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PREVALENCE OF SYMPTOMATIC OPPORTUNISTIC RESPIRATORY MYCOSES AND MYCOBACTERIUM TUBERCULOSIS AMONG HUMAN IMMUNODEFICIENCY VIRUS POSITIVE PATIENTS IN CALABAR, NIGERIA

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ABSTRACT

Objectives: This study was designed to determine the prevalence of symptomatic respiratory mycoses and Mycobacterium tuberculosis among Human Immunodeficiency Virus (HIV) sero-positive patients in Calabar, Nigeria. To correlate the relationship between respiratory mycoses, tuberculosis and cluster of density four (CD4) count levels among these subjects.

Methodology: The study population comprised of 272 known HIV positive and 59 HIV-non reactive subjects with respiratory symptoms, aged 17-70 years who were attending Anti-Retroviral (ARV) clinics and Directly Observed Treatment Short course (DOTS) centers. Sputum and blood samples were collected under standard aseptic conditions from all the subjects enrolled in the study for microscopy, culture and serology. Tuberculosis status was determined by microscopy using the Ziehl Neelsen method.

Results: The overall prevalence of pulmonary mycoses recorded in the study was 31.6%. *Candida albicans* was the most prevalent 32(11.8%) pathogen, while *Candida guilliermondii* was the least prevalent (0.4%). Tuberculosis (TB) infection rates were higher (17.3%) among the Human Immunodeficiency Virus sero-positive subjects than the Human Immunodeficiency Virus negative subjects (controls) (10.2%). Out of the 272 Human Immunodeficiency Virus positive subjects, 86(31.6%) had mycoses, 47(17.3%) had Tuberculosis while 21(7.7%) amongst them had mixed mycoses and Tuberculosis infections. There was a statistically significant relationship (X^2=4.48, p=0.03) between tuberculosis and mycoses among HIV positive subjects.

Conclusion: The importance of respiratory mycoses as an opportunistic infection among HIV patients cannot be over emphasized as subjects suffered more mycoses than tuberculosis infections.

Key words: Mycoses, Respiratory, HIV/AIDS, Tuberculosis.

INTRODUCTION

Opportunistic infections are those which occur when a person's immune system has been impaired by an infection, drugs or other diseases. The infecting agent or opportunist rarely causes infection in immunocompetent individuals[1]. Over the last several decades the advent of the Human Immunodeficiency Virus (HIV) pandemic has dramatically increased the number of persons who are severely immunocompromised. As a result, the incidence of invasive fungal infections of the lungs has risen substantially[2, 3]. This is important because, despite marked advances in antifungal therapy, infections caused by opportunistic fungi continue to be associated with high morbidity and mortality, and poor patient outcomes[3,4].

Mostly, the infections seen in Acquired Immunodeficiency Syndrome (AIDS) patients are endemic to the geographical region, and involve many organs and organ systems simultaneously with a tendency to disseminate[5]. Pulmonary mycoses often precede the appearance of other opportunistic infections, but frequently co-exist with other pathogens[6]. Infections of the Lower Respiratory Tract (LRT), tend to be more severe than those of the Upper Respiratory Tract (URT), and choice of appropriate antimicrobial is very important and may be life saving[2].

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Opportunistic respiratory fungal organisms such as *Candida* species, *Aspergillus* species, *Mucor* species, *Cryptococcus neoformans* and *Pneumocystis carinii* tend to cause pneumonia in patients with acquired defects in their defenses and are ubiquitous in nature[7, 8, 9]. Fungi that are members of the normal microbial flora are endogenous opportunists; *Candida* and related yeasts, while exogenous opportunists are made of fungi that are globally present in soil, water and air[10]. Infection occurs following the inhalation of spores, or conidia, or by the reactivation of a latent infection. Hematogenous dissemination frequently occurs, especially in an immunocompromised host[11, 12].

Nigeria is ranked third in the world in terms of the number of persons infected with HIV[13]. The national prevalence rate dropped from 5.8% in 2001, to 5.0% in 2003, and then to 4.4% in 2005. About 2.9 million Nigerians are estimated to be living with HIV[14]. Nigeria is in a state of generalized epidemic in all the States of the federation[13]. Cross River State with Calabar as the capital city is reported to have the highest number of persons infected with HIV, with infection levels rising from 4% in 1993–1994, to

Table 1 Prevalence of fungal pathogens in clinical samples by age

Age group (years)	No of Pathogens (n=272)	Clinical specimens	
		Sputum	Blood
15-24	18(6.6%)	*P. carinii* (3) *C. albicans*(7) *A. fumigatus* (2) *A. corymbifera*(2) *C. tropicalis* (2)	*C. neoformans* (2)
25-34	40(14.7%)	*P.carinii* (5) *A. flavus* (1) *A. fumigatus* (6) *A. corymbifera*(1) *C. tropicalis* (3) *C. albicans*(15) *A. niger* (2) *R. arrhizus* (2)	*C. neoformans* (5)
35-44	23(8.5%)	*P.carinii* (9) *A. flavus* (1) *A. fumigatus* (3) *R. arrhizus* (1) *C. dubliniensis* (1) *C. guilliermondii* (1) *C. albicans*(3)	*C. neoformans* (4)
45-54	11(4.0%)	*P.carinii* (1) *C. albicans* (6) *C. dubliniensis* (1)	*C. neoformans* (3)
55-64	6(2.2%)	*P. carinii* (2) *C. albicans*(1) *A. fumigatus* (2) *A. niger* (1)	0(0.0)
65-74	0(0.0)	0(0.0)	0(0.0)
Total	**98(36.0)**	**84**	**14**

0 denotes no respiratory fungal pathogen was present.

12% in 2003, although the results of the 2005 sero-prevalence survey showed a reduction from 12% to 6%[13]. The pattern of spread also indicates that young people, especially women below the age of 24 years are among the most vulnerable groups with prevalence rates of 6% higher than the national average of 4.4%[14].

Table 2 Prevalence of pulmonary mycoses among HIV positive subjects by gender.

	Gender		
Mycoses	**Male**	**Female**	**Total (%)**
Positive	30(28.6)	56(33.5)	86(31.6)
Negative	75(71.4)	111(66.5)	186(68.4)
Total	**105**	**167**	**272**

Tuberculosis (TB) is a major public health problem in Nigeria. The country is currently ranked fourth among the 22 high-burden countries of the world; with an incidence of 311/100,000 smear positive cases. The estimated mortality rate for the country was 81 cases per 100,000 populations which ranked second in Africa[15]. The global burden of TB has increased in developing countries of Africa and Asia where about two-thirds of the cases are seen[15].

The Nigerian National Tuberculosis and Leprosy Control Program (NTBLCP) was established in 1988 for effective TB control operating the Directly Observed Therapy (DOT). The goal of the National TB program is to reduce, the burden of TB by 2015 in line with the Millennium Development Goals (MDGs) and the STOP TB Partnership targets. Some of the targets are to detect at least 70% of the estimated infectious (smear-positive) cases and to achieve a cure rate of at least 85% of the detected smear-positive cases[15].

One of the major challenges facing TB control globally is the HIV epidemic[16]. TB is responsible for about 30% of deaths among HIV infected individuals[17]. The increasing frequency of TB/HIV co-infection has considerable impact on TB control in Sub-Saharan Africa making the achievement of the 85% success rate a big challenge[18].

TB has been a co-infection with opportunistic fungi since the seventeenth century. The fungus, Candida was isolated by Bennett in 1884 in the sputum of a tuberculotic patient[19]. Pulmonary cryptococcosis can also occur in concomitance with other infectious pathogens such as, *Mycobacterium species,* and *Pneumocystis carinii*[5]. Shailaja *et al*[6] established a co-infection between TB and opportunistic respiratory mycoses in HIV-infected patients. They recorded 26.6% polymicrobial isolation from their subjects. *M. tuberculosis* had a prevalence of 42.89%. Among the 27 fungal isolates from their subjects, 9 were pathogenic (12.83%) and included *Cryptococcus neoformans, Aspergillus niger* and *Candida albicans.*

Wadhwa *et al*[5] in North India also recorded 30% prevalence for TB, 8.3% for *Pneumocystis* pneumonia and cryptococcal meningitis. Only a few cases of *Candida* pneumonia and invasive pulmonary aspergillosis were established. Those findings were

Table 3 Prevalence of TB among HIV seropositive and seronegative subjects by gender

	HIV positive subjects (Test)		**HIV negative Subjects (control)**	
Gender	No Examined	TB Positive (%)	No Examined	TB Positive (%)
Male	105	22(46.8)	33	2(33.3)
Female	167	25(53.2)	26	4(66.7)
TOTAL	**272**	**47(17.3)**	**59**	**6(10.2)**

important as most fungal infections and TB seen in the patients were AIDS-defining illnesses manifesting at significantly lower cluster of density four (CD4) counts.

Various mycoses form the bulk of opportunistic infections in the respiratory system of these patients and there is an increase in the number of patients co-infected with HIV and TB in developing countries. Studies are needed to define populations at high risk of these infections who might benefit from targeted antifungal and antibacterial chemoprophylaxis, which remains the most promising of the potential preventive strategies. This study is the first of its kind to be carried out in Calabar, Nigeria.

Table 4 Prevalence of mycoses and TB among HIV positive subjects

Type of infection	No(%) positive (n=272)
Mycoses	86(31.6)
TB	47(17.3)
Mixed Mycoses/TB	21(7.7)

MATERIALS AND METHODS

The study area was Calabar, comprising Calabar South Local Government Area and Calabar municipality, both in Cross River State, Nigeria. The city of Calabar (4857N, 8819E) lies in the South-South geopolitical zone of Nigeria. Study participants were recruited from patients with HIV/AIDS attending the antiretroviral clinics at two tertiary hospitals: the University of Calabar Teaching Hospital (UCTH) and Lawrence Henshaw Hospital (LHH) located in Calabar municipality and Calabar South Local Government Area respectively. The study ran from May 2009 to July 2010.

Patients clinically diagnosed to have lower respiratory tract infections (RTIs) attending the University of Calabar Teaching Hospital (UCTH) and Lawrence Henshaw Hospital were included in the study. A total of 331 subjects comprising 272 known HIV seropositive subjects and 59 HIV non reactive subjects with respiratory symptoms (controls) were enrolled for the study. The inclusion criteria were; the ability to produce sputum and determination of CD4 counts. All the patients were initially screened for anti HIV antibodies before being enrolled in the study with the Determine test kit Abbott Japan and Start Pak diagnostic system, Chembio Incorporated, New York, United States of America (USA), according to the manufacturer's instruction. Subjects were enrolled after obtaining due approval from the Ethical Research Committee, University of Calabar Teaching Hospital, Calabar, Cross River State. Informed consent was sought from every subject prior to data collection.

A pre-designed protocol which was approved by the Ethical Research committee of UCTH, was administered by Research Assistants (Medical Laboratory Scientists and Nurses) to every participant to determine their biodata and clinical conditions. Literate subjects completed the questionnaire themselves, while illiterate ones were assisted by the investigator(s) after verbal response to questions.

Table 5 Comparative mean CD4 counts of HIV positive subjects with mycoses and TB

Type of infection	**No. of subjects (n=272)**	**Mean CD4 counts**	**Statistics**
TB	47	260.8±182.7	(t=3.71, p=0.02)
Mycoses	86	142.3±100.1	

Two repeated samples of early morning expectorate sputum[20] and 5ml of blood were collected from all the subjects under universal aseptic precautions in suitable sterile containers by Laboratory scientists and Nurses. The blood samples were used for; re-confirmation of HIV status of the subjects before enrolling them for the studies, CD4 counts determination and serological tests for Cryptococcal antigen by latex agglutination method.

Processing of specimen:

Direct microscopy using 10% Potassium Hydroxide (KOH) mount, India ink preparation, Gomori's methenamine silver stained (Grocott's stain) and wherever indicated Gram stain was carried out for each of the samples. Sabouraud Dextrose agar with and without chloramphenicol ($16\mu gml^{-1}$) in duplicates (incubated at 37°C and 25°C) alongside caffeic agar plates incubated at 37°C were used for isolation of pathogens. Antigen detection by latex agglutination was carried out for *Cryptococcus neoformans*, using the Cryptococcal Latex

Agglutination System (CALAS) (Wampole laboratories, USA) according to manufacturer's instruction[5, 21]. Identification of isolates was based on gross morphological characteristics and detailed study of microscopy and biochemical tests[6, 22]. Identification and speciation of yeast isolates was based on germ tube production, morphology on corn meal agar with Tween 80, rapid urease utilization test and diphenol oxidase production. All *Candida* isolates were identified to specie level with the Microxpress Candida identification kit (Tulip diagnostic Ltd, India) according to manufacturer's instruction[6, 22].

TB diagnosis was limited to sputum smear positive cases by Ziehl-Neelsen (ZN) test because of lack of facility for TB culture. Patients were considered smear positive, if the two sputum specimens were positive for Acid-Fast Bacilli (AFB) by microscopy. Chest radiographic examinations were not carried out. The sputum grading for AFB positive smears were as follows; scanty, (+) when 3-9 bacilli are seen in the entire smear, (++) when 10 or more are seen in the entire smear and (+++) when 10 or more are seen in most of the fields. Microscopy of sputum is of great value in detection of infectious cases of TB[23].

Data analysis

The data obtained in this study were analysed with Epi-Info CDC, 2000 and Microsoft excel data analysis packages. Descriptive statistics were carried out. Frequencies were calculated for categorical variables. The interactions between specific categorical clinical variables were tested for significance using the $\chi 2$ test. T- Test was used to compare the means between two variables. A P-value of less than or equal to 0.05 was considered statistically significant.

RESULTS

Out of the 272 HIV reactive subjects, 105(39.0%) were males, while 167(61.0%) were females with a male to female ratio of 1: 1.6. Subjects had a mean age of 34 ± 11years, with a minimum age of 17 years and a maximum age of 70 years. Most 104(38.2%) of the subjects belonged to age group 25-34 years followed by those aged 35-44 years with 63(23.2%), thus 167(61.4%) of the subjects were within age group 25-44 years.

The commonest clinical conditions were cough 234(86.0%), fever 90(33.1%), sinusitis and chest pain 68(25.5%) respectively. Others were tuberculosis (17.3%) and hemoptysis (11.0%). Other less frequent conditions like, weight loss, pneumonia, headache, asthma, pleural effusion, dryness of throat, supurative lung disease and difficulty in breathing occurred in less than ten percent of the subjects.

The highest prevalence 40(14.7%) of fungal pathogens occurred among subjects aged 25-34 years. Subjects aged 55-64 years had the least prevalence of 6(2.2%), while subjects aged 65-74 years had no respiratory pathogens. The most frequently isolated fungus was *Candida albicans* with a prevalence of 32(32.7%) whose isolation peaked amongst subjects aged 25-34 years. *Aspergillus* species*, Absidia corymbifera, Rhizopus arrhizus,* and non albicans *Candida* infection were not reported amongst subjects aged 45 years and above (Table 1).

A total of 86(31.6%) subjects had pulmonary mycoses of different types (Table 2). Out of these 56(33.5%) were females, while 30(28.6%) were males. This is similar to the report of Aluyi *et al* (2010) with higher infection rates 86(44.1%) in females than 80(41.0%) in males. There was no statistically significant influence of gender on the prevalence of respiratory mycoses (X^2=0.28, p=0.3, df=1).

Out of the 272 HIV seropositive subjects, 47(17.3%) were TB positive. Females were more infected 25(53.2%) than males 22(46.8%). Also among the controls, 6(10.2%) were TB positive; females were more infected 4(66.7%) than males 2(33.3%). There was no statistically significant influence of gender on TB (X^2=1.73, P=0.2) (Table 3).

Out of the 272 HIV positive subjects, 86(31.6%) had mycoses, 47(17.3%) had TB, while 21(7.7%) had mixed mycoses and TB infections. There was a statistically significant relationship between TB and mycoses among HIV positive subjects (X^2=4.48, P=0.03) (Table 4).

The mean CD4 count of HIV positive subjects with mycoses was 142.3±100.1 cells/µl, while that of TB positive subjects was 260.8±182.7 cells/µl. The mean CD4 count of HIV positive subjects with TB was higher than those with mycoses (t=3.71, P=0.02) (Table 5).

The critical value of CD4 count during the research was 250 cellsμl^{-1}, 126 (46.3%) had CD4 counts between 0-250 cells/µl (Fig. 1).

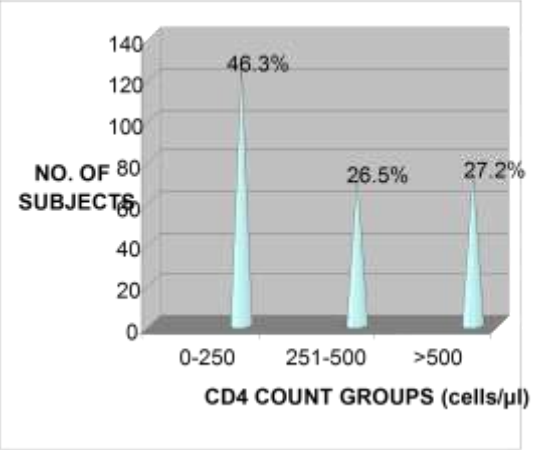

Fig. 1:CD4 count distribution among HIV seropositive subjects.

DISCUSSION

In this study, a higher prevalence of pulmonary mycoses was recorded amongst females than males. This is similar to the report of Aluyi *et al*[24] with a higher infection rate (44.1%) in females than (41.0%) in males. There was no statistically significant influence of gender on the prevalence of respiratory mycoses (X^2=0.28, p=0.5), as both males and females suffered immunosuppresion resulting in opportunistic infections.

We found that pulmonary mycoses were most prevalent in the age group 25–34 years, and least prevalent in those aged 55 years and above. This observation is similar to the report of Aluyi *et al*[24], where the highest prevalence (37.9%) of pulmonary mycoses was seen among subjects aged 21–30 years. The high prevalence of infection associated with this age bracket may be related to the high sexual activity of subjects in this age group, as HIV infection primarily occurs through heterosexual transmission in Nigeria[25].

Although TB was the most common non-fungal etiologic agent encountered in the study with 47 (17.3%) prevalence. This is lower than the 30% reported in Zaria, Nigeria by Onyemelukwe and Musa[26], the 32.8% reported in Ibadan, Nigeria by Awoyemi *et al*[27] and the 42.9% reported in India by Shailaja *et al*[6]. The low prevalence reported in this study may be due to the effectiveness of DOTS to the HIV positive subjects which has been associated with 90% sputum conversion in two months and no relapse after 18 months[28]. It could also be due to the fact that TB culture was not carried out during the study because of lack of facility Among the HIV non reactive group 6(10.2%) of the subjects had TB infection. The lower rates of TB infection among the HIV negative subjects could be due to the absence of TB/HIV co-infection which results in reactivation of latent infection culminating in higher TB burden among HIV patients. It is important to note that most of the HIV positive subjects came from tertiary health facilities where DOTS was administered.

This study also encountered subjects with mixed mycoses and TB infections 18(15.3%). Multiple infections could have been due to severe immunosuppresion by the HIV virus, thus making the patients more susceptible to opportunistic infections.

The CD4 cell count has been used extensively as a surrogate marker for HIV disease progression and an excellent indicator of an HIV-infected patient's risk of developing a specific opportunistic infection or neoplasm[28, 29]. Pulmonary complication of HIV infection studies has demonstrated that respiratory symptoms are increasingly frequent as CD4 counts decline to <200 cells/µl[29]. In Nigeria, the CD4 cell counts in healthy individuals have been found to range from 500-1500 cells/µl of blood[28]. A total of 126 (46.3%) subjects had CD4 counts between 0-250 cells/µl (Fig. 1), signifying a major population with severe immunosuppresion. The WHO recommendation is on earlier treatment for HIV patients, when their CD4 counts fall to 350 cells/µl or less, regardless of symptom[30]. This recommendation was yet to be implemented in the Institutions at the time of the research. In this study HIV positive subjects with mycoses had a mean CD4 count of 142.3±100.1 cells/µl, while those without mycoses had a mean CD4 count of 435.4±249.1 cells/µl. There was a statistically significant difference between the mean CD4 counts of subjects with mycoses and those without mycoses (t=10.5, P<0.05). This is similar to the findings of Njoku *et al*[31] in Nigeria where a mean CD4 count of 369.0±14.0 cells/µl for HIV-asymptomatic patients and 163.0±13.0 cells/µl for symptomatic patients were reported. The difference in the mean CD4 counts among these subjects has revealed that respiratory mycoses may have facilitated the lowering of the CD4 counts among the HIV positive subjects significantly.

Pulmonary TB in HIV patients should be considered at any CD4 count level, but are more

common when the count is <200 cells/µl[28]. In this study the mean CD4 counts of subjects with TB (260.8±182.7 cells/µl) was significantly higher than those with mycoses (142.3±100.1 cells/µl). This implies that aside immune suppression by HIV-TB co-infection, mycoses may have led to further immune suppression of these subjects with resultant increase in morbidity and mortality rates among these subjects.

CONCLUSION

Pulmonary mycoses remains an under diagnosed problem because it is neither given attention nor investigated like other opportunistic infections (e.g. TB) among HIV/AIDS patients. It is a leading cause of morbidity and mortality among these patients. Subjects with mycoses had CD4 counts lower than their counterparts with TB which suggests that respiratory mycosis further depletes their CD4 counts.

RECOMMENDATIONS

1. All HIV positive patients with respiratory symptoms should be routinely investigated in the laboratory for mycoses alongside other opportunistic infections because no constellation of symptoms, physical examination findings and chest radiographic findings is pathognomonic or specific for a particular pulmonary mycosis.
2. ART centers should implement the WHO, 2010 guidelines for therapy, as earlier treatment will boost the immune system, making it less likely that the patient falls sick, with mycoses and other opportunistic diseases which prey on weakened immune systems. It will also prolong and improve quality of life, reduce death rates, morbidity, HIV and TB transmission.

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