presidential emergency plan for AIDS relief) of the United States.

Five hundred consecutive symptomatic HIV-1 -infected patients, on highly active antiretroviral therapy (HAART), who attended the ART clinic between September 2010 and August 2011 were enrolled into the study. Results of physical examination, medical history obtained from each patient and results of routine laboratory investigations (fasting blood sugar, renal function test, liver function test, full blood count) done for HIV-patients and contained in the patient's record, were used to select patients for the study. Inclusion criteria applied were; patients \geq 18 years with symptom of fever, cough ($>$ 3 weeks), chronic diarrhoea ($>$ 3 weeks), symptoms of the central nervous system (headache, meningism, encephalitis, altered sensorium), anaemia, night sweats and rash. Patients on systemic antifungal drugs or medical history of cryptococcal meningitis, and patients with immunosuppressive conditions (diabetes mellitus, chronic kidney disease, malignancy, immunosuppressive therapy and pregnancy) were excluded.

2.2 Ethical Consideration

Written informed consent was obtained from each study participant and ethical approval was obtained from the Ethics and Research Committee of the UBTH. All patients with positive serum CRAG test had their results transmitted to the clinician for possible antifungal treatment.

2.3 Data Collection

Each participant was interviewed and a comprehensive physical examination was carried out by the attending physician. Information sought included socio demographic data,

medical and treatment history. Data were entered into a structured data sheet.

2.4 Laboratory Assays

2.4.1 Serum cryptococcal antigen determination

Twelve milliliter of blood was collected from each participant and aliquot of 5ml was put into a sterile container without anticoagulant for serum cryptococcal antigen testing and another 5 ml into EDTA container for the determination of CD4+ cell count and viral load determination.

Serum cryptococcal antigen determination was carried out using the cryptococcal Latex agglutination test (Wampole laboratories Crypto-LA test New Jersey, USA) following manufacturer's instructions. In brief, the blood in the sterile container was allowed to clot at room temperature and centrifuged at 1000 \times g for 15 minutes to obtain serum. The serum was inactivated by heat in a water bath at 56 $^{\circ}$ C for 30 minutes. 50 μ l of inactivated serum was applied on the agglutination slide followed by addition of 50 μ l Reagent latex (anti-cryptococcal latex). This was mixed using a disposable stirrer. The slide was then rotated for 10 minutes at 160 rpm on a rotator. Control reactions (positive and negative controls) were similarly set up in parallel to each test using Control latex provided in the kit. The slides were observed for agglutination over a dark background and the result of reaction was interpreted as non- reactive (negative) when there was no agglutination, indicated by smooth milky reaction, and as reactive (positive), when there was large clumps or small but distinct clumps. Results for each test was validated using the corresponding controls.

2.4.2 CD4 T-cell count and viral load determination

The CD4 count was determined using flow cytometry (count bit Y-R 1004 Partec Muster Germany). Viral load was determined using Abbot RealTime HIV-1 assay machine (Abbot Molecular Inc Des Plaines, IL60018 USA).

2.5 Data Analysis

All data obtained from each patient, including results of laboratory tests were analyzed using the statistical package for social sciences (SPSS) version 22.0. Continuous variables were reported as means and standard deviations and

differences in mean were tested by student t-test. Categorical variables were described as proportions or percentages. Viral load, CD4 count were categorized, cross-tabulated with serum CRAG results and association was determined using Chi-square or Fisher's exact test where appropriate. All statistical test of significance were carried out at 95% level of confidence and a p value of <0.05 was considered significant.

3. RESULTS

All the 500 HIV-infected patients had been on ART for a mean duration of 28 months. Forty nine (9.8%) had positive serum CRAG. The age and sex distribution of the patients in relation to serum CRAG is shown in Table 1. The age range was 18-81 years, with a mean age of 40.08±11.8. Majority of the patients fell between 20-29 years of age. The difference in age prevalence was not statistically significant (P = .715). The ratio of male to female was 1.1:1 and the difference in sex prevalence was not statistically significant (P= .715).

About two-third (66.8%) of the patients had a CD4 count of less than 100 cells/µl and 45 (13.5%) of these had positive serum CRAG. Only 26 patients had a CD4+ T-cell count of above

200 cells/µl and none of them had a positive serum CRAG (Table 2). The difference in serum CRAG prevalence was statistically significant (P < 0.001).

Viral load was done for only 90 patients. This included all the 49 patients with positive serum CRAG and another 41 patients with negative serum CRAG taken consecutively. Of the 90 patients who had viral load done, forty-six (51.1%) had a high viral load, while 43(47.8%) had a moderate viral load. All patients with a high viral load had positive serum CRAG (Table 3). The association between viral load and positive serum CRAG was statistically significant (P < 0.001).

All the patients had at least one symptom, and fever was the commonest (Table 4). Headache, night sweat, cough, diarrhoea, and altered sensorium were found to have significant association with serum CRAG in univariate analysis. While fever, anaemia and skin rashes did not. Of all the variables that showed significant association with serum CRAG in univariate analysis, only headache, night sweat, and altered sensorium were found to independently predict serum CRAG in a multivariate analysis.

Table 1. Age and Sex Distribution of Patients in Relation to Serum CRAG N = 500

Characteristic	No (%)	Positive (%)	Negative (%)	P - value
Age group (years)				
10 – 19	2 (0.4)	0 (0.0)	2 (100)	0.715
20 - 29	89 (17.8)	12 (13.5)	77 (86.5)	
30-39	168 (33.6)	19 (11.3)	149 (88.9)	
40-49	118 (23.6)	10 (8.5)	108 (91.5)	
50-59	94 (18.8)	6 (6.4)	88 (93.6)	
60-69	26 (5.2)	2 (7.7)	24 (92.3)	
70-79	1 (0.2)	0 (0.0)	1 (100)	
> 80	2 (0.4)	0 (0.0)	2 (100)	
Sex				
Male	266 (53.2)	24 (4.8)	242 (48.4)	0.533
Female	234 (46.8)	25 (5)	209 (41.8)	

Table 2. Prevalence of serum CRAG in relation to CD4 count of patients N=500

CD 4 Count	Serum CRAG			P- value
	Positive (%)	Negative (%)	Total	
< 100	45 (13.5)	289 (86.5)	334	< .001
100-199	4 (2.9)	136 (97.1)	140	
≥ 200	0 (0.0)	26 (100)	26	

Table 3. Prevalence of serum CRAG in relation to viral load of patients N = 90

Viral load (HIV-RNA/ μ L)	Serum CRAG			P- value
	Positive (%)	Negative (%)	Total	
High (\geq 100,000)	46 (100)	0 (0.0)	46	< 0.001
Moderate (1000 - 99,000)	3 (7.5)	40 (92.5)	43	
Low (> 400 - 999)	0 (0.0)	1 (100)	1	
Undetectable (\leq 400)	0 (0.0)	0 (0.0%)	0	

Table 4. Distribution of symptoms among patients in relation to serum CRAG N=500

Symptom	Total	Serum CRAG		Univariate analysis			Multivariate analysis		
		Positive	Negative	OR	(95% CI)	P	OR	(95% CI)	P
Fever	361	49	312	0.09	(0.09-1.01)	0.995	-	-	-
Headache	132	44	88	1.03	(0.01-1.07)	<0.001	14.70	(2.18-18.70)	0.006
Night sweat	103	17	86	2.43	(1.20-4.58)	0.006	4.51	(1.46-20.05)	0.012
Altered Sensorium	334	45	289	1.02	(1.01-1.03)	<0.001	1.48	(0.96-2.99)	<0.001
Cough	239	15	224	2.24	(1.18-4.22)	<0.013	0.79	(0.73-10.67)	0.134
Diarrhoea	226	3	223	14.99	(4.59-48.92)	< 0.001	0.44	(0.09-2.25)	0.324
Anaemia*	200	24	176	0.90	(0.83-2.70)	0.17	-	-	-
Skin rashes	220	34	186	0.85	(0.82-2.68)	0.16	-	-	-

*Defined as PCV<25%

4. DISCUSSION

The prevalence of cryptococcal antigenaemia found in this study was high. This implies a high estimate for disseminated cryptococcosis [18] in the HIV-infected population. This finding is in line with studies done in Zaire (12.5%) [19] and Ethiopia (8.4%) [20] and further affirms the burden of cryptococcosis as an opportunistic infection in HIV-infected individuals in Sub-Saharan Africa. On the contrary, elsewhere in the under developed world, such as Vietnam and South Africa, lower prevalence of 2.0% and 5.0% have been reported respectively [21,22]. The disparity in reported prevalence could be due to the patient selection criteria in the different studies. For instance, in the Ethiopia study [20], the criteria for study entry was a CD4 count of \leq 200 cells/ μ L and all patients were on antiretroviral treatment (ART), while in a previous study done in Nigeria [15], which reported a prevalence of 21%, the study patients were ART naive and CD4 count level was not an inclusion criteria. In this study, patients were not ART naive and patients selection was not limited by their CD4 count level. Another possible explanation for these discrepancies is the differential risk of exposure to *Cryptococcus neoformans* among HIV-patients. As *Cryptococcus neoformans* is associated with soil contaminated by bird guano, environmental and occupational exposure may vary between the different populations [23].

Also observed in this study is the relatively low prevalence of antigenemia (2.9%) associated

with a CD4 range of 100-200 cell/ μ L. This is similar to previous studies done in South Africa [24] and Uganda [25]. This correlation is expected as a higher CD4 count implies greater protection against opportunistic infection in HIV-infected individuals. A sharp contrast to our finding is the study done in Ethiopia [20], which reported a comparatively high prevalence of cryptococcal antigenemia (7.1%) among patients with CD4 counts between 100–200 cells/ml. Although, the Ethiopia study, is similar to our study as it was not based on ART-naive patients, the study did not provide data on the viral load of the patients.

Data on viral load, in this study was limited to only 90 patients because viral load is not routinely done at UBTH where this study was carried out. This included all the 49 patients with positive serum CRAG and another 41 patients with negative serum CRAG taken consecutively. All the 46 patients with viral load \geq 100,000 had a positive serum CRAG test. The significant association between viral load and positive serum CRAG on one hand, and CD4+ T cell count on the other hand, as observed in this study, agrees with the report of Ekwaru et al. [26], that among HIV-patients on ART, opportunistic infection is associated with elevation of HIV RNA viral load to detectable level and decline in CD4 cell count.

Serum cryptococcal antigenaemia is a harbinger of cryptococcal meningitis, which is a leading cause of mortality in HIV infection [27]. It has

been reported that CRAG is detectable in serum for a median period of 3 weeks before the onset of symptoms of cryptococcal meningitis. Therefore, screening for serum CRAG and subsequent treatment of those with a positive test result has been recommended by WHO as a potential means of reducing cryptococcal meningitis-related mortality [9,28]. In 2011, the WHO further provided a conditional recommendation that in resource-poor setting, with high prevalence of cryptococcal antigenemia, routine screening of and treatment for cryptococcal antigenemia might be considered before ART initiation for ART-naïve adults with a CD4 T-cell count <100 cells/ μ L [28].

In this study, lumbar puncture (LP) was not done. This could be used to determine the proportion of patients with cryptococcal meningitis among those with positive cryptococcal antigen test. The decision to carry out LP was left to the discretion of the physician managing the patients. Nonetheless, our findings that headache and altered sensorium independently predicted positive serum CRAG in multivariate analysis suggest that central nervous system (CNS) manifestations, including meningitis are reliable predictors of disseminated cryptococcosis. This is in consonance with the findings in Cambodia by Micol et al. [29] and in Tanzania by Kisenge et al. [30].

Cough and night sweat were also found to be significantly associated with positive serum CRAG in this study. Although, sputum analysis for *Cryptococcus neoformans* and Acid Fast Bacilli was not included in our study, these symptoms may indicate pulmonary cryptococcosis or tuberculosis, since both diseases are recognized opportunistic infections in HIV-infected individuals. Symptoms of disseminated cryptococcosis in the background of HIV infection are difficult to interpret, especially in the absence of specific microbiological evidence. Therefore a definitive diagnosis of disseminated cryptococcosis in HIV - infected patients would warrant further evaluation of clinically relevant specimens such as blood, cerebrospinal fluid and sputum for the presence of *Cryptococcus neoformans*, or cryptococcal DNA, using culture and polymerase chain reaction (PCR) techniques. These technologies are not cost-effective for screening purposes and are not widely available in resource -poor settings, thus the CRAG screening tests (latex agglutination test and Lateral flow assay) which are sensitive, readily

available, affordable, and easy to perform have been recommended [22,25,28,]. In this study, previous history of cryptococcal meningitis was an exclusion criteria; therefore, a positive serum CRAG in a symptomatic HIV-positive patient on ART, may imply a primary cryptococcal meningitis in the background of immune suppression or Immune reconstitution inflammatory syndrome (IRIS) resulting from the initiation of ART, in the background of disseminated cryptococcosis. The former scenario would benefit from the management of the meningitis while the latter would benefit from both meningitis treatment and IRIS treatment.

This study is to our knowledge, the first in Nigeria to provide data on disseminated cryptococcosis among HIV-infected patient on ART. However, there are some limitations. First, being a hospital based study, data obtained from the study should be carefully interpreted as only a select group (symptomatic patients) were evaluated. Some recent observational studies have revealed that 6- 15% individuals entering HIV out-patient care with a CD4+ cell count of \leq 100 cell/ μ l have asymptomatic cryptococcal infection [20,25, 26,31]. Therefore our data might not be representative of the Nigerian HIV-infected population. Second, false-positive serum CRAG results due to cross-reactivity of the cryptococcal antigen with the fungus *Trichosporon beigeli* and DF-2 bacillus have been reported [32,33]. Due to lack of facilities, they were not excluded in this study.

The implementation of CRAG screening programs has begun in several countries in sub-sahara Africa, including South Africa, Rwanda, and Mozambique. Nigeria is among those countries that have not started. Some hindrances to implementation include lack of data on the prevalence of disseminated cryptococcosis among the HIV-infected population and non availability of diagnostic tools. With the recent evaluation of a point- of- care cryptococcal antigen test [34]. which can use serum, plasma or urine, cryptococcal antigen test has become a well-established, rapid and available tool of diagnosing disseminated cryptococcosis.

5. CONCLUSION

The high prevalence of serum CRAG among symptomatic HIV-infected patients as found in this study, implies that disseminated cryptococcosis may be common despite ART implementation among the study population.

There is need therefore to introduce cryptococcal antigen test for all symptomatic HIV-infected patients on ART; while further microbiological investigations for those with positive result are recommended for appropriate medical intervention.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. John EB. Cryptococcosis. In: Kasper DL, Fauci AS, Longo DL, Braunwald E, Hauser SL, Jameson JL, editors. Harrison's principles of internal medicine. 16th edition. New York: McGraw-Hill. 2005;1183-1184.
2. Hung MS, Tsai YH, Lee CH, Yang CT. Pulmonary cryptococcosis: Clinical, radiographical and serological markers of dissemination. *Respirology*. 2008;13(2): 247-251.
3. Perfect JR, Casadevall A. Cryptococcosis. *Infect Dis Clin North Am*. 2002;16:837-874.
4. Capoor MR, Nair D, Deb M, Gupta B, Aggarwal P. Clinical and mycological profile of cryptococcosis in a tertiary care hospital. *Indian Journal of Medical Microbiology*. 2007; 25(4):401-404.
5. Castelnovo B, Manabe YC, Kiragga A. Cause-specific mortality and the contribution of immune reconstitution inflammatory syndrome in the first 3 years after antiretroviral therapy initiation in an urban African cohort. *Clin Infect Dis*. 2009;49(6):965-97.
6. Jean SS, Fang CT, Shau WY, et al. cryptococcaemia clinical feature, and pragmatic factors. *Q J Med*. 2002;95:511-518.
7. Neville S, Dromer F, Morin O, Dupond B, Romin O, Lorthotary O. French Cryptococcosis study group. Primary cutaneous cryptococcus: A distinct clinical entity. *Clin inf Dis*. 2003;36:211-212.
8. Sungkanuparpho S, Tanphaichitra D, Prachaktam R. Chronic diarrhea caused by Cryptococcus neoformans in a non-human immunodeficiency virus-infected patient. *Scan J Infect Dis*. 2003;35:211-215.
9. Jarvis JN, Harrison TS. HIV-associated cryptococcal meningitis. *AIDS*. 2007;21:2119 -2129.
10. Lotholary O, Poizat G, Zeller V, Neuville S, Boibieux A, Alvarez M, et al. Long-term outcome of AIDS-associated cryptococcosis in the era of combination antiretroviral therapy. *AIDS*. 2006;20:2183-2191.
11. Kambugu A, Meya DB, Rhein J, O'Brien M, Janoff EN, Ronald AR, Kanya MR, Mayanja-Kizza H, Sande MA, Bohjanen DR: Outcomes of cryptococcal meningitis in Uganda before and after the availability of highly active antiretroviral therapy. *Clin Infect Dis*. 2008;46(11):1694-1701.
12. Mwaba P, Mwansa J, Chintu C, Pobe J, Scarborough M, Portsmouth S, et al. Clinical presentation, natural history, and cumulative death rates of 230 adults with primary cryptococcal meningitis in Zambian AIDS patients treated under local conditions. *Postgrad Med J*. 2001;77:769-773.
13. Coovadia YM, Solwa Z. Sensitivity and specificity of a Latex agglutination for detection of cryptococcal antigen in meningitis. *S Afr Med J*. 1987;71:510-512.
14. WHO. Global Health Observatory Data Repository. Data on the size of the HIV/AIDS epidemics: Number of people living with HIV; 2011. Accessed May 28, 2014. Available: who.int/gho/data/node.main
15. Osazuwa F, Dirisu JO, Okuonghae PE, Ugbebor O. Screening for cryptococcal antigenemia in anti-retroviral naive AIDS patients in Benin City, Nigeria. *Oman Med J*. 2012;27:228-31.
16. Chukwuanuku R, Manafa P, Iloghalu E, Onyenekwe C, Ifeanyichukwu M, Mbamalu C. *Cryptococcal neoformans* antigenaemia in HIV- positive pregnant women attending a PMTCT clinic in South-East Nigeria. *Journal of Biology, Agriculture and Healthcare*. 2013;3(18). ISBN 2225-093X.
17. Gomerep SS, Idoko JA, Lade NG, Ugoya SO, Obaseki D, Agbaji OA, et al. Frequency of cryptococcal meningitis in HIV-1 infected patients in North- Central Nigeria. *Nigr J Med*. 2010;19(4):395-399.
18. Pongsai P, Atamasirikul K, Sumgkanuparph S. The role of serum cryptococcal antigen screening for the early diagnosis of cryptococcosis in HIV infected patients with different ranges of CD4cell counts. *J Infect*; 2010.
19. Desmet P, Kayembe KD, Devroey C, Institute of Tropical Medicine, Antwerp, Belgium. The value of cryptococcal serum

- antigen screening among HIV-positive/AIDS patients in Kinshasa, Zaire. AIDS. 1989;3(2):77-78
20. Alemu AS, Kempker RR, Tenna A, Smitson C, et al. High Prevalence of cryptococcal antigenemia among HIV-infected patients receiving antiretroviral therapy in Ethiopia. PLoS ONE. 2013;8(3):e58377
DOI:10.1371/journal.pone.0058377.
21. McKenney J, Smith RM, Chiller TM, et al. Prevalence and correlates of cryptococcal antigen positivity among AIDS patients-United States 1986-2012. Weekly. 2014;63(27):585-587.
22. Smith RM, Nguyen TA, Ha HT, et al. Prevalence of cryptococcal antigenemia and cost-effectiveness of a cryptococcal antigen screening program—Vietnam. PLoS ONE. 2013;8:e62213.
23. Chowdhary A, Rhandhawa HS, Prakash A, Meis JF. Environmental prevalence of *Cryptococcus neoformans* and *Cryptococcus gattii* in India: An update. Crit Rev Microbiol. 2012;38:1–16.
24. Jarvis JN, Lawn SD, Vogt M, Bangani N, Wood R. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. Clin Infect Dis. 2009;48:856–862.
25. Meya DB, Manabe YC, Castelnovo B, Cook BA, Elbireer AM, et al. Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count , or = 100 cells/microL who start HIV therapy in resource-limited settings. Clin Infect Dis. 2010;51:448–455.
26. Ekwaru JP, Campbell J, Malamba S, et al. The effect of opportunistic illness on HIV RNA viral load and CD4+ T cell count among HIV-positive adult taking antiretroviral therapy. Journal of the International AIDS Society. 2013;16:17355.
27. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS. 2009;23:525–30.
28. WHO. Rapid Advice: Diagnosis, Prevention and Management of Cryptococcal Disease in HIV-infected Adults, Adolescents and Children. Geneva, World Health Organization; 2011.
29. Micol R, Lortholary O, Sar B, Laureillard D, Ngeth C, Dousset JP, et al. Prevalence, determinants of positivity, and clinical utility of cryptococcal antigenemia in Cambodian HIV-infected patients. J. Acquir Immune Defic Syndrome. 2007;45(5):555-559.
30. Kisenge PR, Hawkins AT, Maro VP, Mchele JP, Swai NS, Mueller A, et al. Low CD4 count plus coma predicts cryptococcal meningitis in Tanzania. BMC Infect Dis. 2007;7:39.
31. Liechty CA, Solberg P, Were W, Ekwaru JP, Ransom RL, Weidle PJ, et al. Asymptomatic serum cryptococcal antigenemia and early mortality during antiretroviral therapy in rural Uganda. Trop Med Int Health. 2007;12(8):929–35.
32. McManus EJ, Jones JM. Detection of a *Trichosporon beigelii* antigen cross-reactive with *Cryptococcal neoformans* capsular polysaccharide in serum from a patient with disseminated trichosporon infection. J Clin Microbiol. 1985;21:681-685.
33. Westerink MAJ, Amsterdam D, Petell RJ, et al. Septicaemia due to DF-2: cause of a false-positive Cryptococcal latex agglutination result. Am J Med. 1987; 83:155-158.
34. Jarvis J, Percival A, Bauman S, Pelfrey J, Meintjes G, Williams G, et al. Evaluation of a novel point-of-care Cryptococcal Antigen Test on Serum, Plasma, and Urine from Patients with HIV-Associated Cryptococcal Meningitis. Clin Infect Dis. 2011; 53(10):1019–23.

© 2015 Joseph et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sciencedomain.org/review-history.php?iid=906&id=12&aid=7636>