Biological and Environmental Sciences Journal for the Tropics
Volume 21, Number 3, December 2024. Pp 130 - 136
https://dx.doi.org/10.4314/bestj.v21i3.12

Research article

Pulmonary Aspergillosis among tuberculosis positive patients attending infectious diseases hospital in Kano-Nigeria

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Submission: 30/08/2024
Accepted: 28/12/2024

Abstract
Tuberculosis is one of the serious lungs-related air-borne diseases that mostly affects people with low economic status in resource limited settings of developing countries, especially in Africa and Asian. The disease is common among poor people with low immunity which pave way for opportunistic pathogens like *Aspergillus* species to set in, resulting in secondary infection. The aim of the research was to determine the prevalence of aspergillosis among TB patients in Infectious Diseases Hospital (IDH), Kano. As methods, sputum samples from the enrolled subjects were cultured onto Sabouraud Dextrose Agar (SDA) and incubated, after which the colonies produced were characterized accordingly. Based on the obtained results, a prevalence of 38(27.3%) was recorded, with three different *Aspergillus* species; *A. niger* 25(65.8%), *A. flavus* 9(23.7%), and *A. fumigatus*, 4(10.5%) been implicated. According to the results, males were more at risk 24(63.2%) than females 14(36.8%) in the study area. Age related infection revealed that age limit of 41-50 had the highest infection rate 11(28.95%) while 51-60 and 71-80 were the least affected with 3(7.89%) each. It can therefore be concluded based on the findings that *Aspergillus* species as opportunistic pathogens gets refuge in TB patients causing secondary infection as a result and male were the most affect by this phenomenon in the study area.

Key words: Tuberculosis, Aspergillosis, Opportunistic pathogens, Secondary infection

Introduction

Tuberculosis still remains a global health problem especially in developing countries (Ganguly, 2000). In this disease, the lungs are primarily involved; however, the infections can also occur in other organs of the body. In tuberculosis (TB) patients the burden of opportunistic infections these days increased significantly due to increase in people with immunocompromised status (Bansod and Rai, 2003). The fungal infections are now becoming more frequent, due to expansion of high risk individuals and the use of treatment measures that prolong the survival tendencies of this group of patients. Pulmonary tuberculosis (PTB) is commonly associated with secondary disease (the spergilloma) in any affected patients (Reichenberger *et al*., 1999).

Increased prevalence of this disease is normally, due to the inefficiency of immune system and the use of Antituberculosis Treatment (ATT), which favors the growth and reproduction of fungal flora at the expense of bacteria, thereby aggravating the underlying disease pathology (Wheat *et al*., 2007). The fungi, like Aspergillus as opportunistic agents, cause disease in immunocompromised individuals with pre-existing disease conditions and with long history of use of antibiotics. Across the globe, more than a million individuals develop Chronic Pulmonary Aspergillosis (CPA) while on TB retreatment (Patterson, 2015).

The major means of contracting Aspergillosis is through spores which are released in large numbers, the spores remain air borne for several hours and they are inhaled through the nose (Patterson, 2015).

In the aspergillosis, the pathologic reactions observed in patients, varies from simple colonization to invasive aspergillosis or allergic alveolitis or aspergilloma among other possible conditions (Fraser *et al*., 1994). According to records, tuberculosis (TB) caused by *Mycobacterium tuberculosis* complex is the leading cause of patients death from a single infectious agent, it also continues to be of serious public health interest worldwide (WHO, 2020). But the good thing about this, is that, if diagnosis is done early enough and correct treatment regimen initiated fast, millions of lives would be spared, as observed in record estimate of 53 to 64 million lives been saved globally from TB alone between 2000 to 2018 (Kyu *et al*., 2018 and WHO, 2020).

The disease pulmonary tuberculosis (PTB), as the most common form of TB, causes serious structural damage to the lung in more than two-thirds of the patients that are shown to persists after TB treatment, even though microbiological cure of active disease has been achieved (Khan *et al*., 2020). These observed residual changes are categorized as airway disease, parenchymal, pleural/chest wall, vascular, and mediastinal pathologies, collectively referred to as post-TB lung disease (PTBLD) (Khan *et al*., 2020).

In PTBLD, complications such as chronic pulmonary aspergillosis (CPA), is usually a common feature (van Kampen *et al*., 2018). The CPA is a progressive respiratory syndrome, which mostly occur usually in inummunocompetent or subtly those that are immunocompromised with underlying known structural lung diseases, which in most cases are treated TB cases (Denning *et al*., 2003). In this aspergillosis cases, residual cavities mostly remain in a range of 20% to 40% of lungs of the patients affected, following treatment for pulmonary tuberculosis. Existence of cavitation and ectatic lesions due to PTBLD allows for the eventual colonisation and growth of *Aspergillus* species following inhalation of its spores from the environment, causing the increase in pre-existing complication (Denning *et al*., 2003). Presence of these cavities can lead to the generation of complex mixture that comprise of inflammatory cells, *Aspergillus* hyphae, mucin and tissue debris known as a fungal ball or the aspergilloma (Smith and Denning, 2011).

In the cases of aspergillosis, progress of the disease and long period of treatment with antibiotics or immunosuppressive drugs makes tuberculosis patients to be more immunocompromised and more susceptible to fungal infections (Rathod *et al*., 2012). If there is decline in body defense capability, the standby opportunistic fungi like *Aspergillus* species may influence the trend of the existing disease and may even lead to fatality (Baradkar *et al*., 2009). By definition, coinfection is usually defined as the simultaneous presence of two or more infections, which leads to the increased severity and duration of one or both conditions in a given host (Stedman, 2012). Based on that, the pulmonary aspergillosis co-infection in TB patients is the simultaneous infection of host's lungs with *Aspergillus* spp. In the presence of *Mycobacterium tuberculosis* in the same patient that leads to increased disease complications (Xerinda *et al*., 2014). Drug-resistant tuberculosis was found to be present in 4.7 percent of follow-uptuberculosis patients in Kano State, Nigeria (Mohammad *et al*., 2017). This study focused on the burden of aspergilosis among TB patients attending Infectious Disease Hospital (IDH) Kano.

Materials and Methods

Study area

The research was carried out at Infectious Diseases Hospital within Kano metropolis, which is situated in the Sahelian geographic region, south of the Sahara. Kano city is located in North-western Nigeria. It lies between latitude 11º30`N and longitude 8º30`E and lies at about 1580/feet above sea level. Kano State borders Nortwestern Sates of Katsina, Jigawa and Kaduna in the region. The total land area of the state is 20,760 sq kilometer with a population of 9,383,682 based on the official 2006 National Population and Housing Census (Ado, 2009) making it the state with the largest population in Nigeria.

Study population

The study population comprised of Pulmonary TB patients with possible co-infection with *Aspergillus* species attending Infectious Diseases Hospital (IDH) Kano, during the period of the study.

Sample size

The sample size (139) was obtained using the formula:

$$n = \frac{Z^2P(1-P)}{d^2}$$; (Cochran *et al*., 1977).

Where:

n = Sample size

Z = Statistic for a level of confidence at 95% =1.96

P = Prevalence 9% (Yahaya *et al*., 2015)

d = allowable error of 5% (d = 0.05).

$$n = \frac{1.96^2 \times 0.09(1-0.09)}{0.05^2} = 126$$.

Applying 10% attrition

n=139

Ethical approval and informed consent

Ethical approval to conduct the research was obtained from Kano State Ministry of Health and the participants' verbal and/or written consents as the case may be were obtained before administration a structured questionnaires to the participants.

Sample collection and processing

Sample collection

Early morning sputum samples were collected in a wide mouth screw-caped and leak proof plastic containers and processed immediately and where delay was anticipated, the samples were stored in the fridge at 8°C.

Microscopy

Ziehl neelsen Staining Technique

Sputum smears were prepared, heat fixed by passing over a burnsen flame, then flooded with carbol fuchsin solution and heated gently until steam rises continuously for 5 minutes, it was then washed with water and decolourized using 3% acid alcohol and then washed with water, it was counterstained with 0.5% methylene blue for 30 seconds and washed with water before allowed to dry, then examined microscopically using 40× and 100× objectives (Ochei and Kolhatkar, 2005).

Potassium hydroxide (KOH) preparation

A drop of 10% potassium hydroxide (KOH) was placed on a clean glass slide, a portion of each sample was added, homogenized and examined with 40x objective (Ochei and Kolhatkar, 2005).

Culture method

The samples collected were individually inoculated onto Sabouraud Dextrose Agar (SDA) supplemented with chlorampenicol for 7 days at 37°C. The fungal isolates were examined morphologically and microscopically (Shahid and Malik, 2000).

Lactophenol cotton blue staining technique

A drop of lactophenol cotton blue stain was placed on a clean slide and a small portion of the growth was placed in it and the preparation was covered with coverslip and examined using microscope under 10× and 40× objectives (Ellis, *et al*., 2007)

Statistical analysis

Data collected were analyzed using statistical package for social sciences SPSS version 20.0 software version 20.0.

RESULTS

All the samples were cultured in Sabouraud Dextrose Agar for isolation of the organism. During the research, a total prevalence of 38(27.3%) was recorded with respect to the *Aspergillus* species, and out of 139 participants 80(57.6%) were males while 59(42.4%) were females. Positivity rate among them showed that 24(63.2%) were positive for males and 14(36.8%) were found to be females infected with the *Aspergillus* species (Table 1). Out of 139 participants, 17 showed the presence of septate hypae.

Out of the 38 fungal culture-positive individuals, *Aspergillus niger* accounted for the highest proportion of the isolates 25(65.8%) followed by *Aspergillus flavus* 9(23.7%) while *Aspergillus fumigatus* has the lowest proportion 4(10.5%) (Table 2).

In terms of positivity, the result on age and gender of the participants revealed age limit of 10-20 years had 4(80%) positive for males and 1(20%) positive for females between, 21 - 30 years had 2(50%) positive each for males and females between 31- 40 years showed 4(57.1%) and 3(42.9%) positive for males and females participants, 41 – 50 years revealed 8(72.7%) and 3(27.3%) positive for males and females, 51 – 60years indicated 2(66.7%) and 1(33.3%) positive for male and females, 61 – 70 years showed 3(60%) and 2(40%) for males and females, 71 – 80 years confirmed 1(33.3%) and 2(66.7%) positive for males and females while there was no positive case among the age bracket of 81-90 in both genders (Table 3).

Table 1: Gender distribution of the participants and infection rate

Gender	NE (%)	NI (%)
Males	80(67.6)	24(63.2)
Females	59(42.4)	14(36.8)
Total	139(100)	38100

Key: NE=Number examine; NI= Number infected; %= Percentage

Table 2: Frequency and percentage of *Aspergillus* species isolated

Aspergillus Species	Frequency	Percentage (%)
Aspergillus niger	25	65.8
Aspergillus fumigatus	4	10.5
Aspergillus flavus	9	23.7
Total	38	100

Table 3: Distribution of *Aspergillus* spp according to age group and gender

Age group Years	Number examined	Male	Female	Total
	NE	NI (%)	NI (%)	(%)
10-20	11	4(80%)	1(20%)	5(13.16%)
21-30	33	2(50%)	2(50%)	4(10.53%)
31-40	23	4(57.1%)	3(42.9%)	7(18.42%)
41-50	26	8(72.7)	3(27.3%)	11(28.95%)
51-60	9	2(66.7%)	1(33.3%)	3(7.89%)
61-70	25	3(60%)	2(40%)	5(13.16%)
71-80	10	1(33.3%)	2(66.7%)	3(7.89%)
81-90	2	0(0%)	0(0%)	0(0.00%)
Total	139	24(63.2%)	14(36.8%)	38(100%)

Key; NE = Number Examine, NI = Number Infected, % = Percentage

Discussion

Pulmonary aspergillosis as opportunistic disease is frequently linked to people with weak immunity like TB patients. In this study, overall prevalence of pulmonary aspergillosis 38(27.3%) was obtained. Also a result reported from similar study revealed 32(19.8%) as the total pulmonary aspergillosis prevalence rate Martha *et al*. (2020). The high prevalent rate obtained could be due to the fact that, the research was conducted in Infectious Disease Hospital (IDH) which usually houses seriously ill and debilitated patients. In another study on chronic pulmonary aspergillosis (CPA) in TB patients, a total prevalence of (22%) was reported among participants confirmed with CPA (Anna *et al*., 2022). However, a higher prevalence 55(44.3%) was recorded by Hedayati *et al*. (2022), in a sero-study on aspergillosis among TB patients in which sero-specific positive cases for IgG against *A. fumigatus* was observed. This result is higher than our findings because they check for antibody against *Aspergillus* which could be found in blood even if the organism could not grow on culture. Also among their participants, out of the 124 TB patients enrolled, 3(2.4%) presented with aspergilloma while 14(11.3%) had chronic cavitary pulmonary aspergillosis (CCPA) features.

Male subjects had the highest burden of the disease 24(63.2) as the most affected than the female counterpart 14(36.8). Similar pattern of infection indicated that males had the highest infection rate 26(73.7%) while female 10(26.3%) are the least infected (Nguyen *et al.,* 2021).

However, a contrary result was reported in 2012 revealed that females are most prone to the disease than males, with 11 and 3 isolation frequencies respectively (Anna *et al*., 2012).

The distribution of the isolates revealed that *Aspergillus niger* had the highest isolation rate of 25(65.8) followed by *Aspergillu flavus* with 9(23.7) while *Aspergillus fumigatus* had the least isolation rate 4(10.5). Also, in another study in 2014, similar isolates were reported but with different isolation frequencies, indicating 2(25%), 3(37.5%) and 3(37.5%) as isolation rates of *Aspergillus fumigates, Aspergillus flavus* and *Aspergillus niger* respectively (Sivasankar *et al*., 2014). But in another similar study, Martha *et al.* (2022), isolated only two *Aspergillus* species in their study, with 38 *A. niger* isolates representing (23.4%) and 13 *A. fumigatus* isolates representing (8.0%) according to their work. In another study in 2015, it was discovered that while screening TB patients for aspergillosis, direct microscopic examination (DME) revealed the presence of septed hyphae and later 55(44.3%) of the samples confirmed serum-specific IgG against *A. fumigatus* which were subsequently isolated (Hedayati *et al*., 2015). It was also found according to report in 2019 by Hamed and colleaques that four different *Aspergillus* species were isolated, with *Aspergillus oryzae* in addition to the three species; *Aspergillus fumigates, Aspergillus flavus and Aspergillus niger* we recovered in this study (Hamed *et al.*, 2019).

In what could be termed as multi-genus targeted co-infection study in TB patients, it was found in 2016 that both *Candida* and *Aspergillus* species were associated with mycobacterium tuberculosis patients with 8 isolates representing (44.4%) for *Candida albicans* isolated, 6 isolates representing (33.3%) for *Aspergillus niger* and 3 isolates representing (16.5%) identified as *Aspegillus fumigatus* and 1 isolate representing (5.5%) as *Aspergillus flavus* isolation rate (Babita *et al*., 2016). Another study, in 2019 on TB patients with known HIV status revealed same types of isolates we recovered in this study, in which *Aspergillus fumigatus* (13%), *Aspergillus niger* (3.2%), and *Aspergillus flavus* (9.8%) were isolated (Vivian *et al*., 2019). Adane and Solomon in 2021 also reported several *Aspergillus* species in their research work with *Aspergillus niger* 41 isolates, *Aspergillus fumigatus* 26 isolates, *Aspergillus flavus* 2 isolates, *Aspergillus glacus* 1 isolate and *Aspergillus terreus* also with 1 isolate (Adane and Solomon in 2021)

In this research, isolates distribution based on age group indicated that 41-50 had the highest infection rate 11(28.95%) followed by 31- 40 with 7(18.42) while 51-60 and 71-80 had 3(7.89) each. According to research conducted by Anna and colleagues in 2012, they found isolation rate of *Aspergillus* species with regards to age range indicating that age limit of 21-30 had the highest infection rate 14(20.59%) followed by 61-70 with 5(41.67%) while 71-80 had the least 1(25.0%) isolation rate (Anna *et al*., 2012). In another study in 2021, it was reported that age range of >10year had 14(42.4%) isolation frequency as the age group with highest infection followed by 5-10 years with 10(30.3%) while age bracket of <5 was the group with least infection rate in their study where they used only 3 age limits (<5years, 5-10years and >10years of age) as reported (Nguyen *et al*., 2021). The high prevalence of aspergillosis in the study suggest the contribution of fungi as secondary compounder in disease complication among turculpsis patients.

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