

## INCIDENCE OF PULMONARY MYCOSES IN PATIENTS WITH ACQUIRED IMMUNODEFICIENCY DISEASES

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### ABSTRACT

**Background:** Fungal infections are common complications of AIDS and pulmonary complications remain a major cause of both morbidity and mortality in immunocompromised patients. Such complications can also result in life threatening meningitis and discomforting if not debilitating thrush. The impact of the Acquired Immunodeficiency Syndrome (AIDS) on the incidence of mycoses is difficult to predict but is likely to be substantial. Retrospective studies in Africa and USA have indicated that 58% to 81% of patients with AIDS develop a mycosis.

**Objectives:** The objectives of the study were to determine the prevalence of pulmonary mycosis in AIDS patients with complications of cough, determine if there is any relationship between AIDS and pulmonary mycoses and determine if there is any difference in the prevalence of pulmonary mycoses in AIDS patients on anti retroviral drugs and those not on drugs.

**Methods:** A total of 195 sputum samples were obtained from patients diagnosed with full blown Acquired Immune Deficiency Syndrome (AIDS) who had been sick between 6 months to 3 yrs with CD4 count less than  $200/\text{mm}^3$  presenting with cough at the University of Benin Teaching Hospital (UBTH) Edo State. 55 (28.2%) of population studied had been on anti-retroviral medication, with the remainder on none during the study period. Forty other sputum samples were obtained from apparently healthy (HIV Seronegative) persons also based in Benin City as controls. All subjects were grouped into  $<20$  (3.1%), 20-30 (32.3%), 31-40 (30.3%), 41-50 (17.4%),  $\geq 51$  (2.1%) age groups. Test and control samples were cultured on Sabouraud Dextrose Agar and Potato Dextrose Agar and incubated at  $37^\circ\text{C}$  and room temperatures respectively with daily observation for growth for 2 weeks. Cultural and morphological characteristics, KOH mount and Lactophenol Cotton Blue staining were used to identify opportunistic fungal pathogens.

**Results:** One hundred and forty (71.8%) of test samples yielded fungal pathogens while 55 (28.2%) yielded no growth. 3 (7.5%) of control samples yielded fungal growth. Fungal organisms isolated were: *Candida albicans* (19.0%), *Candida stellatoidea* (9.7%), *Cryptococcus neoformans* (9.7%), *Candida parapsilosis* (9.7%), *Torulopsis glabrata* (5.6%), *Mucor* spp (7.2%), *Penicillium marneffeii* (4.1%), *Rhodotorula rubra* (3.6%) and *Fusarium* spp (3.1%) in that order. All (9) organisms were isolated from patients within 21-30 and 31-40 age groups; 8 organisms from 41-50 age group and 3 organisms each from  $\leq 20$  and  $\geq 50$  age brackets. *Candida albicans* and *Cryptococcus neoformans* occurred in all age groups.

**Conclusion:** Findings suggest that routine management (treatment) of pulmonary opportunistic mycoses in AIDS patients should include treatment for Candidiasis and Cryptococcosis for all age groups as well as additional antifungal agents if patients fall within 21-45 age group.

**Key Words:** pulmonary mycoses, incidence, HIV/AIDS, illness

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### INTRODUCTION

Acquired Immuno Deficiency Syndrome (AIDS), is a clinical syndrome (a group of various illnesses that together characterize a disease) that results from damage to the immune system caused by infection with the human immunodeficiency virus<sup>1</sup>. The current definition of acquired immune deficiency syndrome (AIDS) include individuals who test positive for human immune deficiency virus (HIV) and have a CD4 (cluster of differentiation antigen) T

cell number of less than  $200/\text{mm}^3$  (the normal count is  $600 - 1000/\text{mm}^3$ ) of whole blood or a CD4 T cell number of  $\geq 200/\text{mm}^3$ .

In addition, there is presence of opportunistic diseases such as fungal diseases including candidiasis, coccidioidomycosis, cryptococcosis, or toxoplasmosis of the brain. Others are bacterial diseases including pulmonary tuberculosis and other *Mycobacterium* spp infections or recurrent salmonella septicemia, as well as diseases including cytomegalovirus infections, chronic ulcers or bronchitis due to herpes simplex or progressive multifocal leuko-encephalopathy, malignant diseases

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such as invasive lymphoma<sup>2</sup>. Besides, some other diseases which may be overlooked, but require consideration, include those caused by *Strongyloides stercoralis*, *Pseudomonas aeruginosa* and measles virus<sup>3</sup>.

The end result of HIV infection is that CD4 cells progressively decline in number. This has serious health consequences. In a normal human, CD4 cells constitute about 70% of the total T cell pool. In AIDS patients, the number of CD4 cells steadily decreases and by the time opportunistic infections set in, CD4 cell may be almost absent. As CD4 cells decline in number, there is a concomitant loss in the cytokines they produce. Since cytokines influence the production and maturation of other lymphocytes, this leads to the gradual reduction of uninfected T-cells and eventually, all other lymphocytes, thus shutting down the immune system in those suffering from clinical AIDS. This loss of both humoral and cellular immune function is readily apparent in the opportunistic infections observed. Systemic infections by fungi and Mycobacteria point to a loss in TH1 cellular immunity. Other opportunistic infections such as the various viral and bacterial infections associated with AIDS, indicate the loss of humoral immunity. Decline in antibody production is due to the loss of TH2 cells necessary to stimulate antibody production by Beta cells.

The overall picture of AIDS progression indicates that during the clinical latency period, a very active infectious process is proceeding. First, there is an intense immune response to HIV of which about 1 billion virions are destroyed each day and HIV number drops. However, this means that HIV is replicating at a very high rate and this replication results in the corresponding destruction of about 100million CD4 T cells each day. Eventually, the immune response is simply overwhelmed, HIV levels increase and the T cells are finally completely destroyed thus crippling the immune responses and allowing the emergence of opportunistic infections<sup>4</sup>. Fungi are well recognized causes (among others), of opportunistic diseases in most, if not all full blown AIDS cases. Most of the diseases they cause (which are superficial), include dermatophytosis (skin, nail and hair infections) and candidiasis. However, extreme deforming and potentially fatal deep systemic infections can also occur<sup>5</sup>. Fungal infections are common complications of AIDS and pulmonary complications remain a major cause of both morbidity and mortality in immunocompromised patients<sup>6</sup>. Such complications can also result in life threatening meningitis and discomforting if not debilitating thrush. The impact of the Acquired Immunodeficiency Syndrome (AIDS) on the incidence of mycoses is difficult to predict but is likely to be substantial. Retrospective

studies in Africa and USA have indicated that 58% to 81% of patients with AIDS develop a mycosis<sup>7</sup>.

The most common is oral candidiasis which can begin during the AIDS related complex (ARC) or later. Cryptococcosis is a potentially lethal infection which has occurred in roughly 68% of patients in the United States<sup>8</sup>. If this prevalence rate is current, the actual incidence of this mycosis should be at least double in the United States, compared to the decade prior to AIDS. Although mycoses exhibit an extraordinary heterogeneity, they have certain features in common. Infection is usually acquired from nature, not from infected humans or animals. Certain types of ringworm and candidiasis are notable entry points for systemic mycoses. Systemic mycoses are fungal infections which involve deep organs. While some often referred to as the endemic mycoses infect healthy individuals, others are opportunistic infections which occur in patients with some underlying predisposition. In recent years, systemic mycoses such as cryptococcosis and histoplasmosis have become prominent as secondary complications in patients with AIDS, although it is of interest that other systemic mycoses have not increased in this population. Generally, while in most developed countries, the opportunistic infections are seen more commonly, the endemic fungal infections are seen more frequently in the tropical areas<sup>4</sup>.

Four stages are recognized in the progression of HIV disease and these are: primary infection/seroconversion, clinically latent period, early HIV disease and late HIV disease/AIDS. In advanced HIV disease with severe immuno suppression, there is a decrease in the number of CD4 T cells to below 400 cells/ml (the normal CD4 cell count in HIV negative adult is about 1000 cell/ml<sup>4</sup>. At CD4 T cell level below 200 cells/ml, fungal diseases including: candidiasis, coccidioidomycosis, cryptococcosis, histoplasmosis, pulmonary aspergillosis, penicillosis and pneumocystis pneumonia are often found in such patients. In a healthy immuno competent individual, these fungal pathogens do not pose any problem because they are always countered by the host immune mechanism. The destruction of the host immune mechanism in AIDS patients makes the affected individual vulnerable to non opportunistic organisms as those responsible for the diseased conditions mentioned above. Pulmonary mycoses are common diseases associated with HIV/AIDS patients<sup>2</sup>.

The aim of this research is to study pulmonary mycoses incidence in patients with acquired immunodeficiency diseases with the following objectives:

(a) To determine the prevalence of pulmonary mycoses in AIDS patients with symptoms of cough.

- (B) Determine if there is any relationship between AIDS and Pulmonary mycoses.
- (C) Determine if there is any difference in the prevalence of pulmonary mycoses in AIDS patients on antiretroviral drugs and those not on Drugs.

## MATERIALS AND METHODS

One hundred and ninety five (195) sputum samples were collected from patients diagnosed with HIV/AIDS and presenting with cough at the University of Benin Teaching Hospital, Edo State, Nigeria. 40 other samples were obtained from apparently healthy persons based in Benin City, Edo State capital. All sputum samples were collected with sterile screw capped plastic containers. Out of the 195 samples, 32 were obtained from patients who were on anti-retroviral therapy. To obtain good samples, sterile screw capped plastic containers were given out to patients a day prior to sample collection. Patients were instructed to cough out sputum upon waking up into the containers and bring them along to clinic same day. Samples were collected from patients and sent immediately to the laboratory for culture and direct microscopy. Patient's biodata were obtained from them by oral questioning.

Primary identification of these opportunistic fungal organisms was based on their cultural characteristics on Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA). These characteristics included the colour, colonial morphology, texture, colony surface and underside. Others were germ tube production, formation of pseudomycelia and chlamydospores.

### Inclusion and Exclusion Criteria for Patient's Selection

Although this study was centred on HIV/AIDS patients, not all of them were eligible for sampling. Because of the difficulty in generating sputum, only debilitated patients that had persistent cough and were positive for tuberculosis (i.e Acid Fast Bacilli positive) as obtained from biodata were sampled while AIDS patients who had no cough and were negative for AFB were excluded.

### Cultural Studies

Sterile Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) were prepared (according to manufacturers instruction), sterilized by autoclaving at 121psi for 15mins, allowed to cool and while in molten form, modified by addition of 0.4g of chloramphenicol, 20,000 units of penicillin and 4,000 units of streptomycin sulphate each added to a litre of molten agar which was then dispensed into sterile Petri dishes, allowed to set and stored in the refrigerator. A plate of each medium was incubated at 37°C for 24hrs to check for purity.

With the aid of a sterile wire loop, the sputum

samples were inoculated on the SDA and PDA media and streaked out to obtain discrete colonies. These plates were then incubated at both room temperature and at 37°C in the incubator. Plates were incubated at room temperature and 37°C to aid growth of dimorphic fungi i.e. those that grow as yeasts (parasitic phase) at 37°C and as moulds (saprophytic phase) at room temperature. Cultures were observed daily for growth for 2 weeks. Gross colonial morphology, colour and texture of the colony surface and underside which can be very distinctive were observed.

### Direct Microscopy

A drop of sodium hydroxide (20% w/v) solution was placed on a slide. An aliquot of each sputum sample was transferred to the drop of sodium hydroxide on the slide and this was covered with a glass cover slip. This was then allowed to stay for 5-10 mins to enable it to clear, after which it was examined microscopically using X40 objective. Characteristic morphological elements such as nature and form of microconidia, macroconidia, spherule, fruiting bodies, sporangium, encapsulated cells, budding cells and broad based cyst, spherical fish like yeast cells were observed.

Direct microscopy of culture isolates was also done. A drop of lactophenol cotton blue was placed on grease free slide and a fraction of culture was transferred to drop of lactophenol cotton blue on the slide and covered with a glass cover slip. This was then examined microscopically using the X10 and X40 objectives with the condenser iris diaphragm closed sufficiently to give good contrast. Structures such as spherules, microconidia, pseudohyphae, encapsulated cells, sporangia were noted.

### Germ Tube Test

Human Serum (0.5ml) was pipetted into a small test tube. Using a sterile wire loop, the serum was inoculated with a yeast colony from the culture. The tube was placed in an incubator at 37°C for 2-3 hrs. With the aid of a Pasteur pipette, a drop of the serum yeast culture was transferred to a glass slide and covered with a coverslip. This was then examined using the X10 and X40 objectives.

### Formation of Pseudomycelia and Chlamydospores

Certain yeasts develop special fruiting forms (called chlamydospores) when grown in glucose free medium like Rice Agar. Isolates identified as yeasts were inoculated onto prepared sterile rice agar plates and incubated at room temperature for 24-48 hrs and observed for pseudomycelia and chlamydospore development.

## RESULTS

Based on the criteria for identification, identified, fungal isolates included *Candida albicans* (20.5%),

*Candida Stellatoidea* (9.2%), *Cryptococcus neoformans* (9.2%), *Candida parapsilosis* (8.2%), *Torulopsis glabrata* (7.7%), *Mucor spp* (7.2%), *Penicillium marneffei* (4.1%), *Rhodotorula rubra* (3.1%) and *Fusarium spp* (2.1%) in that order (Table 1). Of the 195 samples, 163(83.6%) produced fungal growth while the remaining 32(16.4%) samples (from patients on anti retroviral therapy), yielded no growth after two weeks of incubation. Three out of the 40 control samples (HIV Seronegative patients) yielded growth of fungal organisms.

The incidence of pulmonary mycoses was highest in the age group 21-30 with 63 isolates. Of these isolates, *Candida stellatoidea* had the highest occurrence of 10(5.1%) followed closely by *Candida albicans* and *Cryptococcus neoformans* with 9(4.6%) each. The least occurring organisms within the group were *Rhodotorula rubra* and *Fusarium spp* with 2 (1.0%) occurrences. The 31-40 age bracket had the next highest incidence of pulmonary mycoses with 59 isolates of which *Candida albicans* had the highest occurrence of 12(6.2%) followed by *Candida parapsilosis* and *Candida stellatoidea* having occurrence of 8(4.2%)

and 6(3.1%) respectively. The least occurring organisms in this age group were *Penicillium marneffei* and *Fusarium spp* 1 (0.5%) each (Table 1). The ≥ 51 age group patients yielded the least fungal isolates of which *Candida albicans* occurred highest with 2(1.0%) (Table 1). On the whole, *Candida albicans* occurred highest across the whole age groups with 37(19.0%) and then *Candida stellatoidea* 19(9.7%), *Cryptococcus neoformans* 19(9.7%), *Candida parapsilosis* 19(9.7%), *Mucor spp* 14(7.2%), *Torulopsis glabrata* 11(5.6%), *Penicillium marneffei* 8(4.1%), *Rhodotorula rubra* 7(3.6%) and *Fusarium spp* 6(3.1%) in that order (Table 1).

The occurrence of opportunistic pulmonary mycoses was higher in females 86(44.1%) than in males 80(41.0%) of which within the female patients, the highest occurrence was in the age group 21-30. The lowest occurrence was recorded in the ≥51 age group. The highest occurring organisms in females were *Candida albicans*, *Cryptococcus neoformans*, *Candida albicans*, *Candida parapsilosis*, *Candida stellatoidea* and *Cryptococcus neoformans* (Table 2).

**Table 1:** Age Distribution of Opportunistic Fungal Infections among HIV/AIDS Patients.

Age Group (Yrs)	Number of patients in Age Group	Candida albicans	Candida Stellatoidea	Cryptococcus neoformans	Candida Parapsilosis	Torulopsis Glabrata	Mucor Spp	Penicillium Marneffei	Rhodotorula rubra	Fusarium Spp
< 20	6	4(2.1%)	0(0.0)	1(0.5%)	1(0.0)	0(0.0)	0(0.0%)	0(0.0)	0(0.0)	1(0.5%)
21- 30	63	9(4.6%)	10(5.1%)	9(4.6%)	6(3.1%)	4(2.1%)	7(3.6%)	4(2.1%)	2(1.0%)	2(1.0%)
31- 40	59	12(2.2%)	6(3.1%)	4(2.1%)	8(4.2%)	4(2.1%)	4(2.1%)	1(0.5%)	5(2.6%)	1(0.5%)
41 - 50	34	10(5.1%)	3(1.5%)	4(2.1%)	3(1.5%)	3(1.5%)	3(1.5%)	2(1.0%)	0(0.0%)	2(1.0%)
≥ 51	4	2(1.0%)	0(0.0%)	1(0.5%)	1(0.5%)	0(0.0)	0(0.0)	1(0.5%)	0(0.0)	0(0.0)
<b>Total</b>	<b>166(85.1%)</b>	<b>37(19.0%)</b>	<b>19(9.7%)</b>	<b>19(9.7%)</b>	<b>19(9.7%)</b>	<b>11(5.6%)</b>	<b>14(7.2%)</b>	<b>8(4.1%)</b>	<b>7(3.6%)</b>	<b>6(3.1%)</b>

**Table 2:** Age and Sex Distribution of Opportunistic Fungal Pathogens among HIV/AIDS Patients on Nil Treatment and those on Anti Retroviral Therapy.

Age Group (Yrs)	Sex	Number of Patients in Age Group	Candida albicans	Candida stellatoidea	Cryptococcus neoformans	Candida parapsilosis	Torulopsis glabrata	Mucor spp	Penicillium marneffei	Rhodotorula rubra	Fusarium m spp	Duration of sickness (yrs)
< 20	M	2	2	0	0	0	0	0	0	0	0	8MTHS
	F	4	2	0	1	1	0	0	0	0	0	6MTHS
21-30	M	29	8	5	4	3	0	2	2	2	0	3
	F	34	1	6	5	6	2	4	5	1	2	2
31- 40	M	34	8	2	3	6	3	2	1	4	0	1
	F	25	4	3	4	2	3	5	0	3	2	3
41-50	M	14	4	2	0	2	2	1	1	0	0	2
	F	20	6	3	3	0	2	0	1	0	2	3
≥ 51	M	1	1	0	0	0	0	0	0	0	0	10mths
	F	3	1	0	1	1	0	0	0	0	0	6mths
<b>Total</b>	<b>M</b>	<b>80(41.0%)</b>	<b>23</b>	<b>8</b>	<b>7</b>	<b>11</b>	<b>5</b>	<b>5</b>	<b>3</b>	<b>6</b>	<b>0</b>	
<b>Total</b>	<b>F</b>	<b>86(44.1%)</b>	<b>14</b>	<b>12</b>	<b>14</b>	<b>10</b>	<b>7</b>	<b>9</b>	<b>6</b>	<b>4</b>	<b>0</b>	

## DISCUSSION

Of the nine fungal isolates, *Candida* spp, *Cryptococcus neoformans*, *Mucor* spp, and *Penicillium marneffeii* are known fungal pathogens of man irrespective of the immunological status of man. Female patients occurring in the age group 20-30 who had been sick for 2yrs had the highest occurrence of fungal organisms while the least occurred in  $\geq 51$  age group patients who had been sick for 6 months. In the male patients, the highest fungal occurrence was in the 31-40 age group patients who had been ill for 10 months (Table 2). *Torulopsis* spp, *Rhodotorula rubra* and *Fusarium* spp, which generally are not common pathogens in immunocompetent individuals, may become pathogenic in immunocompromised individuals (as in AIDS patients).

Opportunistic fungal infections are usually associated with human Immunodeficiency virus and acquired immunodeficiency syndrome<sup>9</sup>. Out of the 195 sputum samples obtained from AIDS patients with symptoms of cough, 163 (83.6%) of them produced fungal growth. The remaining 32 samples that produced no fungal growth were from patients on anti-retroviral drugs. The absence of fungal growth is due to Anti Retroviral Drug (ARD) therapy, which points to the effectiveness of these drugs in checking HIV/AIDS.

*Candida* species especially *Candida albicans* were the highest implicated fungal pathogens. Generally, *Candida* spp is the most common causative agent of opportunistic fungal infections in HIV/AIDS patients<sup>9</sup>. Candidal infection of the oesophagus, trachea, bronchi or lungs are recognized as indicator diseases of AIDS. Baily *et al.*<sup>10</sup> reported that the incidence of overall rates of candidiasis of the esophagus and respiratory tract among patients infected with HIV is as high as 30-40%. This is similar to our finding of 38.4%. The occurrence of *Cryptococcus neoformans* was found to be 9.7% among samples investigated. Infection rates of this organism in the tropics are not known but it appears to be variable even in patients with AIDS<sup>11</sup>. Also, there is evidence that in Zaire, about 12% of those with AIDS have circulating Cryptococcus antigen indicating active infection while in Great Britain, it has been reported to occur in 3.8% of AIDS patients<sup>12</sup>. Dismusks<sup>13</sup> reported that the incidence of Cryptococcus in AIDS patients in United State of America ranges from 6% to 12%. *Penicillium marneffeii* in this study, shows an incidence of 4.1% much lower than the findings of Supratinyo *et al.*<sup>14</sup> who reported an incidence of 10% of this organism in AIDS patients in Hong Kong. Intense global travel could be responsible for its spread to Nigeria. *Mucor* spp had an occurrence of 7.2%. According to Cheesbrough<sup>15</sup>, *Mucor* infection causes severe

paranasal, pulmonary or disseminated infection in those with reduced host defenses as in AIDS patients. *Rhodotorula rubra* has recorded an incidence of 3.6%. Golden *et al.*<sup>16</sup> reported its isolation in patients with meningitis, endocarditis and sepsis. It is an unusual opportunistic mycosis<sup>17</sup>. *Torulopsis glabrata* which showed an occurrence of 5.6%, is one of the unusual fungal pathogens rarely isolated and finally *Fusarium* spp which are well known as plant pathogens, showed an occurrence of 3.1%. This organism is involved in secondary infection of the lungs of tubercular or immunocompromised patients<sup>17</sup>.

Patients within the age group of 21-30 recorded the highest incidence of pulmonary mycoses. The most probable reason may be due on the way to the high activity of patients in this age bracket and being that AIDS takes 5-10 years to manifest, HIV infection must have been acquired much earlier in life. Patients who had been sick between 1-3 years seemed to have more incidences of mycoses than those just entering the AIDS phase from HIV with few months of illness. This is quite expected because years into the AIDS phase, nearly, if not all the immunity and immune system would have been paralyzed thus making patients highly vulnerable to infection by opportunistic mycoses.

## CONCLUSION

There was a high incidence of pulmonary mycoses in HIV/AIDS patients than in apparently healthy individuals as 163 (83.6%) of patients sampled had fungal infection of the upper and lower respiratory tracks. Female AIDS patients had higher incidence of opportunistic mycoses than males and patients with longer duration of illness in AIDS phase showed higher incidence of mycoses.

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