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Research Article

Prevalence of Cryptococcosis among HIV Patients Attending Infectious Disease Hospital (IDH), Kano

***H. Sule and R. M. Tsadu**

Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Bayero University, Kano-Nigeria

****Corresponding Author's email*: sule.hamza@yahoo.com; Phone: +2348036062021**

ABSTRACT

Cryptococcosis is an opportunistic infection widely associated with people living with HIV. Some even consider it one of the indicators of possible HIV infection during the peak of the HIV epidemic. The study aimed to investigate the incidence of cryptococcosis among HIV patients attending the Infectious Disease Hospital (IDH), Kano. In the study, KOH preparation, Gram staining technique, Culture method, Indian Ink staining techniques and urease test were employed for the isolation and identification of *Cryptococcus* species. In the results, apart from *Cryptococcus* species, *Candida* species and molds like *Aspergillus* and *Penicilium* species were also isolated. The female patients, as well as those within the age group of 30-39 years old, were more at risk of the infection. In conclusion, *Cryptococcus* species remain one of the opportunistic pathogens among HIV patients, with a higher risk among females.

Keywords: Cryptococcosis; *Cryptococcus* species; HIV patients; Immunocompromised individuals; Opportunistic disease

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INTRODUCTION

The disease cryptococcosis evolved from a single case report of 1895 to global epidemic status, per each year, a million or more cases were reported during the peak of HIV epidemic (Perfect and Bicanic, 2015). The disease is mostly caused by the fungus *Cryptococcus neoformans*, which is an opportunistic pathogen (measuring 4-6 micron, round-to-oval yeast, with a polysaccharide capsule coat), they spread by inhalation of aerosols from the environment, and have latency ability for many years, with frequent reactivation in people with low immunity, especially among human immunodeficiency virus (HIV) infected subjects (Diniz-Lima *et al.,* 2022).. The disease cryptococcosis is among the leading cause of morbidity and mortality among HIV-infected individuals, more especially in developing countries particularly of sub-Saharan Africa, where it is said to account for up to 15% of deaths related to HIV and AIDS cases (Oladele *et al.,* 2020).

During the 1980s, the issue of HIV and AIDS epidemic significantly changed the then known clinical profile of this cryptococcal infection, where *C. neoformans* emerged as the then major cause of meningitis and encephalitis among with people with weak immune system (Rajasingham *et al.,* 2017). During the late 20th and early 21st centuries cryptococcosis became a serious opportunistic disease among people living with HIV, particularly in sub-Saharan Africa, which had the highest burden of HIV cases in the world (Brouwer *et al.,* 2013). At one time, almost 300,000 new cases of HIV cases are reported yearly in Nigeria. In 2024, there was report of 3.4 million HIV-positive individuals towards the end of the year. One issue with this disease is that, even though most HIV treatment centres in Nigeria have access to Highly Active Antiretroviral Therapy (HAART), but treatment

for cyptococcosis among HIV patients is not part of HAART, therefore it is not implemented (Mohammed *et al.,* 2019).

Around 1990, the availability of antifungal agents like fluconazole and amphotericin B significantly improved patient treatment conditions, however, mortality rates from the disease (cryptococcal meningitis) were still high as a result of late diagnosis of the disease, especially in resource-limited countries (Lortholary *et al.,* 2011). As a result of that, cryptococcosis persisted as a serious and fatal fungal infection worldwide, leading to over 200,000 deaths annually with people death annually courtesy (cryptococcal meningitis), with greater percentage from sub-Saharan Africa (Rajasingham *et al.,* 2017).

MATERIALS AND METHODS

Study Area

The study was carried out at the Infectious Disease Hospital (IDH) located in Kano metropolis, Kano State, Nigeria. The state lies between Latitude: 12° 00' 0.43" North and Longitude: 8° 31' 0.19" East with total surface area of 137 km2.The population of Kano State is over 13 million which makes it the most populated state in Nigeria (National Population Commission, 2016).

Study Population

The research involved HIV patients attending the HIV Clinic at IDH, Kano.

Sample Size Determination

Sample size was determined using Cochran's formula (Cochran, 1963)

$n = \frac{Z^2pq}{d^2}$

Where; n = sample size

p = Past prevalence (11%=0.11) (Mohammed *et al.,* 2019)

q = negative prevalence (1-p =1-0.11= 0.89)

Z = confidence interval (95%=1.96)

d = Precision (5%=0.05)

$n = \frac{Z^2pq}{d^2}$

$n = \frac{1.96^2 \times 0.11 \times 0.89}{0.05^2}$

$n = \frac{0.3760}{0.0025}$

n = 150 samples

Sample Collection and Processing

Sputum samples were collected aseptically in a properly labelled sterile wide mouth container from each participant.

Potassium Hydroxide (KOH) preparation

Potassium hydroxide preparation is a simple and rapid method used to detect fungal elements, including *Cryptococcus*, in clinical samples (Forbes *et al.,* 2007). Two drops of 10% KOH solution were added to the sputum sample on a glass slide. It was mixed gently using a sterile applicator stick to ensure even distribution and a coverslip was then placed over it. It was allowed to sit at room temperature for 10 minutes to clear the background debris (Murray *et al.,* 2020).

Culture Method

The collected samples were aseptically inoculated onto Sabouraud Dextrose Agar (SDA), a medium that supports fungal growth and inhibits bacterial contamination through the addition of antibiotics (Zhao *et al.,* 2019). The SDA was preferred due to its rich composition that facilitates the growth of fungi, including *Cryptococcus neoformans* and *Cryptococcus gattii* among other fungi. The plates were then incubated at a temperature of 37°C for 24 hours. After incubation, the plates were examined for *Cryptococcus* spp which typically appear as smooth, creamy, and mucoid colonies (Firacative *et al.,* 2022).

Gram Staining

Smear preparation

A loop full of the colonies was placed on a clean glass slide and mixed with a drop of normal saline to make a smear and allowed to air-dry completely. The slide was passed briefly through a flame to heat-fix ensuring the slide did not overheat

Staining technique

The smear was covered with crystal violet for 1 minute and rinsed with water. The slide was covered with Gram's iodine for 1 minute and rinsed with water. A few drops of acetone was then used for decolorization and allowed to stand for 5 seconds and then rinsed immediately with water. The smear was counterstained with Safranin for 1 minute and rinsed with water, and allowed to dry. The mixture was examined under the microscope using 100× objective (Koneman *et al.,* 2005). *Cryptococcus* appear as: large, round to oval Gram-positive (purple) yeast cells. The capsule may appear as an unstained halo surrounding the yeast due to its polysaccharide nature Cheesbrough, 2006).

Urease Test

Cryptococcus produces the enzyme urease, which hydrolyzes urea into ammonia and carbon dioxide, increasing the pH of the medium. A portion of the cultured colony was inoculated onto a urea agar slant and incubated at 37°C for 24hours (Cheesbrough, 2006).

Indian Ink Staining Technique

The Indian ink staining technique is a negative staining method primarily employed to detect encapsulated yeast cells like *Cryptococcus* species in

clinical samples such as sputum and CSF. This method allows visualization of the polysaccharide capsule, which appears as a clear halo surrounding the yeast cell. Adrop of sputum sample was placed on a clean glass slide and one drop of Indian Ink was added to it. It was mixed gently with an applicator stick and covered with a cover slip and viewed under the microscope using 10x and 40x objective lens (Forbes *et al.,* 2007).

Data Analysis

The obtained data was analyzed using statistical package for social sciences (SPSS) version 20.0.

RESULTS

Out of the total of 150 sputum samples, 139 (92.7%) were positive, out of which 25 samples yielded more than one fungal species. Among the isolates, 114 were yeasts (82%) comprising of *Cryptococcus neoformans* (14) and *Candida spp*. (100). The remaining isolates were molds (18%) comprising of *Aspergillus spp*. (12.9%) and *Penicillium* spp. (5.1%) as shown in Table 1. Based on gender of the patients, *Cryptococcus* species (*C. neoformans*) isolated females had 78.6% *C. neufomans* infection as against 21.4% among the males (Table 2). Based on age group of the subjects, 30-39 age bracket had the highest *Cryptococcus* infection rate (57.1%) followed by age group of 40-49 with 21.4% (Table 3).

Table 1: Prevalence of Fungal infections among HIV Patients attending IDH, Kano

Fungal Species	Pure Colonies	Mixed Colonies	Total	Percentage (%)
C. neoformans	3	11	14	10.1
Candida spp	71	29	100	71.9
Aspergillus spp	18	-	18	12.9
Penicillium spp	7	-	7	5.1

Table 2: Distribution of *Cryptococcus neoformans* in relation to gender of the HIV Patients

Sex	Number examined	Number Positive	Percentage (%)
Male	44	3	21.4
Female	106	11	78.6

Table 3: Distribution of the *Cryptococcus neoformans* in relation to age of the participants

Age group (years)	Numbers examined	Number Positive	Percentage (%)
10-19	1	0	0
20-29	22	0	0
30-39	45	8	57.1
40-49	39	3	21.4
50-59	27	1	7.1
60-69	15	1	7.1
70-79	1	1	7.1

DISCUSSION

The study investigated the incidence of cryptococcosis among HIV-positive individuals. From the results, out of 150 sputum samples analyzed, *Cryptococcus* species had a total prevalence of 14 (10.1%). This is similar to findings from a study conducted in Sokoto by Mohammed *et al.* (2019), where a prevalence of 11% was reported. Although *C. neoformans* was less frequent than *Candida* spp., its presence is of significant clinical concern due to its potential to cause severe systemic infections such as cryptococcal meningitis (Rajasingham *et al.,* 2017). The relatively lower isolation rate in sputum may reflect its tendency to disseminate beyond the respiratory tract, particularly in patients with advanced immunosuppression (Jarvis *et al.,* 2012).

The distribution of *C. neoformans* according to gender revealed a higher prevalence among females, the 14 positive cases compared the males. This is comparable to the findings reported by Datir and Azeez (2017) where a prevalence of serum CrAg comprised of 3 females and only 1 male.

Based on age groups, findings from this study show that majority of the positive cases for *Cryptococcus* were within the ages of 30-40 years. This is in accordance with a study by Ezenabike *et al.* (2020), who reported that the age group of 35-44 years had the highest prevalence of *Cryptococcus* in their study. It is also in agreement with Joseph *et al.* (2015) in

similar research where patients aged 30-39 years reported the highest incidence of serum *Cryptococcus* species.

While the reason for this gender and age disparity remains unclear, it may be attributed to a variety of factors including environmental exposure, health-seeking behaviour, or genetics. However, given the relatively small sample size of the male participants (44) compared to the female participants (106), these observations should be interpreted with caution and therefore warrant further study.

CONCLUSION

This study demonstrates *C. neoformans* as the most common *Cryptococcus* species associated with HIV patients and the detection of mixed cultures emphasizes the need for comprehensive mycological investigations in vulnerable population like people living with HIV. The higher occurrence of *C. neoformans* among the females indicate their vulnerability to this disease in the study area.

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