ORIGINAL ARTICLE

Prevalence of Pulmonary Aspergillosis among HIV-positive subjects in a Tertiary health-care institution, Nigeria

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ABSTRACT

Background: In developing countries, the incidence of pulmonary aspergillosis (PA) in HIV-positive subjects has risen persistently and characterised by a significant drop in CD4+ cell count.
Aim: To determine the prevalence of PA amongst individuals presenting with HIV at a tertiary hospital in Nigeria.
Methods: Sputum samples were collected from 200 and 195 HIV- positive (study group) and HIV- negative (control group) subjects respectively aged 15 - 64 years. A structured questionnaire was used for data retrieval. Identification of *Aspergillus* species was done using standard microbiological methods. Furthermore, CD4+ cell count was determined using the Partec new model Cyflow Counter. Data analysis was done using the SPSS Version 21.
Results*:* The mean age (mean ± SD) was 43.65 years ± 12.00 for the HIV-positive subjects. The prevalence of PA was 52% among the HIV-positive subjects. The distribution of *Aspergillus* species revealed *A. fumigatus* as the most specie with prevalence of 49.04% among the HIV-positive subjects. The HIV-positive subjects were significantly associated with PA than the HIV-negative subjects (p= 0.001). The mean CD4+ cell count of the HIV-positive subjects with PA was 194.70 cells/µL ± 64.10. PA was significantly associated with HIV-positive subjects having a CD4+ cell count < 200 cells/µL (p = 0.003) as well as the age (p = 0.003) and gender (p = 0.014) of the HIV-positive subjects.
Conclusion: Majority of the HIV-positive subjects had PA of which decreased CD4+ cell count (< 200 cells/µL) was associated; likewise the age and gender of the HIV-positive subjects.
Keywords: Pulmonary aspergillosis, *Aspergillus* species, HIV, CD4+ cell count

INTRODUCTION

In recent years, fungal respiratory infections are important cause of mortality and morbidity among immunocompromised persons[1]. Pulmonary aspergillosis is a lung infection with the fungus *Aspergillus* as the causative agent. Members of this genus *Aspergillus* are spore- forming which are readily inhaled and thrives in different climates worldwide. In the lungs, the fungus *Aspergillus* demonstrates varied clinical conditions of pulmonary aspergillosis ranging from the acute to chronic stage. *Aspergillus* spp. are prevalent in the environment and mostly isolated from the soil, hospital environment or even plant debris[2].

The rise in the prevalence of pulmonary aspergillosis in the immunocompromised has resulted to a worldwide healthcare problem and if not diagnosed and treated, could disseminate and further leads to devastating invasive disease condition. There are diverse demonstration of symptoms associated with pulmonary aspergillosis. This include: cough, chest pain, arthralgia, fever, difficulty in breathing and headache. Diagnosis of this disease pose a challenge to clinicians due to non-specific symptoms[3]. *Aspergillus* infection can be prevented when both innate and adaptive immune system are very functional and healthy[4].

Human immunodeficiency virus (HIV) infection remains a significant concern globally with a recent estimation of 0.7% among adults aged 15 - 49 years living with the infection worldwide. Reports from the World Health Organization suggests Africa is still burdened with this infection reporting an estimation of 26 million people living with the infection; of which 62% is estimated within the age group 15 – 49 years[5]. The condition symbolises the destruction of the immune system; precisely the white blood cells known as CD4+ cells. White blood cells (CD4+ cells) are indicators for a healthy immune system. As their number drops, the immune system will become feebler to fight infections consequently; opportunistic fungal respiratory infections and other complications occur[6].

The prevalence of pulmonary aspergillosis among HIV-infected in developing countries like Nigeria has been on the rise. Studies have reported an estimated prevalence for pulmonary aspergillosis as ranging from 13.3% - 47.1% among HIV-positive individuals [7, 8]. Significant risk factors associated with pulmonary aspergillosis most especially the invasive form in HIV-infected individuals include: use of corticosteroids, neutropenia, bacterial infection, use of multiple broad-spectrum antibiotics, stay in Intensive Care Unit, use of immunosuppressive agents, solid organ transplant, underlying lung infection such as tuberculosis, smoking, alcohol consumption, age, gender, diabetes, leukocytopenia, and low CD4+ cell count [9-11].

Pulmonary aspergillosis is most often associated with delayed diagnosis by physicians as *Aspergillus* species are underrated in HIV-infected individuals. Consequently, detection of these species is paramount among individuals living with HIV to facilitate prompt commencement of

appropriate and effective antifungal regimen. The aim of the study therefore was to isolate, identify the etiologic agents for pulmonary aspergillosis, and determine the possible risk factors for acquisition of pulmonary aspergillosis among HIV-positive individuals attending a tertiary health-care institution in Nigeria.

METHODOLOGY

This cross sectional, hospital-based study comprised of 200 HIV-positive and 195 HIV-negative subjects aged 15 to 64 years who were attending a tertiary hospital with symptoms suggestive of pulmonary aspergillosis within a six-month period. Here, we assumed a 95% confidence interval with 0.05 degree of accuracy and 14.9% proportion of HIV-positive subjects with pulmonary aspergillosis[12] to obtain the minimum sample size of 200 for the study. The HIV-negative subjects served as control for the study. The HIV-positive subjects were recruited irrespective of their inclusion in Highly Active Antiretroviral Therapy (HAART). The HIV status of the subjects were confirmed in the molecular laboratory of the hospital using two test-kits: Determine kit (Alere, Japan) and Stat-pack kit (USA) according to the guidelines provided by the National AIDS Control Organization (NACO, Strategy III)[13]. The exclusion criteria for the study included subjects under the age of 15 years, and patients with history of antifungal therapy within the last three month from the time of the study.

The subjects were educated on the relevance of the study of which they gave their informed consent prior to sample collection. The convenient sampling method was employed. A sterile sample vessel was used for sputum collection intended for *Aspergillus* microbial screening. Simultaneously, a well-structured questionnaire served as tool to retrieve demographic data such as: gender, age and other variables like HIV transmission route, HIV status of the partner, and duration of HAART as well as clinical symptoms. Data generated were anonymously analysed throughout the study.

The identification of *Aspergillus* species was done using standard microbiological techniques which involved: microscopy, macroscopy and culture. The sputum samples from all the subjects were subjected to a culture-based method using Sabouraud Dextrose Agar (SDA) media containing Gentamicin (20U/mL) and Chloramphenicol (0.2mg/ml). Czapek Dox Agar and Yeast Extract Agar were also used. The inoculated agar were incubated at 25ºC and examined daily for 2 to 5 days. The morphological features of the *Aspergillus* cultures were carefully observed using remarkable macroscopic features like the colony diameter, colony texture, exudates colour (conidia and reverse) and identified using cellulose and starch hydrolysis tests as standard biochemical tests.

Potassium hydroxide (KOH) direct microscopy. A loopful of sputum was mixed with a 10% KOH solution prior to placing a coverslip over the preparation on a microscopic glass slide. The slide was observed under x10 and x40 objectives for the presence of fungal hypha and spore forms under low light intensity.

Staining with Lactophenol cotton blue. This was done using lactophenol cotton blue staining technique with a small fragment of a colony retrieved from the midpoint of the fungal culture and examined under ×10 and ×40 objectives for fungal spores and hyphae.

Starch hydrolysis test. Here, a starch agar medium was used. This medium comprised of: starch 20.0 g/L, peptone 5.0 g/L, yeast extract 3.0 g/L, agar 15.0 g/L with pH 7.0. Inoculation was done with isolated fungal cultures and incubated at 25°C for 5–7 days in an inverted position. The surface was flooded with iodine solution for 30 seconds, and zones around the fungal growth were observed and interpreted as positive for growth when there was a decrease in iodine solution.

Cellulose hydrolysis test. Czapek- mineral salt agar medium containing KCl 0.5 g/L, K_2HPO_4 1.0 g/L, $NaNO_3$ 2.0 g/L, $MgSO4•7H_2O$ 0.5 g/L, peptone 2.0 g/L, carboxymethyl cellulose 5.0 g/L was inoculated with fungal cultures and incubated for 5 days at 25°C in an inverted position. The petri-dish surface was then flooded with 1% aqueous solution of hexadecyltrimethylammonium bromide and left for 35 seconds. Formation of clear zones surrounding the fungal colonies were observed.

CD4+ cell count assay. In addition to the sputum sample, 5 ml of whole blood sample was collected from each subjects for CD4+ cell count assay. Flow cytometry assay was employed in determining the CD4+ cell count. Based on the manufacturer's instructions, the CD4+ cell counts in whole blood sample collected from the HIV-positive subjects were analysed using a Partec™ CyFlow Analyzer (Sysmex, Norderstedt, Germany) Model SL3.

Statistical analysis involved Statistical Package for Social Sciences (SPSS) version 21 for windows and frequency with percentage were used to evaluate the results of this study. The mean values were expressed with the standard deviation. Student's t-test was used to compare the mean values and values at $p < 0.05$ were considered to have significant association.

Ethical consideration entailed an ethical approval with the number COOUTH/CMAC/ETH.C/VOL. 1/FN:94/ 0024 was obtained from the Hospital's Management Research Ethics Committee Board according to the Declaration of Helsinki. All subjects gave an informed consent prior to their participation in the study.

RESULTS

This cross-sectional hospital-based study had 200 and 195 HIV-positive and HIV-negative subjects respectively aged 15 to 64 years. The mean age of all subjects (mean ± SD) was 43.75 years ±11.56 with a standard error of 0.582. The mean ages (mean±SD) of the HIV-positive and HIV-negative subjects were 43.63 years±10.96 and 43.87 years ±12.18 respectively. In addition, HIV-positive subjects aged 40-45 years had the highest distribution 53(26.5%) likewise; the married 143(71.5%) and females 147(73.5%) [Table 1].

The characteristics of the HIV-positive subjects revealed that 55.00% acquired the virus via multiple sexual transmission route. Furthermore, the duration of HAART (less than 24 weeks), unknown HIV status of partner and CD4+ cell count of 200-350 cells/µL were 31.00%, 30.50% and 41.00% respectively [Table 2]. The clinical symptoms presented by the subjects included: cough, fever,

headache, cold and dyspnea of which cough was the most presented clinical symptom (31.39%).

Table 1: Socio-demographic characteristics of all subjects

Sociodemographic variables	HIV status	
	HIV Negative (%)	HIV positive (%)
Gender		
Females (n=265)	118 (60.51)	147 (73.50)
Males (n=130)	77 (39.49)	53 (26.50)
Marital status		
Married (n=276)	133 (68.21)	143 (71.50)
Single (n=119)	62 (31.79)	57 (28.50)
Age (years)		
Mean age±SD	43.87±12.18	43.63±10.96
15-29 (n=48)	26 (13.33)	22 (11.0)
30-39 (n=102)	51 (26.15)	51 (25.5)
40-49 (n=99)	46 (23.59)	53 (26.5)
50-59 (n=87)	37 (18.97)	50 (25.0)
60-64 (n=59)	35 (17.95)	24 (12.0)
Total	**195 (100)**	**200 (100)**

**numbers in bracket represents the percentage prevalence of each group.*

The HIV-positive subjects were significantly associated with pulmonary aspergillosis than the HIV-negative subjects (p=0.001). Diagnostically, pulmonary aspergillosis presented in 104/200 (52%) of the HIV-positive subjects as demonstrated in table 3. The distribution of *Aspergillus species* revealed the most species as *A. fumigatus* with prevalence of 49.04% among the HIV-positive subjects and *A. niger* with prevalence of 42.59% among the HIV-negative subjects. The HIV-positive females 72(69.23%) were more susceptible to pulmonary aspergillosis than males 32(30.77%). The age distribution revealed that HIV-positive subjects of the age group 40-49 years were most susceptible 32(30.17%), followed by those of the age group 30-39 years 25(24.04%).The least age group of the HIV-positive subjects susceptible to pulmonary aspergillosis were those of the age group 15-29 years and > 59 years with prevalence of 12(11.54%) and 15(14.42%) respectively. There was a significant association between the gender (p = 0.014) of the HIV-positive subjects and prevalence of pulmonary aspergillosis equally the age (p=0.003).

Among the HIV-positive subjects, the mean CD4+ cell count (mean ± SD) of subjects with and without pulmonary aspergillosis was 194.70 cells/µL± 64.10 and 222.8 cells/µL ± 59.67 respectively [Table 4]. The HIV-positive subjects with pulmonary aspergillosis had significant association with a decreased CD4+ cell mean count (mean±SD) of 194.70 cells/µL ± 64.10 (p < 0.003).

Table 2. Characteristics of the HIV-positive subjects

Variables	Frequency	%age
HIV transmission route		
Multiple sex	110	55.00
Unknown	17	8.50
Unprotected sex	73	36.50
Duration of HAART		
More than 24wks	38	19.00
No HAART	100	50.00
Less than 24wks	62	31.00
HIV status of partner		
Negative	29	14.50
Nil	54	27.00
Positive	56	28.00
Unknown	61	30.50
Clinical symptoms		
Cough	124	31.39
Fever	81	20.51
Headache	69	17.47
Cold	57	14.43
Dyspnea	22	5.57
CD4 count		
<200	112	56.00
200-350	82	41.00
>350	6	3.00

Table 3: Association of Age, Gender with aspergillosis and distribution of *Aspergillus* species among the subjects

Variable	HIV status (%)		X^2-value	*p*-value
Number = 158	HIV-negative (jn=54)	HIV-positive(n=104)		
Gender				
Female	39(72.22%)	72(69.23%)	12.449	0.014*
Male	15(27.78%)	32(30.77%)		
Age (Years)				
15-29 years	4(7.41%)	12(11.54%)		
30-39 years	22(40.74%)	25(24.04%)	35.947	0.003*
40-49 years	13(24.07%)	32(30.77%)		
50-59 years	8(14.81%)	20(19.23%)		
60-64 years	7(12.96%)	15(14.42%)		
Aspergillus species				
A. flavus (n=26)	8 (14.81%)	18 (17.31)		
A. fumigatus (n=70)	19 (35.19%)	51 (49.04)	71.193	<0.001*
A. niger (n=42)	23 (42.59%)	19 (18.27)		
A. tamarii (n=20)	4 (7.41%)	16 (15.38)		
Total	**54(27.69)**	**104(52.00)**		

**=significant p-value<0.05, *numbers in bracket represents the percentage prevalence of each group.*

Table 4: Evaluation of the CD4 profile among HIV positive subjects with and without *Aspergillosis*

	Positive *Aspergillosis* (Mean±SD)	Negative *Aspergillosis* (Mean±SD)	t-value	*P* -value
CD4+ cell count (cells/µL)	194.70 ± 64.10	228.12 ± 59.67	2.954	0.003*
Range	101-352	111-353		
Median (IQR)	195.5 (128-233)	200 (196-271)		

**=significant p-value<0.05*

DISCUSSION

The prevalence of pulmonary aspergillosis was 52% in the HIV-positive subjects. Following the earlier studies from within and outside Nigeria, the prevalence in our study was higher than Ogba et al[8] and Nasir et al[14] who reported an incidence of 47.1% and 12.7% from Southern and Northern Nigeria respectively. Likewise, Kaur et al[15] who reported 16.9% and Prakash et al reported somewhat similar prevalence of 16.5% from India[16]. This discrepancies might be due to the differences in the sample size and the defining inclusion criteria employed although the methodologies were slightly similar. Clinicians are faced with the rising tide of pulmonary aspergillosis which have been associated with significant morbidity and mortality among immunocompromised patients especially those with HIV infection[1]. Our study highlights *Aspergillus* species as the etiology of pulmonary aspergillosis in HIV-positive individuals. The most prevalent specie was *A. fumigatus* with prevalence of 44.30%. This is similar with reports from within Nigeria[8, 14] and outside Nigeria[16].

Interesting, we reported the prevalence of pulmonary aspergillosis in immunocompetent individuals (HIV-negative subjects) whereas, mostly the immunocompromised patients are susceptible. However, the prevalence is significantly higher when compared to immunocompetent individuals. This is consistent with previous studies with reported similar incidence among immunocompromised or HIV-positive individuals[16-18] who are mostly affected with pulmonary aspergillosis due to a weakened immune system. In recent times, the emergence of reports on pulmonary aspergillosis in immunocompetent individuals have increased[16, 18-21]. The immunocompetent patients are generally asymptomatic unlike the immunocompromised (HIV-positive individuals) with presented symptoms as acute productive cough, fever, headache, cold and dyspnea as recorded in this study. More recently, reports have described patients having normal immunity with invasive lung infections caused by *Aspergillus* species, predominantly *A. niger* followed by *A. fumigatus* as reported in our study. This supports the fact that countries with a tropical climate, *A. niger* is the most common species isolated from immunocompetent individuals[22, 23].

The risk factors for pulmonary aspergillosis in HIV-positive individuals as low CD4+ cell count have been described in earlier study[11,24,25]. Overall, more than half of the HIV-positive subjects positive for pulmonary aspergillosis in our study showed significant risk factor of low CD4+ cell counts of <200 cells/µL. In our study, the associated mean CD4+ cell count of 194.70 $\pm$ 64.10 cells/µL was found in HIV-positive subjects with pulmonary aspergillosis. This is comparable with results of Kaur et al[14] and Roohani et al[19] who reported a mean CD4+ cell count of 194.70 $\pm$116.79 cells/µL and 101.70 $\pm$19.20 cells/µL respectively. Data from previous studies suggests that *Aspergillus* species be considered a potential etiologic in HIV-positive patients with CD4 counts below 200 cells/µL[14,15] which is attributed to the increased prevalence of pulmonary aspergillosis among HIV-positive individuals. As such can be elucidated by the depletion of neutrophils and macrophages seen in the progressive phases of HIV infection.

The prevalence of pulmonary aspergillosis was more in subjects with the age 30-59 years but declined among subjects above 59 years. The change in sexual behavior and economic productivity among individuals of this age bracket could predispose them to HIV and consequently pulmonary aspergillosis. Morbidity and mortality amongst this age group could result to significant loss of man-power. In this study, females were more infected than males which may be due to the differences in their body anatomy[25].

CONCLUSION

The high prevalence of pulmonary aspergillosis; the low CD4+ cell count of the HIV-positive subjects and the predominant *Aspergillus fumigatus* among the subjects emphasises the need for improved diagnosis in pulmonary infection caused by *Aspergillus* species particularly among HIV-positive subjects having low CD4+ cell counts.

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