ISSN: 2581 – 3633
PubMed - National Library of Medicine - ID: 101745081

International Journal of Medical Science and Advanced Clinical Research (IJMACR)

Available Online at:www.ijmacr.com

Volume – 6, Issue – 1, February - 2023, Page No. : 321 - 329

Prevalence of Cryptococcal Anti-genaemia among Newly Enrolled HIV Patients from Two Health Facilities in Rivers State

[1]Erasmus, M. A., Medical Laboratory Department, Rivers State University Teaching Hospital, Port Harcourt, Rivers State, Nigeria.

[2]Lawson, S,Pathology Department, Rivers State University Teaching Hospital, Port Harcourt, Nigeria.

[3]Nwalozie, R.M.,Medical Laboratory Department, Rivers State University, Port Harcourt, Nigeria.

[2]Amadi-wali O., Pathology Department, Rivers State University Teaching Hospital, Port Harcourt, Nigeria.

Corresponding Author: Erasmus, M. A., Medical Laboratory Department, Rivers State University Teaching Hospital, Port Harcourt, Rivers State, Nigeria.

How to citation this article: Erasmus, M. A., Lawson, S,Nwalozie, R.M.,Amadi-wali O,"Prevalence of Cryptococcal Anti-genaemia among Newly Enrolled HIV Patients from Two Health Facilities in Rivers State", IJMACR- February - 2023, Volume – 6, Issue - 1, P. No. 321 – 329.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

This study was carried out as part of the health management packages for the newly enrolled HIV patients. HIV infection weakens the immune systems of those infected and exposes them to opportunistic infections which could be viral, bacterial, fungal or protozoan especially among those with CD4 cells below 200cells/ul. A total of one thousand, one hundred (1100) patients were screened using Visitect® CD4 Advanced Disease Rapid Test kit which is a semi-quantitative method of determining the CD4 values, out of which six hundred (600) patients with CD4 < 200 cells/ul were recruited from Bori Zonal Hospital (BZH) and Rivers State University Teaching Hospital (RSUTH), all n Rivers State. Two milliliters (2mls) of blood wereaseptically collected from each patient into a plain bottle, centrifuged at 1500 rev/min for 15minutes and further screened for Crypyococcal antigen (CrAg) using the CrAg lateral flow assay test kit. The overall prevalence of Cryptococcal anti-genaemia was 7 (1.2%). The prevalence of Cryptococcal anti-genaemia was significantly higher in RSUTH (2.3%) than in BZH (0.0%) ($p<0.05$), with males having higher prevalence (1.5%) than the females (0.9%). The age-related prevalence showed the highest prevalence of (42.9%) among the age bracket of (31-40) and years, with the lowest prevalence of (14.3%) among the age brackets of (21-30) and (51-60) years. Cryptococcal antigen is usually released by Cryptococcus neoformansand if this infection is not handled properly and on time too, the

infected patient can come down with Cryptococcal meningitis. This study aims to determine the prevalence of cryptococcal anti-genaemia among ART-naïve patients.

Keywords: Cryptococcal anti-genaemia, Visitect, Advanced HIV Disease, Nigeria.

Introduction

The United Nations Programme on AIDS (UNAIDS) in 2019 reported that about 37.9 million deaths resulting from AIDS-related morbidity within the same year globally. Cryptococcosis is a common cause of morbidity and mortality among HIV-infected populations, even in the era of Highly Active Anti-Retroviral Therapy (HAART) [1].Cryptococcosis an opportunistic infection caused majorly bya capsulated yeast,Cryptococcus species found in the environment and is mostly associated with pigeon droppings, soil and avian excreta globally. Cryptococcus neoformansis the majorcauses of infection among the immunocompromised, though C. gattimay rarely cause infection [1]. In HIV/AIDS, cryptococcus is commonly affects the central nervous system (CNS) and the lungs sometimes, the skin and other organs of the body could be affected upon inhalation of the spores [2]. The commonest cryptococcal disease, cryptococcal meningitis is associated with an estimated 15-20% of AIDS- related deaths globally. Recently, an updated analysis of cryptococcal infection revealed an incidence of 223,100 global cases with about 73% occurring in sub-Saharan Africa. Out of the 181,100 deaths associated with cryptococcal disease, 75% is said to also occur in sub-Saharan Africa.Nigeria has been estimated to have 25,000 annual cases of cryptococcal anti-genaemia[3]. In the absence of intervention, mortality from Cryptococcal meningitis is 100%. In settings where fluconazole monotherapy is the routine care, estimated 3-month mortality is 70%, Cryptococcus is a fungus found in soil throughout the world. Sporesare usually inhaled from the environment by humans and there is no person-to-person transmission of Cryptococcus.After inhalation, the fungus can cause an acute lung infection, or often no symptoms at all, and stay dormant in the body for months to years although, reactivation of disease can occur in immune-suppressed people like; people living with HIV(PLHIV) [4].

Not all cases of Cryptococcal meningitis (CCM) are reactivations, some may be new CCM infections, adult HIV/AIDS patients with a CD4 count < 100 are at highest risk for reactivation. When Cryptococcus reactivates in the body, it can cause disease in the brain, lungs, skin, and bones. Cryptococcal meningitis is a common cause of death among PLHIV**,** even when patients are treated with antiretroviral medications and anti-fungal therapy, 30 to 70 per cent die from their cryptococcal disease. Cryptococcal disease should be suspected in all advanced HIV disease (AHD) patients who complain of headache.

Studies have shown that screening of patients at the time of entry into ART programmes using point-of-care tools like Visitect for CD4 cells and cryptococcal antigen Lateral Flow Assay (CrAg LFA)is very effective in early identification of patients at risk of having cryptococcal meningitis.

Diagnosis of cryptococcal meningitis requires the implementation of the cerebrospinal fluid (CSF) culture of Cryptococcus species, microscopic examination with Indian ink staining, or CrAg testing in the CSF or serum[5]. Serum cryptococcal antigen (CrAg) is recognized as a marker for an invasive or disseminated cryptococcal infection, as well as the most sensitive and

specific indicator for systemic cryptococcus is[1]. It is known that CrAg can be detected in blood some weeks or months prior to the development of overt clinical symptoms with sensitivity and specificity close to 100% [3]. Therefore, the screening of CrAg in serum provides an opportunity to identify people with an asymptomatic disease at an early stage.

Materials and Methods

Study Area

This study was carried out at Bori Zonal Hospital (BZH) and Rivers State University Teaching Hospital (RSUTH), both within Rivers State, Nigeria.Bori is an ancient town with farming as the major occupation of the people, located within the South-East Senatorial District at 7°21'0"E and4°39'0"N. The Zonal Hospital is positioned within Bori town and it is owned by the Rivers State government. Rivers State University Teaching Hospital (RSUTH) formally known as Braithwaite Memorial Specialist Hospital is owned by the Rivers State /

government and located in the heart of Port Harcourt (7°3¹0¹¹E and 4°48'0"N). It is a tertiary health institution mainly for referrals from other secondary health facilities. It is officially recognized by the Federal Ministry of Health and ranked among the largest hospitals in the Niger Delta [6].

Study Design and Participants

This study was a hospital-based cross-sectional study made up of 600 ART-naive patients of both sexes and different age groups with $CD4^+$ cells <200, out of the 1100 HIV positive patients that were newly enrolled from February, 2021 to July, 2022. These participants were recruited from Bori Zonal Hospital and Rivers State University Teaching Hospital, all in Rivers State.

Determination of Sample Size

The minimum sample size was calculated using the formula [7] at 95% confidence level and the prevalence of cryptococcosis in Calabar for the year 2016 which was 5.1% [8].

$$N = \frac{Z^2 Pq}{d^2}$$

Where N = Sample size

Z = Statistic corresponding to level of confidence level, 1.96

P = Expected prevalence 5.1% (0.051)

d = Level of significance (allowable error) = 5% (0.05)

q = 1 - P

$$N = \frac{(1.96)^2 \times 0.051 \times (1-0.051)}{0.05^2}$$

$$= \frac{3.8416 \times 0.051 \times (0.949)}{(0.05)^2}$$

$$= \frac{3.8416 \times 0.051 \times 0.949}{0.0025}$$

= 74.4 (minimum sample size).

Inclusion / Exclusion Criteria

Only newly diagnosed HIV positive patients with CD4<200 or patients at stage 3 or 4 of the disease were recruited for this study while patients on ART were excluded.

Ethical Approval and Consent

Approval for this work was given from the Rivers State Ministry of Health, Port Harcourt and a verbal consent of the patients also taken.

Sample collection and Preparation

The consent and socio-demographic information of participants were verbally taken and participants were first screened to determine their $CD4^+$ values using 30ul of capillary blood and the VISITECT kit. Those with

$CD4^+$ values <200 or those at stage 3 or 4 of the HIV disease, that is; those with advanced human immunodeficiency virus (HIV) disease (AHD), were further screened for Cryptococcosis usingCryptococcal Antigen Lateral Flow Assay(CrAg LFA) kit.

Determination of CD4 count using VISITECT ®CD4 Advanced Disease Rapid Test kit.

VISITECT ® CD4 Advanced Disease is a rapid, semi-quantitative lateral flow assay for the estimation of CD4 in human whole blood, mostly used at point-of-care.

Principle of the test

VISITECT ® CD4 Advanced Disease rapid test is an immunochromatographic assay that estimates the full length CD4associated with $CD4^+$ T cells in human whole bloodand is directly correlated with $CD4^+$ T cell level. A capture monoclonal antibody (MAb) specific for the cytoplasmic domain of CD4 is applied as a line on the nitrocellulose membrane.

Whole blood is directly added to the VISITECT ® CD4 Advanced Disease Rapid Test where red blood cells and monocytes are retained in the blood collection pad and following the addition of buffer, other white blood cells (including $CD4^+$ T cells) migrate to the reaction site where lysis occurs, resulting in the release of full length CD4 for capture in the test strip. Colloidal gold-labeled MAb conjugate against CD4 and forms a test line. These complexes are visualized as a pink/purple line. A reference line (200 line) is included to allow estimation of CD4 levels by comparison to a set cut-off (equivalent to the signal level generated by samples containing 200 $CD4^+$T cell**).**

Test Procedure

The test kit was allowed to come to room temperature after checking the expiry date. The pouch was opened, the test device removed and 30µl of capillary blood added into well A. After 3 minutes, a drop of buffer was added to the same well A and allowed for 17mins. Finally, 3 drops of buffer was further added to well B, allowed for 20 minutes and the result was read as either < or >200 (below or above reference).

Cryptococcal Antigen Detection using Lateral Flow Assay

Detection of cryptococcal antigen was accomplished using the lateral flow assay (LFA) by means of the Immy Latex-Crypto Antigen kit (Immuno-Mycologic, Inc., Norman, Oklahoma). This method involves the impregnation of gold-conjugated, monoclonal anti-bodies onto an immunochromatographic test strip to detect cryptococcalcapsular polysaccharide glucuronoxylomannan antigen for all four *C.* neoformans serotypes (A-D)

Principle of Cryptococcal antigen test

The CrAg Lateral Flow is a dipstick sandwich immunochromatographic assay. Specimens and specimen diluents are added into appropriate reservoir, such as a test tube, and the lateral flow device is placed into the reservoir.

The test uses specimen wicking to capture Gold conjugated, anti-CrAg monoclonal antibodies deposited on the test membrane. If CrAg is present in the specimen, then it binds to the gold conjugated, anti-CrAg antibodies. The gold labeled antibody-antigen complex continues to wick up the membrane where it will interact with the test line, which has immobilized anti-CrAg monoclonal antibodies.

The gold labeled antibody-antigen complex forms a sandwich at the test line causing a visible line to form. With proper flow and reagent reactivity, the wicking of any specimen, positive or negative, will cause a gold – conjugated control antibody to move the control line.

Immobilized antibodies at the control line will bind to gold-conjugated control antibody and form a visible control line. Positive test result forms two lines (test and control). Negative test results form only one line (control). If a control line fails to develop then the test is not valid.

Test Procedure

The cryovial tube and plain bottle containing the patient blood to be analyzed was labeled and **a** drop of Lateral Flow (LF) specimen diluents and 40µl of serum were added into the labeled cryovial tube.An LF test strip was taken from the vial and inserted into the mixture, LF test strip vial was firmly recapped with desiccant cap immediately. Test was allowed for 10 minutes before reading the test result as either positive or negative.

Quality Control

Quality controls for CrAg test and Visitect were done weekly before running samples and results were confirmed by another scientist.

Statistical Analysis

Data generated from this study was analysed usingthe Statistical Package for Social Sciences (SPSS)and Excel (Model 22). The statistical tool used was one-way ANOVA with 5% (0.05) level of significance and data were represented using figures.

Results

Prevalence of cryptococcal anti-genaemia by Location

A total of six hundred (600) subjects were examined for cryptococcal infection. Three hundred

(300) subjects were from RSUTH, Port Harcourt, while three hundred (300) were from BZH,

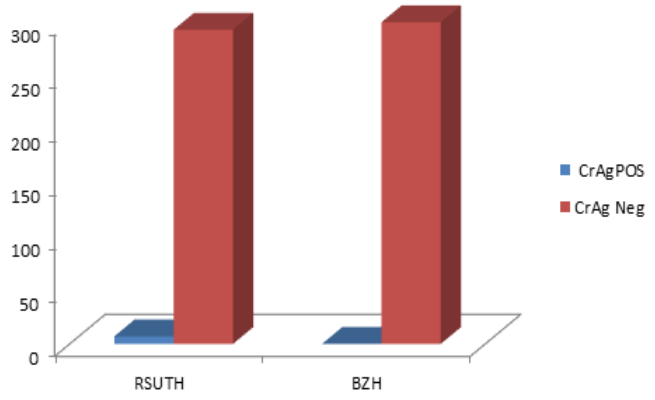
Bori. Out of the 300 subjects from RSUTH, 7(2.3%) were positive for cryptococcal antigen, whileout of the 300 subjects of BZH, 0(0.0%)were positive for cryptococcal antigen. A total of 7(1.2%)subjects were positive for cryptococcal antigen, with p-value ≤0.05 using one-way ANOVA.

The Gender- Related Prevalence of cryptococcal anti-genaemia among the patients

Out of the 260 males that were screened in thestudy, 4(1.5%) were positive for cryptococcal anti-genaemiawhereas for the females, out of the 340 subjects screened, 3(0.9%) were positive for cryptococcal anti-genaemia. There was no statistical significance in their prevalence with p-value ≤ 0.05using one way ANOVA.

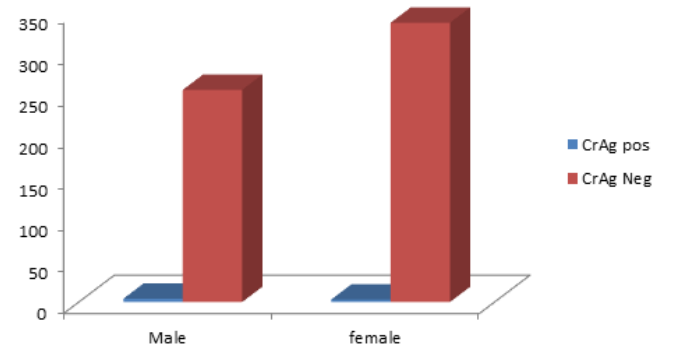
Age-Related of cryptococcal anti-genaemia among the patients

The result of this study showed the age bracket of (31-40) years as having the highest prevalence of cryptococcal anti-genaemia, 3(42.9%) each while (21-30) and (51-60) years had the lowest prevalence of 1(14.3%).



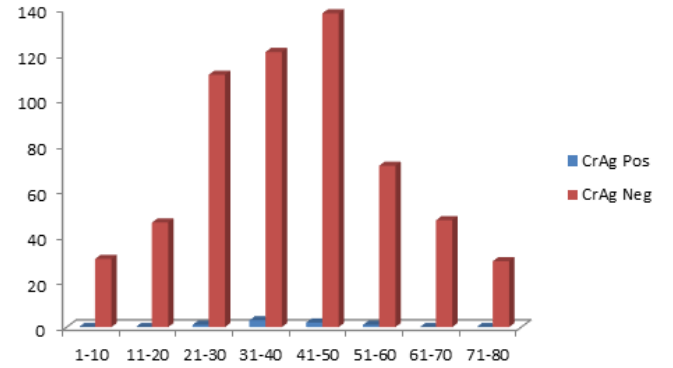
Legend: RSUTH- Rivers State University Teaching Hospital, BZH- Bori Zonal Hospital, CrAg-Cryptococcal antigen.

Figure 1: Location- Related Prevalence of Cryptococcal anti-genaemia.



Legend: cryptococcal antigen (CrAg)

Figure 2: Gender-related prevalence of cryptococcal anti-genaemia among patients.



Age bracket (Years)

Legend: cryptococcal antigen (CrAg)

Figure 3: Age-related prevalence of cryptococcal anti-genaemia among patients.

Discussion

Cryptococcal meningitis is a common but serious opportunistic infection among patients with advanced HIV disease (AHD) especially in developing countries. The high mortality rate among these patients despite the antiretroviral therapy, had led to the need for better management strategies of which CrAg screening is one of them [9].

The overall prevalence of cryptococcal anti-genaemia positivity in this study was 1.2% which contrasts the earlier studies with higher prevalence rates of 36% in Jos[10], 20.9% in Ethiopia by[11],12.7% by[8] in Benin City among ART-naive patients, 9.2% by [12] in Senegal, 8.9% in Lagos by [4] and 7.7% by [2] in Ethiopia. Different prevalence rates are noted in various countries of the sub-Saharan Africa ranging from 2-21% [9]. The differences in their population sizes, the study techniques, $CD4^+$ T-cell count and improved health awareness campaign may have contributed to the differences observed. The presence of Cryptococcus species in HIV was suggested to be the result of reactivation of latent infection in the event of immunosuppression [13]. The result also lower than other researchers like [14,8,9] in Uganda, Calabar and Tanzania, who reported prevalence rates of 5.8%, 5.1% 3.7% respectively.

Although, the result agrees with those of earlier researchers [15] with 2% prevalence in Ghana. In Asia, a prevalence of 4% was observed in Vietnam, in Europe, a prevalence of 5% was recorded in London and 1.6% in Germany [16,17].

From this study, Rivers State University Teaching Hospital (RSUTH) having a higher prevalence ($p \leq 0.05$) of CrAg positivity 7(2.3%) than Bori Zonal Hospital (BZH)0(0.0%). This could probably be because, RSUTH is a referral center where most cases from other facilities in the State sent and of course, the number of poultry in the State has also increased drastically. The difference in the observed prevalence could probably be due to environmental conditions and does not really depend on the prevalence of HIV disease in the location because, Khana local government has the highest HIV burden in the state and yet, the prevalence of cryptococcal anti-genaemia is low. For instance, pigeon raring is very common in the Northern part of Nigeria than the South and thus, it will never be strange if the Northerners are having higher prevalence of cryptococcal anti-genaemia,

Although more females than males were recruited for this study, males were observed to have higher prevalence than their female counterparts. Out of the 260 males that were screened in the study, 4(1.5%) were positive for cryptococcal anti-genaemia whereas for the females, out of the 340 subjects screened, 3(0.9%) were positive for cryptococcal anti-genaemia (p=0.04).There was a statistical difference in their prevalence ($p \leq 0.05$) using one-way ANOVA. This higher prevalence might be due to the facts that males are more involved in outdoor works that could exposed them to birds' droppings leading to cryptococcal infection, the poor health-seeking behaviour of men to care and the interaction of Cryptococcus with testosterone, which results in increased capsular polysaccharide release and Cryptococcus-mediated macrophage death [18,19]. The result from this study agrees with the findings of [2,20] who also observed higher prevalence of cryptococcosis among the males than the females but in contrast to [21,22] where the females had higher prevalence of cryptococcosis.

Most of the positive cases from this study were in the age bracket of (31-40) years with a prevalence of 42.9%, the least was found in the age brackets of (21-30) and (51-60) years. This is similar to the findings of [8,21] who observed higher prevalence or cryptococcosis among age groups >25 years. This contradicts the study of [22] where infection was found among all the age groups.

The limitation of this study was that no lumber puncture was performed to obtain cerebrospinal fluid for confirmatory tests.

Conclusion

The results of this study revealed the likelihood of the progression of cryptococcal disease among HIV-infected individuals with a CD4+ T-cell count of < 200 cells/µl in the area under investigation. Therefore, the routine screening for cryptococcal antigen should be performedfor all patients attending the ART clinics in Rivers State, as well as across the country and also, the provision of adequate antifungal regimen for the treatment of those with cryptococcal anti-genaemia will make early intervention and better management of cryptococcal meningitis cases possible.

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