

The Association between Pulmonary Aspergillus infections and the Immune Status of HIV/AIDS Subjects with Respiratory Symptoms

Ofonime M. Ogba^{1*}, Lydia N. Abia-Bassey¹, James Epoke¹

¹Department of Medical Laboratory Science, Faculty of Allied Medical Sciences,
University of Calabar, Nigeria

Abstract: Fungal respiratory disease results in high mortality among the immunocompromised, such as HIV/AIDS patients. An early diagnosis of invasive aspergillosis based on mycological evidence is difficult to establish.

Methods: HIV-positive subjects with respiratory symptoms were enrolled for the study. Subjects selection was based on HIV screening and the ability to produce sputum. A structured questionnaire was administered to all the subjects after obtaining their informed consent for demographic data. Ethical approval was obtained from the ethical research committee, UCTH, Calabar, Nigeria. Blood samples were obtained for CD4 count determination to ascertain the immune status of the patients. Sputum samples produced early in the morning were obtained twice from the subjects and subjected to macroscopy, microscopy and culture. The immune status of the subjects was assessed by CD4 count levels. Identification to the species complex level was performed by macroscopic and microscopic morphology.

Results: *Aspergillus fumigatus* was the most prevalent isolate (76.5%). Pulmonary aspergillosis (47.1%) occurred mostly in subjects aged 20 – 30 years. Mixed infections with other opportunistic respiratory pathogens occurred (1.5%) at CD4 counts levels lower than 200, but there was no statistically significant effect of co-infection on the immune status ($H= 4.4, p = 0.22$). HIV positive subjects with *Aspergillus* infections had lower mean CD4 counts (168.0 ± 120.7) than those without this infection (354.4 ± 255.7) ($t = 2.97, p = 0.003$)

Conclusion: Pulmonary aspergillosis is a public health challenge among HIV/AIDS patients in our locality. There was a strong association between CD4 count and pulmonary *Aspergillus* infection among HIV-seropositive patients. These infections seem to reduce CD4 counts. The CD4 count could be used alongside non culture methods for prompt and early diagnosis which is essential for effective patient management and a better patient outcome.

Keywords: *Aspergillus* infections; HIV/AIDS; pulmonary; CD4 counts.

AUTHOR'S SUMMARY

Pulmonary aspergillosis is an opportunistic infection (OI). It is a devastating opportunistic infection in the severely immunocompromised. Nigeria is ranked third in the world in terms of the number of persons infected with HIV and as such immunosuppressed with OIs. Data on the spectrum of pulmonary aspergillosis are not available in Calabar, Nigeria. Clinical and immunological profile of infected patients is scarce. This work generated data on these areas and is the first of its kind. In this study an infection rate of 6.3% was recorded among the subjects. The infections were associated with mean CD4 counts of 168.0 ± 120.7 cells/ μ l of blood. The CD4 count could be used alongside non culture methods for prompt and early diagnosis. Further research is needed to understand if other immune defects such as impaired cytokine production are associated with pulmonary aspergillosis.

1. INTRODUCTION

Exposure to *Aspergillus* infection is global but aspergillosis is uncommon unless drugs, other infections, or malignancy have altered the host immune defenses. Aspergillosis is a fungal infection which is life threatening among the immunocompromised, especially human immunodeficiency virus (HIV) subjects [1].

Pulmonary aspergillosis is an opportunistic infection. Opportunistic infections (OIs) are main reason for diseased states and death among HIV/AIDS patients [2]. Data on the spectrum of pulmonary

aspergillosis are not available in Calabar, Nigeria. Clinical and immunological profile of infected patients is scarce. This work generated data on these areas and is the first of its kind.

HIV status in the Locality: HIV/AIDS is a huge problem in Nigeria whose population is about 160 million. Nigeria is ranked third in the world in terms of the number of persons infected with HIV [3]. An estimated 3.3 million people were living with HIV/AIDS in 2010, with 281,180 new AIDS cases and 215,130 AIDS-related deaths. About 25% of tuberculosis cases occur in HIV positive patients (51/100,000) and 210/100,000 occur in the total population annually for HIV negative and HIV positive cases respectively [4]. Cross River State was reported to have the highest number of persons living with HIV in Nigeria. The infection levels rose from 4% to 12% between 1993 and 2003. However, the results of the sero-prevalence survey carried out in 2005 revealed a reduction from 12% to 6% [2].

Relationship between HIV and CD4 counts: The HIV usually infects CD4 T-lymphocyte cells. These cells are crucial elements of the immune system as they are involved in the fight against infections. The HIV infects these cells and reproduces copies of viruses. Immunosuppression makes the patients liable to different respiratory syndromes including aspergillosis [5].

Acute invasive aspergillosis is a devastating opportunistic infection in the severely immunocompromised. These diseases predominate in those with low CD4 cell counts ($\leq 100/\mu\text{l}$), non-suppressed HIV ribonucleic acid (RNA) levels, and among subjects not receiving antiretroviral therapy (ART) [6]. Invasive aspergillosis principally involves the Sino pulmonary tract via inhalation of *Aspergillus* spores. Symptoms of pulmonary aspergillosis are non-specific includes: fever, cough, and dyspnea [7].

The gold standard in the diagnosis of IPA is by the microscopic demonstration of fungal hyphae in histologically stained lung tissue obtained by open-lung biopsy or thoracoscopy. The diagnosis is difficult and involves a positive culture results from normally sterile samples (e.g., pleural fluid) [8]. This prospective cross-sectional study was designed to assess: the spectrum and prevalence of pulmonary *Aspergillus* infections among HIV positive subjects in our locality and to correlate the relationship between probable invasive pulmonary aspergillosis and CD4 count levels among these subjects.

2. METHODS

Ethical approval was obtained from the ethical research committee, UCTH, Calabar, Nigeria. Subjects' enrollment was based on HIV-positivity, complaints of respiratory symptoms and the ability to produce sputum. All adult subjects provided written informed consent for sampling in addition to providing consent for the structured questionnaire. Parents or guardian of children participant provided informed consent on their behalf. A structured questionnaire was administered to all the subjects for demographic data, after obtaining their informed consent.

Study population: Human immunodeficiency virus positive subjects of both gender numbering 272 with respiratory symptoms were enrolled for the study. The HIV positive subjects without respiratory symptoms and those who were on antifungal chemotherapy in the past three weeks were excluded from the study.

Sample collection: Convenient sampling method was used. Sputum samples produced early in the morning were obtained twice from the subjects and subjected to macroscopy, microscopy and fungal culture. Five mls of venous blood samples were obtained for CD4 count determination to ascertain the immune status of the patients. Identification to the species complex level was performed by macroscopic and microscopic morphology.

Data analysis: Epi Info 2010 statistical software was used to analyse the data. The summary of the continuous variables were presented as means and standard deviation using one sample t-test. Frequencies were calculated for categorical variables. Interactions between specific categorical clinical variables were tested for significance using the χ^2 test.

3. RESULTS

Positive cultures were confirmed in the second samples submitted by subjects. Three *Aspergillus* species were encountered among subjects. *Aspergillus fumigatus* was the most prevalent isolate (76.5%) among subjects (Fig. 1).

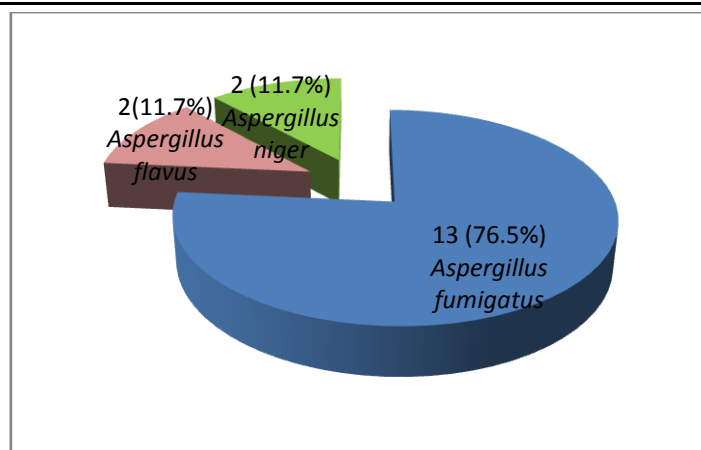


Fig 1. Types of *Aspergillus* species encountered among subjects

Fig 2 shows the distribution of *Aspergillus* infection among subjects by age. Pulmonary aspergillosis (47.1%) occurred mostly in subjects aged 20 to 30 years.

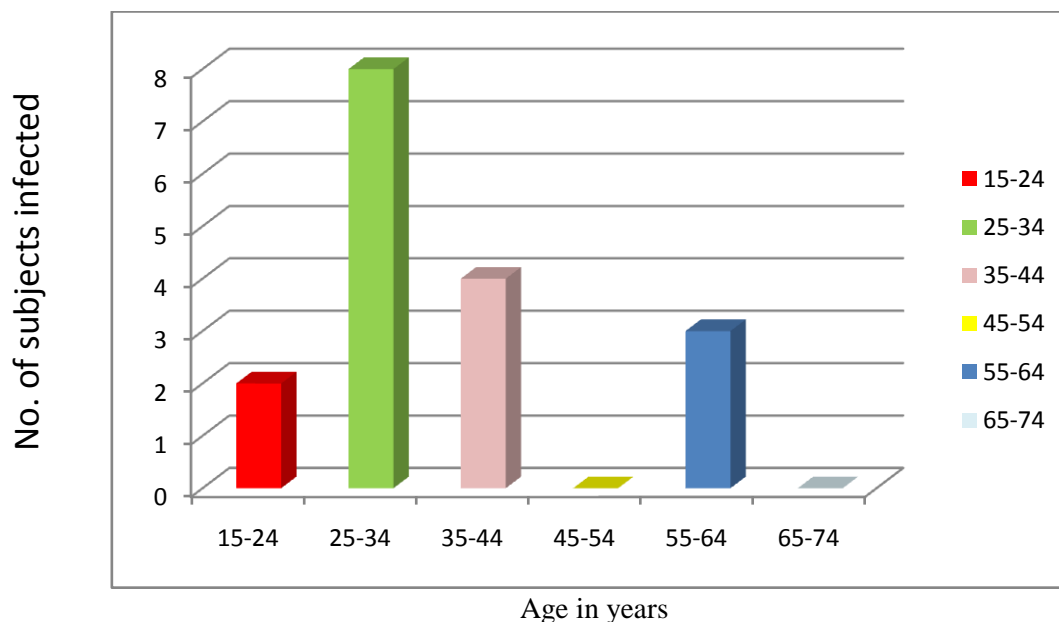


Fig 2. Distribution of *Aspergillus* infection among subjects by age.

The most common presenting symptoms among subjects positive for aspergillus infections were cough (17/17, 100%) and chest pain (8/17, 47.0%); other symptoms include; fever (6/17, 35.2%), hemoptysis (5/17, 29.4%), weight loss (5/17, 29.4%) and sinusitis (2/17, 11.7%). There was a statistically significant relationship between presenting symptoms and type of *Aspergillus* isolates among subjects ($\chi^2=106.3$, $p = 0.01$) (Table 1).

Aspergillus fumigatus was the most prevalent species (76.4%) among subjects with mean CD4 Counts 173.8 ± 131.9 . All *Aspergillus* infection occurred at CD4 count lower than 200/ μ l of blood. There was a statistically significant effect of the immune status of subjects on *Aspergillus* infection rates (Kruskal-Walis $H = 11.1$, $p = 0.01$) (Table 2).

Table 1. Presenting symptoms/complaints and types of *Aspergillus* isolates among subjects

Presenting symptoms	No (%) with <i>Aspergillus</i> infection (n=17)	Types of <i>Aspergillus</i> isolate associated with symptoms		
		Fumigates	flavus	niger
Cough	17(100)	12(70.6)	3(17.7)	2(11.7)
Chest pain	8(47.0)	6(75.0)	1(12.5)	1(12.5)
Fever	6(35.2)	3(50.0)	1(16.7)	2(33.3)
Hemoptysi	5(29.4)	3(60.0)	2(40.0)	0(0.0)

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Weight loss	5(29.4)	3(60.0)	1(20.0)	1(20.0)
Sinusitis	2(11.7)	2(100)	0(0.0)	0(0.0)
Pneumonia	3(17.6)	2(66.7)	0(0.0)	1(33.3)

Table 2 Frequency of occurrence of *Aspergillus* isolates with mean CD4 counts of subjects.

Aspergillus species	No (%) of isolates (n=272)	Mean CD4 counts of subjects	Statistics
A. flavus	2(11.8)	156.5±30.4	Kruskal-Walis H=11.1, p = 0.01
A. fumigatus	13(76.4)	173.8±131.9	
A. niger	2(11.8)	144.5±141.2	

Subjects with pulmonary aspergillosis (6.3%) had lower mean CD4 counts (168±120.7) than those without the infection (354.4±255.7). There was a statistically significant association between the immune status of subjects and the infection rates ((t = 2.97, p = 0.003) (Table 3).

Table 3. Comparative mean CD4 counts of HIV positive subjects with and without *Aspergillus* infection.

Aspergillus infection status	No. (%) of subjects	Mean CD4 counts	Statistics
Positive	17(6.3)	168.0±120.7	(t = 2.97, p = 0.003)
Negative	255(93.7)	354.4±255.7	

Mixed infections with other opportunistic respiratory pathogens occurred (1.5%) at CD4 counts levels lower than 200, but there was no statistically significant association between co-infection on the immune status (H= 4.4, p = 0.22) (Table 4).

Table 4. *Aspergillus* co-infections among subjects

Types of co-infections	No. of subjects	Mean CD4 counts	Statistics
<i>Aspergillus</i> & <i>Pneumocystis jiroveci</i>	2	125.5±79.9	(H= 4.4, p = 0.22)
<i>Aspergillus</i> & <i>Pseudomonas aeruginosa</i>	1	40.0±0.0	
<i>Aspergillus</i> and TB	1	199.0±0.0	

4. DISCUSSION AND CONCLUSION

The diagnosis of pulmonary aspergillosis remains challenging, early diagnosis is carried out with a high index of suspicion. In this study our subjects did not undergo lung surgery but high suspicion index comprising the symptoms, microscopic demonstration of septate branching *Aspergillus* hyphae accompanied by a positive culture result in sputum collected twice from the subjects.

All the subjects presented with productive cough, this is different from the report of Denning et al [9] whose subjects presented more frequently with non-productive cough. Chest pain, hemoptysis and fever were the most presenting symptoms among subjects with *Aspergillus fumigatus* infection. Subjects with *A. fumigatus* infections were more symptomatic than other subjects which may indicate a more invasive nature of these fungi.

Aspergillus species infections have been associated with significant morbidity and mortality among immuno compromised patients. In this study an infection rate of 6.3% was recorded among the subjects. This is lower than the 27.9% *Aspergillus* infection rate reported by Ochiabuto et al [10] in South Eastern Nigeria. The lower infection rates in our study could be due to the effectiveness of HAART therapy among the subjects as all subjects were enrolled from treatment centers.

Aspergillus fumigatus has been the most implicated in pulmonary infections. In this study, *A. fumigatus* accounted for 76.4% of the isolates followed by *Aspergillus flavus* and *A. niger* with 11.8% infection rates each. This is in agreement with the reports of Ochiabuto et al [10] in South Eastern Nigeria who also reported *Aspergillus fumigatus* as the most prevalent 41.2% species among their subjects. Patterson et al [11] also stated that *Aspergillus fumigatus* causes 60-90% invasive aspergillosis. On the other hand Bulpa et al [12] reported that 3.6% invasive pulmonary aspergillosis (IPA) were due to *A. niger*. Their report is lower than the 11.8% prevalence in this study which may have been due to the depressed immune status of the subjects.

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The mean age of subjects with infection was 28.4 ± 16.8 , with 70.6% in their second and third decade of life. Subjects in this age group were more infected probably because of their high sexual activity which may have resulted in high HIV transmission rates and the resulting opportunistic infections. On the other hand, subjects in this age bracket are also economically productive, therefore morbidity and mortality amongst them results in loss of man hours which affects their household and the Nation.

Holding and colleagues [13] reported the association of aspergillosis with CD4 lymphocytopenia among people with HIV infection. In this study all *Aspergillus* infection occurred at CD4 count lower than 200 cells/ μ l of blood. There was a statistically significant effect of the immune status of subjects on *Aspergillus* infection rates (Kruskal-Wallis $H = 11.1$, $p = 0.01$).

In the present study 17(6.3%) subjects with pulmonary aspergillosis were associated with mean CD4 cells of 168.0 ± 120.7 cells/ μ l of blood. This is in agreement with the reports of Khan et al [14] who stated that when the CD4 count is below $150/\text{mm}^3$, fungal infections such as *Aspergillus* should be considered invasive.

5. LIMITATIONS OF STUDY

Radiological diagnosis were not carried out which could have led to a more specific diagnosis. We did not carry out patients follow up for final patient's outcome. This could have revealed the mortality rate amongst our subjects.

6. CONCLUSION

There was a strong association between CD4 count and pulmonary *Aspergillus* infection among HIV-seropositive patients. The CD4 count could be used alongside non culture methods for prompt and early diagnosis which is essential for effective patient management and a better patient outcome. Further research is needed to understand if other immune defects such as impaired cytokine production, are associated with pulmonary aspergillosis.

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