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Full Length Research Paper

Cytologic Assessment of Pulmonary Aspergillosis in Immunocompromised Subjects in Maiduguri North Eastern, Nigeria

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Abstract

Immuno-suppression due to underlying disease states such as HIV/AIDS, immune-modulator therapy for the prevention of rejection in solid organ and hematopoietic cell transplantation and cancer therapies has lead to increase incidence of fungal infections (Libanore et al., 2002). Fungal infections of the lung are among the most feared infections in immune-compromised patients. Aspergillus is the most common ubiquitary opportunistic pathogen affecting the lung (Bernhard et al., 2008). *Aspergillus* forms a genus of ubiquitous, dimorphic molds present in soil, various types of organic debris, water, indoor environment, and many other sites (Al-Alawi et al., 2005; Zmeili and Soubani, 2007). The study was a cross sectional and prospective study. Seventy-seven (77) subjects made up of fifty (50) Human Immunodeficiency Virus (HIV) positive patients and Twenty-seven (27) HIV- negative patient from Direct Observed Treatment Shot course (DOTS) Clinic, University of Maiduguri Teaching Hospital, Nigeria. The sex distribution of Aspergillosis was highest in females in age group 21-30years and in males in age group 31-40years.

Keywords: Immuno-suppression, immune-compromised, *Aspergillus.*

INTRODUCTION

Immuno-suppression due to underlying disease states such as HIV/AIDS, immune-modulator therapy for the prevention of rejection in solid organ and hematopoietic cell transplantation and cancer therapies has lead to increase incidence of fungal infections (Libanore et al., 2002). Fungal infections of the lung are among the most feared infections in immunocompromised patients. Aspergillus is the most common ubiquitary opportunistic pathogen affecting the lung (Bernhard et al., 2008). *Aspergillus* forms a genus of ubiquitous, dimorphic molds present in soil, various types of organic debris, water, indoor environment, and many other sites (Al-Alawi et al., 2005; Zmeili and Soubani, 2007). Airborne *Aspergillus* spores are present virtually everywhere in the atmosphere and are small enough (2-3μm) to be regularly inhaled into the lower airways (Rafal and Elzbieta, 2011). However, due to efficient natural antifungal defense mechanisms (i.e., mucosal barriers, macrophage and neutrophil function) symptomatic pulmonary infections in otherwise healthy subjects are extremely rare. Conversely, the impairment of these mechanisms (local or systemic) significantly increases the risk of airway colonization and progression to various *Aspergillus*-related pulmonary disorders (Roilides et al., 1998; Farouk et al., 2002; Hartemink et al., 2003).

Aspergillus fumigatus is by far the most common pathogen involved in 50–60% of all *Aspergillus* infections. Three other species which are a relatively common cause of human diseases are: *A. flavus*, *A. niger*, and *A. terreus.* Each of these species may be responsible for 10–15% of invasive human diseases (Patterson et al., 2000; Marr et al., 2002; Maschmeyer et al., 2007).

Aspergillus species were isolated from 9(13.8%) patients in a study carried out by Shahid et al., 2007 in broncho alveolar larvage of (65) pulmonary tuberculosis patients. Malik et al., 2001, isolated Aspergillus in culture from 13(14.7%) cases of chronic lung disease (CLD) while 30.6% cases showed anti-aspergillus antibodies by serological methods. They concluded that prevalence of Aspergillus is quiet high in CLD, culture and serological

test should be performed in conjunction to confirm diagnosis. Also a prevalence of aspergillosis was conducted (Malik et al., 2003), in 420 patients with bronchogenic carcinoma using samples of bronchoaveolar larvage and sera of patients analyzed for the presence of anti-aspergillus antibody by immunodiffusion, Enzyme link immunoassay and Dot blot assay. Aspergillus was isolated from 6(1.42%) patients with BGC

Aspergillus species are gradually becoming a serious concern among immune-compromised population. The respiratory challenges of HIV Patients is not tuberculosis alone, it includes other infections like aspergillosis. Effective management of the patient must take into cognizance this opportunistic infection. This study therefore seeks to bring to the fore, the role of Apergillus species as an opportunistic infection in HIV/AIDS Patients especially in the North-Eastern Nigeria. It also seeks to improve diagnosis of respiratory tract infections in immune-compromised patients

MATERIALS AND METHODS

The study was a cross sectional and prospective study. Seventy-seven (77) subjects made up of fifty (50) Human Immunodeficiency Virus (HIV) positive patients and Twenty-seven (27) HIV- negative patient from Direct Observed Treatment Shot course (DOTS) Clinic, University of Maiduguri Teaching Hospital, Nigeria. Forty-one (41) of HIV positive patients did not have Tuberculosis (TB), while nine (9) also had a concurrent TB infection. The Twenty- seven (27) Control samples are all HIV Negative and also TB Negative. Sputum samples were collected into sterile wide mouth bottles from all the subjects whose ages range from 21-55years. The patients were appropriately instructed to collect the sputum samples prior to mouth washing. None of the participants in this study had been placed on any anti fungal therapy.

The sputum samples were digested to eliminate the slimy nature of sputum and enhance harvesting of epithelial and non-epithelial cellular elements. This was carried out by adding 10% percent Potassium hydroxide (10% KOH) to the sputum in the ratio of one part to ten part 10%KOH. The setup was allowed to stand for 30minutes and then centrifuged at 1500 rmp for 5 minutes. The supernatant was discarded and the deposit smeared on an albumenized slide and fixed in 95% ethanol immediately (Chakraborty and Nishith, 2008). Four smears were made for each sample and stained by: Heamatoxylin and Eosin (H&E) for general morphology, Periodic acid Schiff(PAS) for sugar coat on fungi and Grocott's methanamine Silver(GMS) for Hyphae of fungi (Ochei and Kolhatkar,2000). All stained smears were examined microscopically with x10 and x40 objectives.

The *Aspergillus* spp were identified by their various morphological features associated with their characteristic angulated hyphae and sporing heads (Collier et al., 1998).

This study was carried out after due ethical clearance was obtained from the Ethical committee of the University of Maiduguri Teaching Hospital and obtaining an informed consent from each of the participants.

RESULTS

The sex and age frequency distribution of all the participants is as presented in figure 1. The age range of participants was 21-55 years with mean age of 34.6years.

The sex distribution of Aspergillosis was highest in females in age group 21-30years and in males in age group 31-40years. The prevalence of the disease in ages above 41yrs was relatively lower. (Table1).

Aspergilosis in HIV positive subjects infected with TB is most prevalent in ages 21-40years as shown in table 2. Ages 41years and above only accounts for 32.1% of subjects.

Comparing the methods of staining used, the Gomori's Methanamine silver method is the most sensitive picking 78% of Aspergillus infection and the H&E method as the least sensitive with 60 %. (Table 3).

DISCUSSION

Invasive aspergillosis has been associated with patients with AIDS (Libanore et al., 2002; Murtagh et al., 2008). In our study of the incidence of aspergillosis in HIV patients, we observed a high incidence (46%), higher than those seen in most publications (Denning et al., 1991;Miller Jr et al., 1994; Moreno et al., 2000). The controls were all negative for aspergillosis and this is in line with the findings of Shahid et al., 2007, where none of the 10 volunteers showed growth of *Aspergillus* species. This reaffirms previous studies that HIV/AIDS is associated with increased risk for Aspergillosis. The difference between the incidence in this study and what was reported in several others may be due to the environment. The sanitary condition of the study area is extremely poor. Poverty associated with poor nutrition may also play roles in lowered immunity of subjects. This may also predispose for the development of pulmonary aspergillosis by impairing pulmonary macrophage function. Intravenous drug use is also considered a risk factor for *Aspergillus* infection (Minamoto et al., 1992; Shetty et al., 1997; Denning, 1998) and cannot be ruled out for some of the subjects. Other factors identified in literature associated with this infection but not within the preview of this study are use of high doses of corticosteroids and presence of other opportunistic infections especially those involving the lungs such as

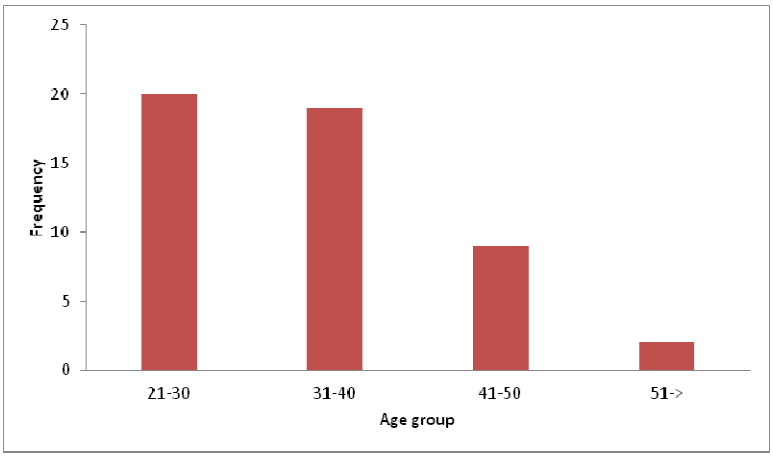

Figure 1. Frequency Distribution of Subjects according to Age groups

Table 1. Prevalence (%) of *Aspergillus sp* by Age and Gender

Age group (years)	Total Sampled (%)	Male		Female		Total (%) +ve (%)	-ve (%)
		+ve (%)	-ve (%)	+ve (%)	-ve (%)		
21-30	20(40)	5(10)	0	15(30)	0	20(40)	0
31-40	19(38)	12(24)	1(2)	5(10)	1(2)	17(34)	2(4)
41-50	9(18)	4(8)	2(4)	3(6)	0	7(14)	2(4)
51->	2(4)	2(4)	0	0	0	2(4)	0
Total	50(100)	23(46)	3(6)	23(46)	1(2)	46(92)	4(8)

Table 3. Frequency Distribution of Aspergillosis in HIV positive Subjects according to Various Methods of Staining

Staining Method	Frequency(%)	
	Positive(%)	Negative(%)
H&E	30(60)	20(40)
PAS	37(74)	13(26)
GMS	39(78)	11(22)

Key:
H&E – Haematoxylin and Eosin
PAS – Periodic Acid Schiff
GMS – Gomori Methinanine Silver

Pneumocystic carini pneumonia and cytomegalovirus (PCP and CMV).

The sex distribution of Aspergillosis in both male and female subjects was generally similar in the HIV positive population (46% each). The sex differences are only observed when age groups are considered. In the 21-30 years age group, the highest prevalence was among females (30%) while in 31-40 years age group, the highest prevalence was among males (24%). Relatively much lower prevalence were observed in age groups 41 years and above in both sexes. The overall prevalence in this study (46%) is slightly higher than that reported by Ofonime et al., 2013, 36% and 35% by Wadhwa et al., 2007, Prevalences are observed to vary widely among researchers, but the very high prevalence in this study area may be due to the very poor environmental factors and the fact that three different methods were used on all the samples thereby increasing the sensitivity of the procedure. The major factors that affect the amount of Aspergillus spacies in the environment are humidity and temperature. Within certain limits (temperature 30-40^{0}c) there is a linear relationship between these factors and the prevalence of Aspergillosis. At the time of this study i.e. the raining season, both humidity and temperature were at the favorable peak in the study area.

The difference in the distribution between males and

females in age group 21-30 years and 31-40 years is different from that reported by other authors. The sex difference observed in this study i.e. higher prevalence in females in age group 21-30 years and higher prevalence in males in age group 31-40 years, suggest that age and hormonal factors may play a role in aspergillosis. The fact that in both male and female subjects the prevalence dropped significantly in age groups above 41 years and above affirms this assumption. This finding is similar to that of Aluyi et al., 2010 and Ofonime et al., 2013.

The prevalence of Tuberculosis in the immune-compromised subject is 18% and the incidence of Aspergillosis among these TB subjects in this study is 89% while concomitant TB and aspergillosis is 8(16%). Prevalence of aspergillosis in immune-compromised subjects that do not have TB is 93%. This is similar to that of those that have TB and suggest that the presence of TB infection does not significantly increase susceptibility to Aspergillosis specy. In this study, the record of 16% concomitant infection is similar to 18% reported by Shome et al., 1976 and 4.5% by Anna et al., 2012. However, our study also showed that the rate of Aspergillosis remarkably increased (89%) when it co-exists with TB in immune-compromised subjects.

Comparing the 3 methods of demonstrating the fungi (Table 3) shows that all three of them leave a wide margin of error individually. However, sensitivity in picking the fungi is significantly increased by a combined use of two or all three methods. The use of these methods was intended in part, to assert that the absence of microbiological (cultural) methods should not hinder the diagnosis of Aspergillosis when necessary. This is in addition to the fact that this approach to the diagnosis of Aspergillosis is cheaper and faster requiring essentially the microscope which is more affordable to the average laboratory. The cultural method takes a longer time.

REFERENCES

Al-Alawi A, Ryan CF, Flint JD, M¨uller NL (2005). “Aspergillus-related lung disease,” *Canadian Respiratory Journal.* 12, (7). 377–387.

Aluyi HAS, Otajevwo FD, O Iweriebor (2010). Incidence of pulmonary mycoses in patients with acquired immunodeficiency diseases *Nigerian Journal of Clinical Practice.* 13 (1):78-83

Anna LN, Anselm AE, Lucien-Henri FK, Dickson SN, Jules-Clement N, David AN, Tebit EK (2012). Respiratory Tract Aspergillosis in the Sputum of Patients Suspected of Tuberculosis in Fako Division-Cameroon *Journal of Microbiology Research.* 2(4): 68-72

Bernhard CD, Vassilios D, Hilmar D, Dimitrios M, Georgios B, Friedrich AS (2008). Surgical treatment of pulmonary aspergillosisymycosis in immunocompromised patients. *Interactive CardioVascular and Thoracic Surgery* 7:771–776.

Chakraborty P, Nishith KP (2008). Laboratory Diagnosis of Fungal Infections. Manual of Practical Microbiology and Parasitology. pp 186

Collier L, Balows A, Sussman M (1998). Topley and Wilson’s Microbiology and Microbial Infections, 9th ed. London: Arnold

Denning DW (1998). Invasive aspergillosis. *Clin Infect Dis*; 26: 781- 805

Denning DW, Follansbee SE, Scolaro M, Norris S, Edelstein H, Stevens DA (1991). “Pulmonary aspergillosis in the acquired immunodeficiency syndrome,” *The New England Journal of Medicine*, 324(10): 654–662,.

Farouk AM, Serrano del Castillo A, D´ıaz-Molina C, Fern´andez-Crehuet Navajas R (2002). “Invasive pulmonary aspergillosis: identification of risk factors,” *Scandinavian Journal of Infectious Diseases*, 34, 11, 819–822.

Hartemink KJ, Paul MA, Spijkstra JJ, Girbes ARJ, Polderman KH (2003). “Immunoparalysis as a cause for invasive aspergillosis?” *Intensive Care Medicine*, 29,11, 2068–2071.

Libanore M, Prini E, Mazzetti M, Barchi E, Raise E, Gritti FM, Bonazzi L, Ghinelli F (2002). Invasive Aspergillosis in Italian AIDS Patients. *Infection* 30, (6) 341-345.

Malik A, Shahid M, Bhargava R (2001). Prevalence of Aspergillosis in chronic lung disease., *Ind. J. Med.Microbiol.*, 43(3): 231-235

Malik A, Shahid M, Bhargava R (2003). Prevalence of Aspergillosis in bronchogenic carcinoma., *Ind. J. Pathol. Microbiol.*, 46: 507-510

Marr KA, Carter RA, Crippa F, Wald A, Corey L (2002). “Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients,” *Clinical Infectious Diseases*, 34, 7, 909–917.

Maschmeyer G, Haas A, Cornely OA (2007). “Invasive aspergillosis: epidemiology, diagnosis and management in immunocompromised patients,” *Drugs*,67,11,1567–1601.

Miller Jr WT, Sais GJ, Frank I, Gefter WB, Aronchick JM, Miller WT (1994). “Pulmonary aspergillosis in patients with AIDS: clinical and radiographic correlations,” *Chest*,105(1): 37–44,

Minamoto GY, Barlam TF,Van der Els NJ(1992). Invasive aspergillosis in patients with AIDS. *Clin Infect Dis*; 14: 66–74.

Moreno A, Perez-Elías M, Casado J, Navas E, Pintado V, Fortún J, Quereda C, Guerrero A (2000). “Role of antiretroviral therapy in long-term survival of patients with AIDS-related pulmonary aspergillosis,” *Eur. J. Clin.Microbiol. and Infect. Dis.*19(9): 688–693

Murtagh RD, Post MJD, Bruce JKK (2008).Post Spinal Epidural Aspergillosis in a Patient With HIV Resulting From Long-Standing (3 Years) Lung Infection AJNR *Am J Neuroradiol* 29:122–24

Ochei J, Kolhatkar A (2000). Histopathology Staining Technique. Medical Laboratory Science Theory and Practical 1162-1163.

Ofonime MO, Lydia NA, James Epokeb (2013). The relationship between opportunistic pulmonary fungal infections and CD4 count levels among HIV-seropositive patients in Calabar, Nigeria *Trans R Soc Trop Med Hyg* 107(3): 170-175.

Patterson TF, Kirkpatrick WR, White M (2000). “Invasive aspergillosis. Disease spectrum, treatment practices and outcomes. I3 Aspergillus Study Group,” *Medicine*, 79, 4, 250–260.

Rafal K, Elzbieta MG (2011). Tracheobronchial Manifestations of *Aspergillus* Infections. *The Scientific World JOURNAL* 11, 2310–2329.

Roilides E, Katsifa H, Walsh TJ (1998). “Pulmonary host defences against Aspergillus fumigatus,” *Research in Immunology*, 149, (4-5), 454–460.

Shahid M, Malik A, Bhargava R (2007). Secondary Aspergillus in Bronchoalveolar Lavages (BALs) of Pulmo-nary Tuberculosis Patients from North-India., *Ameri-can-Eurasian Journal of Scientific Research*, 2 (2): 97-100

Shetty F, Giri N, Gonzales CE (1997). Invasive aspergillosis in human immunodeficiency virus infected children. *Pediatr Infect Dis* J; 16: 216–221.

Shome SK, Upreti HB, Singh MM, Pamra SP(1976). Mycoses associated with pulmonary tuberculosis. *Ind J Tubercul*; 23:64-68.

Wadhwa A, Kaur R, Agarwal SK, Jain S, Bhalla P(2007).AIDS-related opportunistic mycoses seen in a tertiary care hospital in North India. *J.Med.Microbiol.*, 56(Pt 8): 1101-1106.

Zmeili OS, Soubani AO (2007). “Pulmonary aspergillosis: a clinical update,” *Quarterly Journal of Medicine*, 100,(6) 317–334.