Original Article

Cryptococcal Meningitis among HIV-Infected and HIV-Uninfected Patients in Jos, North Central Nigeria

Okolo M O,¹ Onyedibe K I,¹ Dabe F³, Obishakin E F⁴, Envuladu E A², Egah D Z¹, Hawkins C⁵

¹Department of Medical Microbiology and ²Department of Community Medicine, College of Health Science, University of Jos, Nigeria.

³Department of Family Medicine, Plateau State Specialist Hospital, Jos, Nigeria

⁴Biotechnology, National Veterinary Research Institute, Vom, Plateau state, Nigeria.

⁵Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

*Correspondence: Okolo Mark Ojogba

Email: okolomark@gmail.com, Phone: +234(0)8027022223

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ABSTRACT

Cryptococcal meningitis is a global opportunistic infection caused by several serovars of *Cryptococcus neoformans* and *Cryptococcus gattii* with significant morbidity and mortality particularly in immunocompromised patients. Management principles involve early and specific diagnosis followed by appropriate antifungal therapy. The study was conducted among in-patients with clinical features of meningitis admitted into tertiary healthcare facilities in Jos, north central Nigeria from November 2017 to December 2018. Cerebrospinal fluid and blood samples were collected from the patients for culture and sensitivity, serology and genomic studies. Univariate and bivariate analysis was carried out using STATA (version 14IC). The serologic prevalence of cryptococcal meningitis among patients with clinical features of meningitis was 16.8%. The prevalence among HIV-infected and HIV-uninfected patients were 13.4% and 18.8% respectively. The antifungal susceptibility of the *Cryptococcus neoformans* isolated from sample cultures at MIC90ug/ml to Amphotericin B, Fluconazole and Itraconazole were all within the reference range. Gene accession numbers of cryptococcal isolates deposited in the gene bank include MK886574.1, MK886568.1, MK886570.1, MK886563.1, MK886560.1, MK886573.1, MK886562.1, MK886558.1. *Cryptococcus neoformans* are a major cause of meningitis in both HIV-infected and HIV-uninfected patients. This study highlights the fact that routine surveillance should be put in place for culture, antifungal susceptibility tests and genomic studies to ascertain the antifungal susceptibility pattern and genotypes of cryptococcus in circulation.

Keywords: Cryptococcus, meningitis, antifungal agent, HIV-infected, HIV-uninfected

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INTRODUCTION

Cryptococcal meningitis is a serious systemic fungal infection (mycosis) that mainly affects immunocompromised patients causing high morbidity and mortality.¹ It is a life-threatening opportunistic infection that is normally observed in the late stages of acquired immunodeficiency syndrome (AIDS).^{2,3} The vast majority of cryptococcal meningitis are caused by two pathogenic species, *Cryptococcus neoformans* and *Cryptococcus gattii* which differ in host range, virulence as well as antifungal susceptibility.⁴⁻⁶ The disease affects approximately one million persons in the world annually resulting in more than 400,000 deaths within three months following the onset of the disease.^{1,5,7}

Cryptococcal meningitis causes high mortality rates in HIV-infected patients (9%-55%) and HIV-uninfected patients (15%-44%), despite optimal care.^{8,9} There is a decline in the incidence of cryptococcal infection in the developed nations of the world due to effective antiretroviral therapy.^{2,3,10}

Several classes of antifungal drugs effectively treat cryptococcal infections, but the organisms can develop resistance to these drugs.^{6,11,12} Many in vitro studies have shown that *Cryptococcus species* are highly susceptible to amphotericin B at a minimum inhibitory concentration (MIC) of 1 µg/ml, susceptible to flucytosine at an MIC of 4 µg/ml, fluconazole at 8 µg/ml.^{6,11} Fewer studies carried out were mainly on serology to determine the prevalence of cryptococcal meningitis.^{8,13} Molecular methods such as polymerase chain reaction (PCR) and sequencing^{6,11,14} will identify the fungus. Hence the diagnosis and treatment of cryptococcal meningitis remain challenging.

This study aims to determine the cryptococcal agents of meningitis in HIV-infected and HIV-uninfected patients in Jos, Nigeria. We also sought to determine the antifungal susceptibility pattern of the *Cryptococcus species*.

MATERIALS AND METHODS

This cross-sectional study was conducted among adult patients with clinical features of meningitis admitted into two tertiary health care facilities in Jos. The patients were recruited based on the following criteria; the presence of meningitis, acceptance of lumbar puncture, and consent to participate. The exclusion criteria for enrollment included: patients with sepsis due to neurologic infection or metabolic abnormality, patients with psychosis and peripheral neuropathy, patients with neurosurgical illnesses. All the HIV-infected patients were diagnosed with HIV infection before presenting with features of meningitis and were already on antiretroviral therapy.

Cerebrospinal fluid analysis

All cerebrospinal fluid samples were collected via lumbar puncture in aseptic conditions into sterile tubes and sent immediately to the laboratory. Each sample was split into two cryovial tubes; one of each pair of samples was used for microscopic examination by Indian ink, latex agglutination and culture and the second for serologic and genomic study.

Serology/Molecular identification

Cryptococcal antigen enzyme immunoassay (CrAg EIA) was carried out to detect capsular polysaccharide antigen of *Cryptococcus species* (IMMY; Inc. U.S.A).

Total genomic DNA extraction from the *Cryptococcus species* isolated on the cultured plates was performed using Zymo Quick-DNA kits reagents (Zymo Research Corporation, USA) per the manufacturer's instructions. Polymerase Chain Reaction (PCR) using specific primer sequence for the internal transcribed spacer 1 and 4 (ITS 1 and ITS 4) regions were used. The PCR products were utilized for sequencing with a Big Dye Terminator Reaction Kit v3.1 (Applied Biosystems, Inc., Foster City, CA, USA) according to the manufacturer's instructions. After the purification and denaturation of the products, the samples were run on an automated ABI 3500XL genetic analyser (Applied Biosystems, Inc., Foster City,

CA, USA). The sequences obtained were aligned and compared with sequences deposited in a genomic database (GenBank, NCBI, USA). To ensure high reliability of the results obtained using the nucleotide sequence alignment tools,^{15,16} an e-value of less than 10^{-5} and a maximum identity of equal to or higher than 98% were considered for the correct identification of *Cryptococcus* at the species level and the phylogenetic analysis was carried out using Bio Edit, and MEGA 07.

RESULTS

During the study period, a total of 184 (67 HIV-infected and 117 HIV-uninfected) patients with features of meningitis were recruited and 31 (16.8%) of these had cryptococcal meningitis {9 HIV-infected (13.4%) and 22 HIV-uninfected (18.8%)}, and male gender was predominant in both groups as shown in Table 1

Table 1 : Demographic and laboratory characteristics of HIV-infected and HIV-uninfected patients with cryptococcal meningitis in Jos, North-Central Nigeria

Parameter	HIV+	HIV-	P
Age mean no years ^{\$}	30.2	49.3	<0.001
Number recruited	67	117	NA
Male/Female	7/2	16/6	>0.999
Positive serology	9	22	0.416
CSF culture positive/positive serology	7/9	19/22	0.613
CSF CrAg titer 1:512 ^{\$} /positive serology	5/9	20/22	0.043
CSF Indian Ink positive/positive serology	7/9	13/22	0.429
CD4/mm ³ Median (range)	32.5(8-10.9)	NA	NA
HIV viral load log ₁₀ copies/ml	6.18 ± 0.62	NA	NA

P < 0.05; \$ students *t* test, CSF: Cerebrospinal fluid, CrAg: Cryptococcal antigen, NA: not applicable

Table 2: Antifungal susceptibility of *Cryptococcus neoformans* isolated from HIV-infected and HIV-uninfected patients with meningitis in Jos, North-Central Nigeria

Antifungal agents	MIC range (µg/ml)	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)	GM(µg/ml)
HIV-uninfected:				
Amphotericin B	0.0625-1	0.5	1	0.5163
Fluconazole	0.125-3	1	4	1.1578
Itraconazole	0.0312-0.5	0.125	0.25	0.1185
HIV-Infected:				
Amphotericin B	0.125-1	0.5	1	0.3410
Fluconazole	0.5-8	4	8	2.3123
Itraconazole	0.0313-3	0.125	0.5	0.2816
Total HIV- and HIV +				
Amphotericin B	0.0625-1	0.5	1	0.4173
Fluconazole	0.125-8	2	4	1.4385
Itraconazole	0.0313-3	0.125	0.25	0.1321

MIC₅₀= Minimum Inhibitory Concentration required to inhibit the growth of 50% of organisms, MIC₉₀= Minimum Inhibitory Concentration required to inhibit the growth of 90% of organisms, GM= Geometric mean MIC

The HIV-infected patients were younger compared with HIV-uninfected patients (mean age 30.2 years against 49.3 years respectively) and had CD4 counts $<200/\text{mm}^3$. The median CD4 count among the HIV-infected patients was $32.5/\text{mm}^3$ (range: $8-109/\text{mm}^3$) and their median viral load was 6.18 log copies/mL.

Cryptococcus neoformans isolated showed a uniform antifungal susceptibility pattern to the three antifungal

agents. The individual Minimum Inhibitory Concentration (MIC) ranges and MIC which inhibits 90% of the isolates (MIC_{90}) were $0.0313-3\mu\text{g}/\text{ml}$ and $0.25\mu\text{g}/\text{ml}$ for itraconazole, $0.065-1\mu\text{g}/\text{ml}$ and $1\mu\text{g}/\text{ml}$ for amphotericin B, $0.125-8\mu\text{g}/\text{ml}$ and $4\mu\text{g}/\text{ml}$ for fluconazole. The MIC ranges and MIC_{90} for itraconazole, amphotericin B and fluconazole were all within the reference range for the *Cryptococcus neoformans* isolated from HIV-infected and HIV-uninfected patients as presented in Table 2.

The phylogenetic analysis of *Cryptococcus neoformans* as well, as their gene accession numbers, is as shown in Figure 1

Figure1: Evolutionary relationships of *Cryptococcus neoformans* isolated from HIV-infected and HIV-uninfected patients with meningitis in Jos, North-Central Nigeria

DISCUSSION

The prevalence of cryptococcal meningitis varies from one population to another.^{4,5,7,10,14,17} In our study, 31 (16.8%) of our patients with meningitis were all infected by *Cryptococcus neoformans*, whereas the prevalence among HIV-infected and HIV-uninfected patients were 13.4% and 18.8% respectively. Worldwide there is a variation in the distribution of *Cryptococcus* species causing meningitis^{1,2,9} but many of these studies have shown that *Cryptococcus neoformans* is the leading cause.^{7,14,18} It is now well established from a variety of studies that the prevalence of cryptococcal meningitis among HIV-infected patients is decreasing as compared with HIV-uninfected patients because the prevalence of HIV infection is equally decreasing and most of the HIV-infected patients are doing well on antiretroviral drugs.^{4,9,19} In keeping with several other studies,^{19,20} HIV-infected patients were significantly younger than HIV-uninfected patients in our study. In both groups, males presented more with cryptococcal meningitis. Several other studies^{2,19,21} reported that HIV-infected patients with cryptococcal meningitis were mostly young males with Acquired Immunodeficiency Syndrome (AIDS), which may reflect a difference in the exposure of the patients rather than a difference in host susceptibility.

Amphotericin B has remained the mainstay of treatment for *Cryptococcus neoformans* for many years in both HIV-infected and HIV-uninfected patients.^{9,12,22} It targets the ergosterol in the plasma membrane of the fungus to form a channel that ultimately results in the disruption of the proton gradient. There are no defined breakpoints by the Clinical and Laboratory Standard Institute (CLSI) for amphotericin B and *C. neoformans*, it has been suggested that a Minimum Inhibitory Concentration (MIC) value of 2 µg/ml is the resistance threshold for amphotericin B and the susceptibility pattern of *C. neoformans* strain is predictable, with MICs ranging from 0.12-0.5 µg/ml.²³ Although our study did not identify any isolates with amphotericin B resistance, our result describes elevated average MICs to amphotericin B, which was rarely

observed in other studies.^{6,11} It was reported that treatment with amphotericin B may induce the development of clinical and invitro amphotericin B resistance. The MIC values for fluconazole and itraconazole for both groups observed in this study were similar to those reported in some studies^{6,11,12,23} while a few others studies showed higher MIC values and increasing rates of resistance.¹² Several phylogenetic studies evaluating the phylogeny of *Cryptococcus* species used single genomic loci such as (SSu), nuclear ribosomal large subunit (nucLSU), 5.8S or ITS region,^{6,11,14} but our study utilized the ITS region which can also differentiate between the different species of *cryptococcus*. For accurate identification and therapeutic purposes, DNA-based techniques¹² have been used to differentiate *Cryptococcus species*. Worthy of note is the fact that all of the ITS sequences used for the analysis in the study were generated from the PCR products. Regular epidemiological surveys that determine the genetic relationship among species of *Cryptococcus* and susceptibility patterns enables the identification of resistant strains. Our study also reinforces the utility of routine antifungal susceptibility studies for evaluating the patterns among *Cryptococcus neoformans* and its application for future drug therapeutic studies.

CONCLUSION

Cryptococcal meningitis is a treatable cause of death in both HIV-infected and HIV uninfected patients but it is often undiagnosed. A high index of suspicion in patients with known HIV-diagnosis, that are young and with features of meningitis should alert the possibility of cryptococcal meningitis and trigger aggressive diagnosis workups to rule out the disease and to consider empiric antifungal therapy.

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