Medical Science and Discovery
ISSN: 2148-6832

Cryptococcal antigenaemia amongst HIV infected children in Owerri, South East Nigeria

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ABSTRACT

Objective: Cryptococcosis is an opportunistic infection caused by Cryptococcus and can result in cryptococcal meningitis, which is an AIDS-defining condition associated with high morbidity and mortality. Although a high prevalence of Cryptococcal antigenemia (CrAg) preceding cryptococcal disease has been reported in HIV-infected Nigerian adults, it is more common in patients with low CD4, stage 3 or 4 HIV. However, there is only one study on Cryptococcal antigenemia in HIV-infected Nigerian children. Therefore, this study aimed to determine the burden of cryptococcosis in HIV-infected children.

Material and Methods: This study was a hospital-based cross-sectional study conducted between October 2018 and January 2019 at the Pediatric Infectious Disease Clinic of the Federal Medical Centre, Owerri. The study population consisted of HIV-infected children (aged 2-16 years) attending the clinic. The subjects were recruited consecutively and underwent a thorough physical examination and anthropometric measurements before a single blood sample was taken. Cryptococcal antigen was assayed using the Lateral Flow Assay method, and CD4+ counts were assayed using cyflow.

Results: A total of 100 children were involved in the study, of which 51 (51%) were female and 49 (49%) were male (M:F, 1:1). The average age of the participants was 9.73 ± 3.12 years. Among the 100 children tested, there was no cryptococcal antigenemia (0%). Statistical analysis was limited to simple description.

Conclusion: Cryptococcosis is not a common opportunistic infection among HIV-infected children attending the Pediatric Infectious Disease Clinic in Federal Medical Centre Owerri. Therefore, it may not be considered as a differential diagnosis for HIV-positive children with meningoencephalitis.

Keywords: Cryptococcosis; HIV; Children; Immunesuppression

Research Article

Received 15-03-2023

Accepted 04-04-2023

Available Online: 10-04-2023

Published 30-04-2023

INTRODUCTION

The Human Immunodeficiency Virus (HIV) and acquired immunodeficiency syndrome (AIDS) epidemic has become one of the greatest threats to human health and development, particularly in less developed countries (1). It accounts for 36% rise in under-five mortality in Africa (2). A lot of these mortality(s) results from HIV-related opportunistic infections. The basic effect of HIV on the immune system is depletion and destruction of Cluster of Differentiation 4 (CD4) positive cells. This together with other immunologic defects, lead to immunosuppression which sets the stage for opportunistic infections (3).

Cryptococcal disease is one of the most important opportunistic infections, and a major contributor to this mortality (4, 5). It is a rare disease in healthy individuals, but it is a common fungal infection affecting immunocompromised individuals such as people with AIDS, and those on chemotherapy and steroids.

Global estimates suggest a 6% prevalence of cryptococcal antigenemia in HIV infected patients (6). A South African survey reported an incidence of 47 cases per 100,000 HIV-infected children (7).

The true burden of cryptococcosis among HIV-infected children in Nigeria is difficult to describe. Anigilaje et al, 9) in Markudi North Central Nigeria, recorded zero prevalence of CrAg among 88 HIV infected children. However, in a HIV-infected child presenting with fever, headache, and neck stiffness, with positive Kerning and Bruzinski signs, cryptococcal meningitis, a major sequel of opportunistic infection in a HIV infected child needs to be considered (7). Therefore CrAg needs to be screened for in this type of patients. CrAg is detectable at a median of 22 days before the onset of symptoms (9) and has been shown to be 100% sensitive in predicting the development of Cryptococcal Meningitis in the first year of antiretroviral therapy (10). Routine serum or plasma CrAg screening in ART naive adults, followed by pre-emptive antifungal therapy if CRAG is positive to reduce the development of Cryptococcal disease was advocated by WHO in 2011 (11) Early diagnosis and prompt treatment is key to reducing mortality due to Cryptococcal disease (12, 13).

The authors searched several databases using different search engines, but only one study on Cryptococcal antigenemia in HIV-infected Nigerian children was found.. However, non was seen for studies in South Eastern Nigeria. Furthermore, the scarcity of facilities for fungal culture makes it important to pay attention to screening HIV patients, especially those with signs and symptoms of meningitis. This may help to reduce the loss of HIV patients. Therefore, the authors aimed to determine the prevalence of cryptococcal antigenemia in HIV-infected children attending the Pediatric Infectious Disease Clinic at Federal Medical Centre Owerri, in southeastern Nigeria.

MATERIAL and METHODs

Study design and setting: this cross-sectional study was carried out amongst HIV-infected children at the Paediatric Infectious disease care/treatment clinic of the Federal Medical Centre, (FMC) Owerri Imo State between October 2018 and January 2019. FMC Owerri is a tertiary health care facility serving majorly as a referral centre for health facilities in Imo State and an alternative facility for the contiguous states of Abia, Rivers and Anambra.

Inclusion and exclusion criteria: All HIV positive clinic attendees (HAART naive or have been on HAART for one year or less), 2-16years were enrolled consecutively until the study sample size was attained. Those children who less than 2 or more than 16 years, those unwilling to participate or whose caregivers declined consent were excluded others who were on fluconazole at the time of the study were also excluded and allowed to access care without hindrance.

Sampling procedure: for estimation of the sample size, we used the formula for prevalence studies (14).

$n = Z^2pq/d^2$

Where

n = desired sample size.

Z = standard deviation set of 1.96 which corresponds to 95% confidence interval.

P = population in the target population estimated to have co-infection with HIV and Cryptococcus (13%) (15).

q= 1–P

d= degree of accuracy desired shall be taken to be 5% or 0.05

$= [(1.96)2 \text{ X } 0.13 \text{ X } 0.5]/(0.05)^2$

= [3.84 X 0.13 X 0.5]/0.0025= 99

The calculated sample size is 99 for population greater than 10,000

For Study which population is less than 10,000;

nf = the desired sample size for the study if population is less than 10,000

nf = n/1 + (n/N)

N = Estimate of population size of HIV positive children registered in FMC Owerri = 273

nf= 72

The attrition rate of 10% was factored into the sample size calculation. It was calculated using the formula (16).

n = N/(1 – F)

=72(1-0.1)= 80

n = Adjusted sample size

N = Calculated sample size

F = Attrition rate

However, 100 children were recruited for the study. 20 subjects were added for nonresponse. [prevalence of 13% is in adults because local studies have a prevalence rate of 0% in children]. Eligible subjects at FMC Owerri paediatrics infectious disease clinic were conveniently sampled.

Data collection: A structured interviewer administered questionnaire was developed and administered to the parents or guardians by the lead author; to capture the child's socio-demographic data (age, parents educational level, occupation, place of residence and others) Risk factors for cryptococcal infestation, steroid use, antifungal use, History of HAART use, whether or not parents are involved in farming activities (birds rearing {including pigeon}/tendering of fruits and vegetables),history of ownership of farm animals, past history of cryptococcosis, current or past history of other opportunistic infections and symptoms and signs suggestive of meningitis were some of the pertinent information obtained. WHO Clinical Stage was also determined. About 4 millilitre (ml) of venous blood was collected from each subject; 3 ml was put in ethyl diamine tetra acetic (EDTA) bottle for CD4 count and 1 ml into plain bottle container for CRAG LFA. All the samples were analyzed at the immunology laboratory of Federal Medical Centre Owerri. The cryptococcal antigen Lateral Flow Assay (Cr Ag-LFA) method was used. Instructions as stipulated by the manufacturer regarding the storage, handling, validity and procedure of the test were observed (17). A drop of specimen diluents was added to a test tube; 40µl of patient's specimen (whole blood) was also added to the tube. The CrAg LFA strip was then inserted into the tube. This was incubated for 10 minutes after which results were interpreted (18). A positive test result will create two lines [test and control] while a negative test result created one line [control].

http://dx.doi.org/10.36472/msd.v10i4.916

If control line fails to develop, the test is invalid. When compared to the gold standard, diagnosis of cryptococcosis (culture and/or Indian ink), the test has a sensitivity and specificity of 99%, 83% for plasma antigen.

Data analysis: The data collected was coded and imputed into Statistical Package for Social Sciences (SPSS) version 20.0. Frequency tables and figures were used to present relevant variables. Characteristics were summarized using median and standard deviation for quantitative variables (age and social class) and proportions for qualitative variables. Bivariate logistic regression analysis was not performed to assess risk factors since cryptococcal antigenemia was not detected..

Ethical Approval: Ethical approval for the study was sought and obtained from the Ethics Committee of Federal Medical Centre Owerri (FMC/OW/HERC/182. Informed consent was obtained from the parents or caregivers, and assent was obtained from children aged 7 years and older. To ensure anonymity of the subjects, data were coded.

RESULTs

Sociodemographic data: 100 patients that satisfied the inclusion criteria were recruited for the study between October 2018 and January 2019. The socio-demographic information (Table 1) shows that of the total 100 subjects, 49 were Males and 51 were Females (M: F, 0.9:1). The average age of participants was 9.73 ± 3.12 years. 65 (65%) are rural dwellers. The majority of the subjects (59%) were in WHO Stage 1, followed by Stage 3 (24%). More than half of the subjects 57(57%) had CD4 count above 500cells/mm3 and 17 (17%) had CD4 count less than 200cells/mm3 . The proportions of the CD4 counts are as shown in Table 1.

Table 1: Demographic and immunological characteristics of subjects.

Characteristics	**Frequency (%)**
Gender	
Female	51(51%)
Male	49(49%)
Social class	
Upper	20(20%)
Middle	44(44%)
Lower	36(36%)
Place of residence	
Rural	65(65%)
Urban	35(35%)
WHO staging	
1	59(59%)
2	16(16%)
3	24(24%)
4	1(1%)
CD4 count	
<200	17(17%)
200-349	13(13%)
350-499	13(13%)
>500	57(57%)

Crag Test: None of the participants tested positive for Cryptococcal antigen giving a zero prevalence among subjects as displayed in table 2.

Table 2: prevalence of cryptococcal antigenemia among study participants

CRAG Serology	Frequency	Percentage (%)
Positive	0	0
Negative	100	100.0
Total	100	100.0

Associated risk factors for cryptococcal antigenemia: a total of 45 participants had exposure to farming. However, it was not possible to determine the relationship between farming, a potential risk factor for cryptococcal antigenemia, due to the absence of any cases detected in the study population.. 33 participants owned pets, and 93(93%) were HAART experienced. The association could also not be determined because of cryptococcal antigenemia zero prevalence. This is shown in **Table 3**.

Table 3: Association between Risk factors and Cryptococcal Antigenaemia

Risk factors	N (%)
Farming	
Yes	45(45%)
No	55(55%)
History of ownership of domestic animal (pet)	
Yes	33(33%)
No	67(67%)
HAART Experience	
Yes	93(93%)
No	7(7%)

DISCUSSION

The prevalence of cryptococcal antigenaemia among HIV-infected children aged 2-16 years attending the Paediatrics Infectious disease clinic of the Federal Medical Centre Owerri, South Eastern Nigeria was 0%. Our study that determined the prevalence of cryptococcal antigenaemia among South Eastern Nigerian children with HIV-infection, happens to be only one and second in Nigeria. Our 0% prevalence tend to support the WHO recommendation to exclude children from Crag screening routinely. Anigilaje et al's study (8) in Markurdi, North Central Nigeria had zero-prevalence, Meiring et al in South Africa reported a prevalence of 0.00047% (17) among HIV-positive children; 2.97% reported by Likasitwattanakul et al (19) in Thailand over an eight-year period among hospitalized HIV-infected patients, 0.8% prevalence by Gonzalez et al (20) and 0.066% by Sara et al (21) both in USA; all the studies cited above tend to epidemiologically show that cryptococcosis is uncommon in HIV-infected children.

In this study, the prevalence of CrAg was zero despite the fact that a substantial number of subjects were exposed to potential sources of Cryptococcus. The fact that a considerable number of subjects had CD4 count >200cells/ml could have accounted for the observation. However, Anigilaje et al had similar findings in severely immunocompromised subjects. Speed et al (22) demonstrated that the lower exposure of children to cryptococcus maybe responsible for the findings in our study and Anigilaje`s. Abadi et al (23)

additionally posited that in addition to lower exposure in children, high CD4+ counts also contributed to the low prevalence.

Though a large number of our children have been on HAART, it may not explain the 0% Cr Ag prevalence. as studies in adult population have reported a high prevalence of Cr Ag even among patients on ART (24). The 0% prevalence of Cr Ag in our study would appear to suggest that the risk for Cryptococcal meningitis amongst HIV infected patients 2-16years of age is quite low.

Limitations:

This study is a single center, hospital-based study with small sample size. A multicenter, multi-regional study may need to be done to be able to generalize on our findings.

CONCLUSION

Cryptococcosis is not a common opportunistic infection in HIV infected children seen in Federal Medical Center Owerri Imo State. Despite potential exposure to infective agents, the prevalence of cryptococcal antigenemia is low among children attending the Paediatric Infectious Disease Clinic at the Federal Medical Centre Owerri. Cryptococcal meningitis has a high mortality, however, with the zero-incidence recorded in this study it may not be plausible to embark on screening for Cr Ag routinely in our setting.

What is known about this topic?

There is a need for screening for CrAg among HIV positive children.

Despite the need, data on the prevalence in South Eastern Nigeria is scarce.

What this study adds:

The observed prevalence of cryptococcal antigenemia among children with HIV attending the Paediatric Infectious Disease Clinic in Federal Medical Centre Owerri, Imo State was 0%. There may be no need to advocate for regular cryptococcal antigen screening.

Acknowledgments: The authors appreciate the staff of the Paediatric infectious disease clinic of FMCO for their support in carrying out this work. The authors are also grateful to Dr Emerenini F for the statistical analysis.

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and a specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions: Charles Emeka Nwolisa, Joseph Ezeogu, and Vivian Chinelo Okeke conceived, designed the study and wrote the manuscript. **Vivian Chinelo Okeke** did the data collection and contributed in checking the data. **Joseph Ezeogu, Charles Emeka Nwolisa and Ifeoma Egbuonu** participated in interpretation and manuscript writing. All the authors have read and agreed to the final manuscript.

Ethical approval: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and/or with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for being included in the study. Written consent was obtained from each patient to use their hospital data.

REFERENCES

1. AVERT: Global HIV and AIDS epidermic. 2012. Available from www.avert.org/global-hiv-aids-epidermic.htm. Accessed 12 October 2019.
2. Tindyebwa D, Kayita J, Musoke P, Eley B, Nduati R, Coovadia H et al (Eds): Introduction Handbook on Paediatrics AIDS in Africa. African network for the care of children affected by AIDS. 2006.
3. Tindyebwa D, Kayita J, Musoke P, Eley B, Nduati R, Tumwesigye N et al, editors. HIV virology, pathogenesis and natural history. In; Handbook on paediatrics AIDS in Africa.
4. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, et al. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS 23: 525-530. 2009
5. World Health Organization Rapid Advice. Diagnosis, Prevention and Management of Cryptococcal Disease in HIV-infected Adults, Adolescents and Children. 2011
6. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM et al. Global burden of disease of HIV associated cryptococcal meningitis. An updated analysis. The Lancet 2017;17:873-81.
7. Center for disease control and prevention. Fungal disease. CDC [online] 2019 available from https//www.cdc.gov/fungal/disease/cryptococcosis-neoformas/definition.html. Accessed 12 June 2019.
8. Anigilaje EA, Olutola A, Dabit O, Adeoti AO, Emebolu AJ, Abah J. There is no cryptococcal antigenemia among a cohort of children with advanced HIV infection in an antiretroviral therapy programme in Markurdi Nigeria. J AIDS Clin Res 2013; 4:12.
9. French N, Gray K, Watera C, Nakiyingi J, Lugada E, et al. (2002) Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. AIDS 16: 1031-1038.
10. Jarvis JN, Lawn SD, Vogt M, Bangani N, Wood R, et al. (2009) Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. Clin Infect Dis 48: 856-862.
11. World Health Organisation. Rapid Advice: Diagnosis, prevention and management of Cryptococcal disease in HIV – infected Adults, adolescents and children. Geneva: 2011.HIV/AIDS [online] Available from https//www.who.int/hiv/pub/cryptococcal_disease2011/en/. Accessed 12 October 2019.
12. Jarvis JN, Lawn SD, Vogt M, Bangari N, Wood R, Harrison TS. Screening for Cryptococcal antigenemia inpatients accessing an antiretroviral treatment programe in South Africa. Clin infect Dis 2009; 48:856 – 62
13. Liechty CA, Solberg P, Were W, Ekwarm JP, Ransom RL, Weidle PJ et al. Asymptomatic serum Cryptococcal antigenemia and early mortality during antiretroviral therapy in rural Uganda. Trop Med Int. Health2007; 12:929 – 35
14. Araoye MO, Sample size. In: Araoye MO, Editor. Research methodology with statistics for health and social sciences. 2nd Ed. Ilorin: Nathadex: 2004, 115 –129.
15. Chukwuanukwu IR, Manafa PI,Iloghalu E, Onyenekwe C, Ifeanyichukwu M , Mbamelu C. Cryptococcus neofromas antigenemia in HIV patients pregnant women attending PMTCT clinic in South east Nigeria. J of biol, Agric and health care. [online] 2013. Available from https://www.researchgate.net/publication/281556498 (Accessed 2019 June).

16. Bamgboye AE. Subject selection in: Bamgboye AE editor. A comparison of medical statistics. Ibadan: Ibi press and publishing Co; 2000. P. 160 -164.

17. Meiring ST, Quan VC, Cohen C, Dawood H, Karstaedt AS, et al. (2012) A comparison of cases of paediatric-onset and adult-onset cryptococcosis detected through population-based surveillance, 2005-2007. AIDS 26: 2307-2314.

18. CDC. Cryptococcal screening programe training modules for laboratories.2013. [online] Available on-line https // www.cdc.gov> fungal> pdf>cryptococcal screening. Accessed 18 July 2018.

19. Likasitwattanakul S, Poneprasert B and Sirisanthana V. Cryptococcosis in HIV – infected children, South East Asia.J Trop med public health2004;35: 935 – 9.

20. Gonalez CE, Shetty D, Lewis LL, Mueller BU, Pizzo PA, Walsh TJ. Cryptococcosis in human immunodeficiency virus infected children. Pediatric Infect Dis J 1996; 15:796-800.

21. Sara AM, Maureen P, David R, Edward G, Richard H, Mary E et al. The changing epidemiology of Cryptococcus; An update from population based active surveillance in 2 large metropolitan Areas 1992-2000.Clin Infect Dis 2013;36: 789-94.

22. Speed B, Dunt D. Clinical and host differences between infections with the two varieties of Cryptococcus neoformas. Clin infect Dis 1995; 21:28 – 36.

23. Abadi J, Nachwam S, Kressel AB, Pirofski L. Cryptococcosis in children with AIDS. Clin infect Dis 1999; 28:309-13

24. Jarvis JN, Boulle A, Loyse A, Bicanic T, Rebe K, et al. (2009) High ongoing burden of cryptococcal disease in Africa despite antiretroviral roll out. AIDS 23:1182-1183.