



PULMONARY ASPERGILLOSIS AMONG HIV PATIENTS WITH TB SMEAR NEGATIVE ATTENDING AMINU KANO TEACHING HOSPITAL

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ABSTRACT

Aspergillosis is a life threatening disease especially among the immunocompromised, like human immunodeficiency virus (HIV) infected individuals and *Mycobacterium tuberculosis* infected patients, whether they are smear positive or negative. The aim of this study was to determine the prevalence of Aspergillosis among HIV patients with TB smear negative results. Early morning sputum samples were collected from HIV patients with TB smear negative results. The samples were screened using Ziehl-Neelsen staining technique before direct Gram's staining was done and cultured onto Sabouraud Dextrose Agar (DCA) supplemented with chloramphenicol and incubated for 48hrs at 37°C. The fungal isolates were examined morphologically using macroscopy and microscopically identified using lactophenol cotton blue staining under the microscope. One hundred and eighty seven (187) participants were included in the study in which 81(43.3%) were males and 106(56.7%) were females, out of which a prevalence 32(17%) was obtained. Higher percentage of 17(53.1%) was found in females, according to age ranges, higher prevalence of 11(34.4%) was found in the age range of 29-39, Based on isolation rates, *Aspergillus fumigates* accounted for the highest proportion of the isolates 29(90.6%) followed by *Aspergillus niger* 3(9.4%) according to the study, which implies the need for concurrent treatment with mycotic agents, apart from the antiretroviral and anti TB drugs in these category of immunocompromized individuals for better management.

Keywords: *Pulmonary aspergillosis, HIV patients, Tuberculosis, Aspergillus species*

INTRODUCTION

Aspergillosis is an opportunistic disease associated with some fungal species of the genus *Aspergillus*, the disease utilizes any chance of immune suppression associated with some conditions like organ transplant or others diseases like HIV and tuberculosis, and can lead to chronic situations referred to as chronic pulmonary Aspergillosis (CPA) and other conditions.

The condition, chronic pulmonary aspergillosis (CPA) is referred to a progressive pulmonary disorder which usually complicates several other pulmonary/respiratory diseases, such as HIV and pulmonary tuberculosis (TB) and it affects about 3 million people globally (Denning *et al.*, 2016).

It was estimated worldwide that, prevalence rate of chronic pulmonary aspergillosis (CPA) secondary to tuberculosis, lies in the *range* of 0.8 to 1.37 million cases, with up to 43 cases per 100, 000 of population in Nigeria and Congo (Al-shair *et al.*, 2016). Incidentally, according to some reports, just about 57% of pulmonary tuberculosis cases reported to the World Health Organization (WHO) were really bacteriologically confirmed and chronic pulmonary aspergillosis (CPA) both mimics and complicates treated pulmonary tuberculosis cases (Ohba *et al.*, 2012)

The condition, called Chronic Cavitory pulmonary Aspergillosis (CCPA) is the most common form of CPA (Richardson and Denning, 2016). It presents with progressive cavitation and symptoms that includes weight loss, fatigue, cough, breathlessness, chest discomfort and haemoptysis, even among TB smear negative patients are common presentations (Denning *et al.*, 2016). The term chronic pulmonary aspergillosis (CPA) which can appear in HIV patients that are both TB smear positive or negative, is a compound term which encompasses four entities: As the *Aspergillus* nodule, the simple aspergilloma, the chronic cavitory pulmonary aspergillosis (CCPA) and the most severe form known as chronic fibrosing pulmonary aspergillosis (CFPA) (Denning *et al.*, 2016). Cases of aspergillosis mostly appears in patients that are usually immunocompromised, with current/or prior underlying lung conditions, presenting with prominent systemic and pulmonary symptoms that are clinically indistinguishable from pulmonary tuberculosis (Denning *et al.*, 2003; Kosmidis and Denning 2015).

Patients with HIV that are ether smear positive or negative for pulmonary tuberculosis (PTB), can present with complications, such as persistent pulmonary symptoms, progressive loss of lung function and chronic pulmonary aspergillosis (CPA) among others (Nam *et al.*, 2010). According to records, in the 1960s, the Research Committee of the British Thoracic and Tuberculosis Association, was the first to establish the presence of (CPA) in patients who were identified to have residual cavity of at least 2.5 cm on the chest radiograph, after been treated for pulmonary tuberculosis (Massard *et al.*, 1993).

As has been documented over time, in HIV/AIDS patients, in the presence or absent of tuberculosis, aspergillosis in most cases affect the lungs, with a variety of distinct manifestations, such as ulcerative tracheobronchial disease, diffuse unilateral or bilateral infiltrates, obstructive bronchitis and thick-walled cavitory disease of the upper lobes (Latge, 2009).

In most cases, aspergillosis in HIV patients are only diagnosed after death, (at postmortem) and heavily relies upon histologic or microbiologic identification of the fungus in infected tissues and mostly, pulmonary involvement in patients with clear clinical presentations can best be documented most reliably using bronchoalveolar lavage (Latge, 2009). Patients with advanced HIV and *Aspergillus* infections are generally treated with itraconazole or voriconazole, caspofungin and amphotericin B, eventhough the relative efficacies of these drugs has not been established and the median survival rate of AIDS patients with cases of invasive aspergillosis (IA) is mostly 3 months, with only 26% surviving for more than 1 year (Holding *et al.*, 2000).

Some autopsy studies from Europe, Asia and Africa, (Italy, India, Japan and Uganda) indicated that aspergillosis is present in 3-11% of all AIDS deaths and 84% of AIDS-related aspergillosis cases identified at autopsy were missed during patients' life (Lanjewar and Duggal 2001).

On the smear negative tuberculosis, in 2015 World Health Organization annual report, noted that ≈ 2.2 million representing ($\approx 43\%$) of about 5.2 million of incident cases of pulmonary TB were either clinically diagnosed or smear-negative (WHO 2015), but also only 21–40% of smear-negative pulmonary TB cases were culture positive according to records (Hargreaves *et al.*, 2001; Nakiyingi *et al.*, 2015). Unlike histoplasmosis, paracoccidioidomycosis and coccidioidomycosis that are believed to be regionally distributed, aspergillosis is globally spread. It was observed that every year, an average of 373,000 new cases of CPA complicates the normally treated pulmonary tuberculosis within 12 months of completion of the usual anti-TB regiments (Denning *et al.*, 2011). Due to none stable published data on cavitation after treatment of pulmonary TB, at the same time, lack of correct estimate of CPA prevalence among patients without cavities and unclear knowledge of the effects of concurrent HIV and *aspergillus* infections, leads to speculations that, correct incidence and prevalence of CPA are not known but are probably underestimated, in part because CPA occurs in patients with active pulmonary TB, whom may either be smear positive or negative (Smith and Denning 2011), or as a complication of other pulmonary disorders with symptoms similar to those of pulmonary tuberculosis, and is incorrectly diagnosed and treated as pulmonary TB (Evel, 2016).

MATERIALS AND METHODS

The study was carried out at Aminu Kano Teaching Hospital (AKTH), Kano, situated in Kano metropolis. Kano lies between latitude 11°30'N and longitude 8°30'E. Kano state borders Katsina to the North-west, Jigawa state to the north-east, Bauchi state to the south-east and Kaduna state to the south-west. The total land area of Kano state is 20,760 square kilometers with a population of 9,383,682 based on the official 2006 National Population and Housing Census (Ado, 2009). The research was cross-sectional prospective study with population of HIV patients with TB smear negative, attending Aminu Kano Teaching Hospital (AKTH)

The sample size of this study was determined using the following formula:

$$n = \frac{z^2 p(1-p)}{d^2} \text{ (Cochran, 1963).}$$

Where:

n = number of samples

z = statistic for level of confidence at 95% = 1.96

p = prevalence of Pulmonary Aspergillosis = 12.7% (Nasir, et al., 2018)

d = allowable error of 5%, (0.05)

$$n = \frac{1.96^2 \times 0.127(1 - 0.127)}{0.05^2} = 170.4$$

Considering 10% Attrition rate

The total sample size is $170 + 17 = 187$

Simple random sampling technique was used

Ethical approval to conduct the research was sought from the Research Ethics Committee of AKTH. The participants' (subjects) consents, was also sought, prior to the administration of the questionnaires.

Sample collection was done early in the morning, in which early morning sputum was collected in a wide mouth screw-capped and leak proof plastic containers. Samples were processed immediately, but in the event of delay in processing, the samples were stored in the fridge at 2-8°C before use. Direct gram staining technique was carried out by putting a drop of the sputum sample on a labeled clean grease-free glass slide using a sterile wire loop emulsified to make a thin smear. The smears were then allowed to air dry and then heat fixed. The slides were then flooded with crystal violet (primary stain) for 60s after which the stain was rinsed with water. The smears were later flooded with iodine (mordant) for 60seconds and rinsed with water, after which it was flooded with a decolourizer (acetone) and rinsed off immediately. Counter stain (neutral red) was finally applied for 60s and rinse off with water. The stained smears were allowed to air dry, and then observed under the microscope using oil immersion objective (Ochei, 2005)

Wet preparation (potassium hydroxide mount)

A drop of 10% potassium hydroxide solution was placed on a clean glass slide. A small quantity of the specimen was transferred onto the slide and homogenized. The preparation was covered with a clean cover slip avoiding air bubbles. The slide was kept at room temperature for 5 minutes and observed under 10x and 40x objectives (Ochei, 2005)

The samples were individually inoculated onto Sabouraud Dextrose Agar supplemented with chloramphenicol and incubated at 37°C to 48 hours. Colonies were examined macroscopically for morphologic features and microscopically using oil immersion objectives (Shahid and Malik, 2002).

A drop of Lactophenol cotton blue stain was placed on a clean microscope slide and a small portion of the growth midway between the colony center and edge was removed gently using an inoculating needle and placed in the stain on the slide, using sterile inoculating needles and teased and thinly spread out in the Lactophenol. Coverslips were placed on the edge of the Lactophenol and slowly lowered to avoid trapping air bubbles under it and examined (Ellis, et al., 2007). The results obtained were analyzed using statistical package for social sciences software (SPSS version 20.0)

RESULTS AND DISCUSSION

One hundred and eighty seven (187) participants were included in the study in which a total prevalence of 32 (17%) was obtained. Out of the culture-positive samples, *Aspergillus fumigates* accounted for the highest proportion of the isolates 29(90.6%) followed by *Aspergillus niger* (9.4%) Table1. With respect to the participants enrolled, the samples of 32 of them were found to be positive for *Aspergillus* species while the other 155(83%) samples were found to be negative as shown in Table 2. Out of the total participants recruited 81(43.3%) were males and 106(56.6%) were females as shown in table 3. Infection rates according to age groups and gender of the participants were categorized as follows, 18-28 years with 1(20%) in male and 4(80%) in females, 29-39 years with 3(27.3%) in male while 8(72.7%) in females, 40-50 years with 6(60%) in males while 4(40%) in females, 51-61 years with 3(75%) in males while 1(25%) in females and 62 years above with 2(100%) in males while 0(0%) in females as shown table 4.

Also based on gender alone, 15(46.9%) of males were found infected with *Aspergillus* species while 17(53.1%) of the females participants were also found positive as shown in table 4.

Table 1: The Aspergillus species Isolated

Aspergillus specie	frequency	Percentage (%)
<i>Aspergillus fumigates</i>	29	90.6
<i>Aspergillus niger</i>	3	9.4
Total	32	100

Table 2: Prevalence of the disease among the study subjects

Subjects' status	Frequency (n)	Percentage (%)
Positive	32	17.1
Negative	155	82.9
Total	187	100

Table 3: Gender distribution of the participants

Gender	Frequency(n)	Percentage (%)
Males	81	43.4
Females	106	56.6
Total	187	100

Table 4 Distribution of Aspergillus species according age group and gender

AGE GROUP	TOTAL	MALE	FEMALE	TOTAL
(Years)	No. Examined (NE)	NI +ve(%)	NI +ve(%)	+ve(%)
18-28	35	1(20%)	4(80%)	5(100%)
29-39	68	3(27.3%)	8(72.7)	11(100%)
40-50	44	6(60%)	4(40%)	10(100%)
51-61	25	3(75)	1(25%)	4(100%)
≥ 62	15	2(100%)	0(0)	2(100%)
Total	187	15(46.9%)	17(53.1%)	32(100%)

Key; NE = Number Examine, NI = Number Infected = Positive (+ve) Sample % = Percentage

According to the results obtained, *Aspergillus fumigatus* had the highest isolation rate of 90.6% as against *Aspergillus niger* with 9.4%. This was however different from the findings of Oladele and colleagues in 2017 that got *Aspergillus fumigatus* 15, *Aspergillus flavus* 10 and *Aspergillus niger* 3, in their work titled, chronic pulmonary aspergillosis as a cause of smear-negative TB and/or TB treatment failure in Nigerians. In Lagos, in the same Year, 2018, Titilola, et al. presented a case report of a patient diagnosed of chronic pulmonary aspergillosis without evidence of an aspergilloma in January, 2017, which was made based on his *Aspergillus* IgG result (>40 mg/L), his sputum smear was negative for AFB and GeneXpert result was also negative but a plane chest x-ray done on the patient revealed walled apical cavities without fungal balls consistent with chronic cavitory pulmonary aspergillosis (Titilola, et al., 2018).

According to Iain, in Uganda, Differentiating

smear-negative TB and sub- acute invasive pulmonary aspergillosis is near impossible as indicated in their study, they found 54% of Ugandan HIV positive out-patients commencing TB therapy as having smear- negative. At the same time, when sputum TB culture was performed on these patients only 35% had confirmed TB case. For that, they postulate that, some of these patients with smear and culture negative TB are in fact suffering from misdiagnosed sub- acute invasive pulmonary aspergillosis. They were able to demonstrate that using *Aspergillus*-specific IgG that has a sensitivity of 96% and specificity of 98% for the diagnosis of chronic pulmonary aspergillosis in Ugandans (Iain et al., 2017).

In this study, a prevalence of 32(17.1%) was obtained, this is in agreement with findings of Muhammad et al., (2017), in Maiduguri, who got 18.6% as their total prevalence rate among HIV patients attending University of Maiduguri Teaching Hospital, in Borno state.

Based on our study also, 43.4% and 56.6% were male to female ratio of the disease in the study area, this is similar to the findings of Jabbari et al., (2018) in Ethiopia, which had males with higher infection rate 8 than females 4 in their study. But this differs from the findings of Muhammad et al., (2017) that had 59.8 and 40.2 as males to females infection ratio respectively, this implies that males were the most affected in their study as against females with highest rate in our study.

Based on age groups, 29-39 had the greater proportion of the infection, with total of 11 isolates followed by 30-40 years of age with 10 isolates while the least infected were ≥ 62 years of age with only 2 isolates identified. Anna et al., (2012) in Cameroon, also had the same pattern of results with age limit of 21-30 having the highest infection rate followed by 31-40 and the least infected was 70-80, with 14, 4 and 1, as the number of isolates per each group respectively.

Conflict of interest

We want declare that, there is no conflict of interest in which ever form in this research

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CONCLUSION

It can be concluded based on the study that, mycotic agents like *Aspergillus* species are among the causes of secondary infection in HIV and TB smear negative patients in the study area, and *Aspergillus fumigates* remain the most prevalent agent.

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