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Research Article

Frequency of Pulmonary Aspergillosis among Clinically Suspected and Under Treatment Tuberculosis Patients, Khartoum State, Sudan

Abubaker Agbash Baraka*, Salma Ali Alabid, Mohammed Abdulgadir Mohammed, Nagla Mohammed Ahmed
*Department of Medical Microbiology, Faculty of Medical Laboratories Sciences, Al-Neelain University, Khartoum, Sudan.
Corresponding author: abubakaraghbash96@gmail.com

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ABSTRACT

Background: Respiratory tract aspergillosis is a pulmonary disease cause by aspergillus species which are opportunistic fungi that mainly infect immuno-compromised patients. *Objectives:* The present study aimed to detect the frequency of pulmonary aspergillosis among clinically suspected and under follow up tuberculosis patients conducted at Tropical Diseases Teaching Hospital, Omdurman, Khartoum State, Sudan during the period from December 2019 to November 2020.
*Subjects and Methods:*One hundred and fifty sputum samples were collected from suspected cases of pulmonary tuberculosis and under follow up tuberculosis patients. All specimens were examined using 20% KOH and cultured on two sets of Sabouraud′s Dextrose agar slope media with chloramphenicol.
Results: Asperigillus species were isolated from 9/150 (6%) patients. They represented 5(3.3%) A.fumigatus, 3 (2%) A. niger and 1 (0.6%) A. Flavus. It was found that all Aspergillus isolates were revealed from Nonacid fast bacilli (Non AFB) patients 9/137(6.6%) with the most common frequency among asymptomatic patients 3/35(8.6%) , patients who were under tuberculosis treatment (8.5%), those who were under Rifampicin (RIF) and Isonizide (INH) treatment (10%) and with duration of less than three month. Also, the higher percentage (13%) was represented among the age group range from 61-80 year and slightly among males 6/97 (6.2 %) than females 3/53 (5.7%). There was statistically non-significant association between the frequency of aspergillosis and the studied variables in this study.
Conclusion: The frequency of pulmonary aspergillosis among patients with anti-tuberculosis treatment reflects the need for routine screening and diagnosis of those patients since the use of anti- tuberculosis treatment can promote the growth and reproduction of fungi.

Introduction

Opportunistic respiratory mycoses are found all over the world. They are large set of fungal diseases with etiologic agents that are usually potential infections in individuals who are immune-compromised or debilitated. Candida and Aspergillus species are well known examples of opportunistic pathogenic fungus (1).

Aspergillus spp cause a wide range of disorders in immune-competent as well as immune compromised hosts including allergic, colonizing and invasive diseases (2).

Aspergillus pulmonary infection causes a wide spectrum of diseases according to host immunity. There are two major entities; invasive pulmonary Aspergillosis and chronic pulmonary Aspergillosis (CPA) (3), and three syndromes; the first one is

invasive Aspergillosis (IA), which is now known to happen in individuals with critical illness who do not have a neutropenia and in those who have modest immunosuppression. The second syndrome is chronic pulmonary Aspergillosis that includes simple aspergilloma, and the third syndrome is Allergic Bronchopulmonary Aspergillosis (ABPA) which is a hypersensitivity reaction to Aspergillus mycelia that colonize the bronchi (4).

Invasive Aspergillosis has become more common in recent decades all over the world. According to the WHO report in 2011, around 1.2 million people within the world are estimated to possess chronic pulmonary Aspergillosis (CPA) as a sequel to tuberculosis and the majority of infections occur in South-East Asia, Western Pacific and African regions (5).

The majorities of infections of chronic and allergic Aspergillosis are misdiagnosed as tuberculosis and treated incorrectly (6).

Diagnosis of pulmonary Aspergillosis is usually missed as tests of their detection cannot be undertaken in routine diagnostic laboratories (7).The diagnosis of pulmonary Aspergillosis relies on a combination of criteria related to patient characteristics, thoracic computed tomography (CT) scan findings and mycological analysis by detection of Aspergillus precipitins IgG in the serum and/or the isolation of Aspergillus spp. from respiratory samples.

Because fungal lung infection has no specific clinical manifestation and signs and is often complicated by other diseases such as tuberculosis, many physicians have overlooked it, resulting in high rates of morbidity and mortality. As a result, accurate detection of the opportunistic fungal pathogen is crucial particularly in tuberculosis patients. (8).

Aspergillosis of the respiratory tract includes clinical and radiological characteristics that are strikingly similar to tuberculosis, making it easy to misdiagnose and mistreat the disease as tuberculosis.(1). This encouraged us to look further into the prevalence of Aspergillus spp. in the sputum of individuals with suspected pulmonary tuberculosis. Therefore the present study aimed to detect the frequency of pulmonary Asperigillosis among clinically suspected and under treatment tuberculosis patients attended Tropical Diseases Teaching Hospital, Omdurman, Khartoum State, Sudan., since there is a few studies handling this problem in our country.

Subjects and Method

Study design and participants: -

This descriptive cross-sectional study was conducted during the period from December 2019 to November 2020. The study involved 150 Sudanese patients (either they were with signs and symptoms of pulmonary tuberculosis or under treatment of pulmonary tuberculosis) referred to Tropical Diseases Teaching Hospital, Unit of tuberculosis, Omdurman, Khartoum State, they were seeking confirmatory diagnosis and follow up respectively, the study participants included 97 males and 53 females (mean age, 46.5-year-old).

Informed consent and structural questionnaire including demographical data, presence or absence of symptoms (follow up patients may be asymptomatic), type of antit-tuberculosis intake and their duration was obtained from each patient.

The ethical approval was obtained from Medical Microbiology Department, Faculty Medical Laboratories Sciences, Al-Neelain University, Khartoum, Sudan.

Sample collection:

Early morning deep cough sputum specimens were collected from each patient (n=150) in sterile clean dry containers; the specimens were processed for direct microscopy and culture without delay.

Direct Microscopy:

With the use of Pasteur's pipette, a large drop of KOH was placed on the centre of a clean glass slide. A small portion of the sputum was transferred into the KOH drop with a sterile wire loop and mixed well. The preparation was flattened under a cover slip, placed in a moist chamber and kept at room temperature for 30 minutes. The slide was then examined under low power (10x and 40x objective) for the presence of fungal elements. (9).

Culture and Isolation:

All sputum samples were cultured on two sets of Sabouraud's Dextrose Agar slope media with chloramphenicol, the cultures were incubated at 37 C°, aerobically up to 7 days with daily examination as previously mentioned by authors. (10).

Macroscopic Examination of Culture:

The isolates were identified by macroscopic appearance (Surface topography, texture and pigment). The diameter of the colony, the color of the conidia, the mycelia, the reverse colony texture and shape are all characteristic of the colony (10). The laboratory proof of bronchopulmonary aspergillosis is provided by a positive finding on both microscopy and culture (11).

Microscopic Examination of Culture:

Lactophenol cotton blue preparation was used to identify the cultures microscopically. Conidial heads, color, size, and length, vesicles form, serration, and roughness were the microscopic criteria used to identify them (10).

Data Analysis:

The collected data with the laboratory results were analyzed by the statistical package of social science (SPSS) soft program version 20, with reference P-value (0.05), P-value $\leq$.05 consider as significant result. Frequencies and percent obtained in frequency tables, chi-square test for goodness of fit used to test these frequencies. The relations between variables tested using cross tables and chi-square (Fisher exact) test for independence.

Results

All samples (n= 150) were cultured on slope of Sabouraud's dextrose agar and subjected to 20% potassium hydroxide. The correlation of theses finding represented that out of the total specimens, 15 (10%) were positive for Aspergillus growth, whereas only 9 (6%) were positive for both wet preparation and culture and 6 (4%) showed positive culture and negative finding in wet preparation. P. value = 0.001 (Table 1). Accordingly, the present study reported that out of the total 150 examined sputum specimens, 9 (6%) were positive for the presence of Aspergillus species (Figure 1).

The isolates were identified by macroscopic and microscopic cultures characteristics as follow; 5 (55.6%) A.fumigatus , 3 (33.3%) A.niger and 1(11.1%) A.flavus , as in Figure 2.

According to signs and symptoms of pulmonary infections (many patients who had anti-tuberculosis treatments may be asymptomatic) the participants were classified either symptomatic or asymptomatic patients. Table 2 shows that 6/115 (5.2%) of the isolates were from symptomatic patients and 3/35 (8.6%) of isolates were from asymptomatic patients, P. value = 0.464.

The present study reflects that all the examined Zn positive patients (n = 13) showed no presence of Aspergillus isolates whereas among the total non-acid fast bacilli patients (n = 137) there were 9 (6.6%) showed presence of Aspergillus isolates , P. value = 0.341 (Table 3).

The patients who considered new cases and admitted to hospital with suspected infection of pulmonary tuberculosis were 103(68.7%) patients and those who were under follow up with known infection of tuberculosis and under treatments were 47(31.3%) patients. Out of 9 Aspergillus isolates, 5 (4.9%) were isolated from new cases patients and 4 (8.5%) were isolated from follow up tuberculosis patients P. value = 0.382 (Table 4). Among the four positive pulmonary Aspergillosis treated patients 2/27(7.4%) were under treatment of combined drugs (Isoniazid (INH), Rifampicin (RIF), Pyrazinamide (PZA) and Ethambutol (EMB)) while 2/20(10%) of them were under treatment of both Rifampicin (RIF) and Isoniazid (INH). According to duration of treatment it was found that Aspergillus spp. Were most frequent among those with duration of less than three-month (10%) than those of duration more than three-month (7.4%) P. value = 0.637 (Table 4).

According to age group the participants in the present study were classified into five groups. It was found the most common isolates of Aspergillus spp (13%) were from the age group (61-80) followed by the age group (0-20) (11.5%). As shown in Table (5) which also it reflects that the frequency of Aspergillus isolates was slightly common among males 6/97 (6.2 %) than females 3/53 (5.7%). It was found there was no statistically significant association between age, gender and the frequency of Asprrgillus isolates, P. value were (0.356) and (0.897) respectively.

Table 1: Comparison between the results of direct microscopy and results

Variable		Culture		Total
		positive	Negative	
Wet preparation	Dichotomous hyphae seen	9 (100%)	0 (00.0%)	9 (100%)
	No dichotomous hyphae seen	6 (4.2%)	135 (95.8%)	141 (100%)
P. value = 0.001		15 (6%)	135 (94%)	150 (100%)

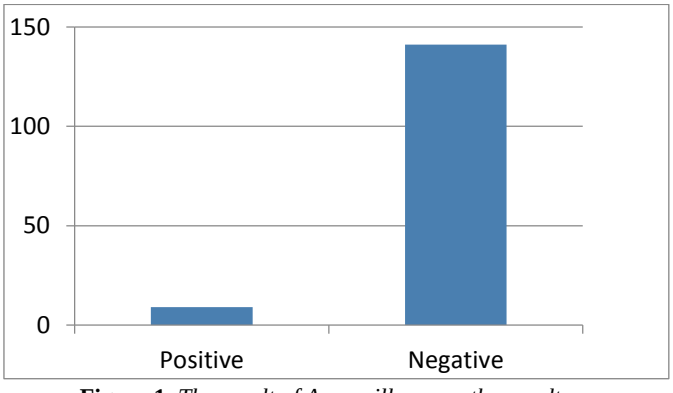

Figure 1. *The result of Aspergillus growth on culture*

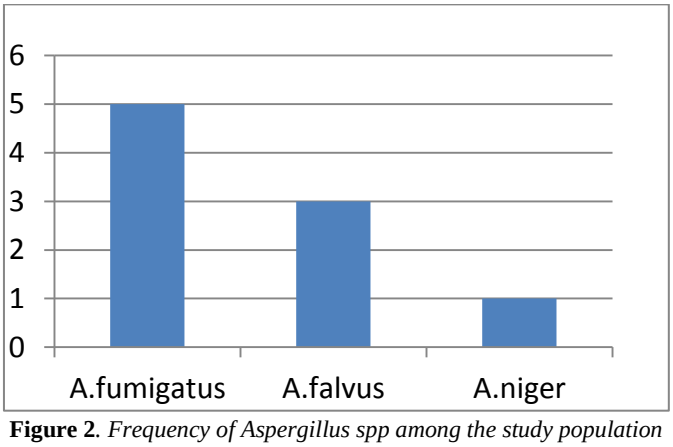

Figure 2. *Frequency of Aspergillus spp among the study population*

Table 2:Frequency of aspergillosis among symptomatic and asymptomatic patients

Variable		Aspergillosis		Total
		Infected	Not infected	
Symptoms	Symptomatic patients	6 (5.2%)	109 (94.8%)	115 (100%)
	Asymptomatic patients	3 (8.6%)	32 (91.4%)	35 (100%)
P. value = 0.464		9 (6%)	141 (94%)	150 (100%)

Table 3: Frequency of aspergillosis among Zn positive and Zn negative patients

Variable		Aspergillosis		Total
		Infected	Not infected	
Zn	AFBS	0 (00%)	13 (100%)	13 (100%)
	No AFBS	9 (6.6%)	128 (93.4%)	137 (100%)
P. value = 0.341		9 (6%)	141 (94%)	150 (100%)

Table 4: Frequency of Aspergillus spp among the study population according to anti-tuberculosis treatment

Variable		Aspergillosis		Total
		Infected	Not infected	
Treatment	Take treatment (follow up)	4 (8.5%)	43 (91.5%)	47 (100%)
	Not take treatment (new cases)	5 (4.9%)	98 (95.1%)	103(100%)
P. value = 0.382		9 (6%)	141 (94%)	150 (100%)
Type of taken treatment	INH+RIF+ PZA+EMB	2 (7.4%)	25 (92.6%)	27 (100%)
	INH+RIF	2 (10%)	18 (90%)	20 (100%)
P. value = 0.637		4 (8.5%)	43 (91.5%)	47 (100%)
Duration of treatment	More than 3 month	2 (7.4%)	25 (92.6%)	27 (100%)
	Less than 3 month	2 (10%)	18 (90%)	20 (100%)
P. value = 0.637		4 (8.5%)	43 (91.5%)	47 (100%)

Table 5: Frequency Aspergillosis among the study population according to age and gender

Variable		Aspergillosis		Total
		Infected	Not infected	
Age	0 — 20	3 (11.5%)	23 (88.5%)	26 (100%)
	21 — 40	2 (2.9%)	68 (97.1%)	70 (100%)
	41 — 60	2 (5.9%)	32 (94.1%)	34 (100%)
	61 — 80	2 (13.3%)	13 (86.7%)	15 (100%)
	81— 100	00 (00.0%)	5 (100%)	5 (100%)
P. value = 0.356		9 (6%)	141 (94%)	150 (100%)
Gender	Males	6 (6.2 %)	91 (93.8 %)	97 (100%)
	Females	3 (5.7%)	50 (94.3 %)	53 (100%)
P. value = 0.897		9 (6%)	141 (94%)	150 (100%)

Discussion

The number of opportunistic infections rise as the immune system of the host become more compromised, particularly in those with tuberculosis. As a result, effective care of opportunistic fungal infections is critical. Patients with tuberculosis are immunocompromised and require long-term antibiotic and vitamin treatment. i.e immunosuppressive drugs, as a result, fungal infection arises in the early stages of infection. But doctors and physicians prescribe only antibacterial drugs, so patients require for cure more time due to infection of fungus (12).

One hundred and fifty patients either who had suspected pulmonary tuberculosis or who were undergoing treatment of pulmonary tuberculosis were enrolled in this study.

Examination of sputum specimens using 20% potassium hydroxide revealed that 9/150 (6%) of samples were positive for fungal elements, while 141/150 (94%) were negative, direct microscopy failed to detect 6 samples which were later found to be positive with culture, similar study done by Anna et al. (2012) (1) in Cameroon that found examination by direct microscopy failed to detect 3 samples which were later found to be positive with culture. This may be due that in exceptional circumstances up to 1000 colonies/ml of sputum may result from inhalation of spores from a heavy contaminated atmosphere and the laboratory proof of bronchopulmonary Aspergillosis is provided by a positive finding of both microscopy and culture.(11).

The present study found the frequency of Aspergillus species among the study population was 6% this result showed that patients with pulmonary tuberculosis are mainly at risk of infection by pathogenic Aspergillus spp. Finding in consistent with several studies: One of these studies done by Saeed et al (2018) (13) in Sudan who found closely similar result (7%) and reported that there was significant correlation between the frequency of Aspergillus spp in patients with pulmonary tuberculosis. Also, in Cuba, Beltran et al. (2019) (14) revealed that Aspergillus and Mycobacterium tuberculosis are ecologically connected since the first takes advantage of the latter's lung injury. Another study done by Anna et al. (2012) (1) in Cameroon, that reported there was a relation between Aspergillosis and patients with pulmonary tuberculosis whereas they found higher result (15%) when compared with our finding. It's possible that this is related to population differences.

The dominant species in this study were A. fumigatus (55.6%), A. Niger (33,3%), A.flavus (11.1%), similar result obtained by Mwaura et al. (2013) (15) in Kenya who found three cases of pulmonary Aspergillosis from Kenya due to A. fumigatus, also similar result obtained by Anna et al.(2012) (1) in Cameroon who found A. fumigatus was the common causes of Aspergillus infection followed by A. niger, A. flavus and then A. terrus , on the other hand this result disagree with studies in Sudan and Cuba (13,14) that reported A. flavus was the commonest cause of Aspergillus infection , followed by A. fumigatus and then A. niger.

The present study reflects that the symptoms were insignificantly (P .value > 0.05) associated with the frequency of Aspergillus isolates and that most of them were from asymptomatic patients (8.6%) vs. (5.2%) from symptomatic patients. This finding reflects that those patients may have Aspergilloma which is the most common form of pulmonary Aspergillosis and it is usually develops in a pre-existing cavity in the lung of patients with these cavity forming diseases in which tuberculosis is the most common. Other study reported that most patients with aspergilloma are a symptomatic (16). Less commonly, patients may develop cough, dyspnea that is probably more related to the underlying lung disease and fever that could be secondary to the underlying disease or bacterial super infection (17).

All isolates in the present study 9(6.6%) were revealed from non AFB patients and most of them were represented among those who were under treatment of anti- tuberculosis (8.5%) as shown in table (3 and 4) respectively. This explains the finding of other study that

reported that, the reason for increased prevalence of disease is the insufficiency of immune system and the use of anti-tuberculosis drugs that increase fungal flora development and reproduction, which in turn aggravating the underlying pathophysiology (18).

It was found that the type and duration of treatment was insignificantly affects the frequency of Aspergillus isolates. This may be due to small sample size of patients in this study who were under tuberculosis treatments.

The present study showed the highest frequency of Aspergillus had been isolated from the age group (61-80) years. This result is similar to finding that reported the highest prevalence of Aspergillus was found in the age group (61 to 70) years (1). This reflects the facts that elderly are more prone to infection due to suppression of their immunity.

Conclusion

This study concluded that out of 150 fresh sputum samples, Asperigillus isolates were 9 (6%). And the most common of them was Asperigillus fumigates (55.6%)

It was found that most of isolates were from asymptomatic patients (8.6%) vs. (5.2%) symptomatic patients. So absence of symptoms may not exclude the probability of infection. All isolates in the present study were revealed from non AFB patients 9(6.6%) and most of them were represented among those who were under treatment of anti- tuberculosis. The high frequency of pulmonary aspergillosis among patients with anti-tuberculosis treatment reflects the need for routine screening and diagnosis of those patients since the use of anti-tuberculosis treatment can promote the growth and reproduction of fungi.

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Conflicts of interest

The authors declare no conflicts of interest.

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