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Research Article

Pulmonary mycoses among the clinically suspected cases of pulmonary tuberculosis

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ABSTRACT

Background: This study was carried with the main objectives: (1) to find out the occurrence of pulmonary mycoses in clinically suspected pulmonary tuberculosis cases at central referral hospital, Tadong, Sikkim. (2) To find out the various fungi causing pulmonary mycoses in clinically suspected pulmonary tuberculosis cases.
Methods: 200 clinically suspected pulmonary tuberculosis cases who visited the department of microbiology for the diagnostic microscopic examination of sputum sample for acid fast bacilli were included in this cross sectional study, carried out under the department of microbiology, Sikkim Manipal institute of medical sciences, over one year. Smears of sputum samples were examined microscopically for acid fast bacilli and fungal elements. Sputum samples were also plated onto different fungal culture media.
Results: Out of 200 patients, various types of pathogens were detected in 54 (27%) patients. Fourteen (7%) patients were positive only for AFB, while fungus as a primary etiological agent was detected in 16(8%) patients. Fungus as a secondary etiological agent was detected in 4 (2%) patients [AFB with fungus in 2 (1%), AFB with fungus and bacteria in 1 (0.5%) and bacteria with fungus in 1 (0.5%) patient].
Conclusion: Pulmonary mycosis can be a primary infection in non- tuberculosis cases or co-infection in pulmonary tuberculosis cases. Investigation for fungal cause in clinically suspected cases of pulmonary tuberculosis will prevent misdiagnosis and mistreatment of cases.

Keywords: Pulmonary mycosis, Primary mycosis, Fungal lung infection

INTRODUCTION

Pulmonary mycosis is a fungal infection of the lungs. It can be caused by either endemic or opportunistic fungi or a combination of both. Case mortality in pulmonary mycosis can be as high as 90% in immune-compromised patients though immune-competent patients generally respond well to antifungal therapy.[1,2] Fungal infections in lung often pose a difficult diagnostic challenge due to lack of any pathognomic clinical syndrome and characteristic radiological features. In India and other developing countries, the problem is further confounded by preponderance of pulmonary tuberculosis and paucity of diagnostic mycology laboratories.[3] Clinical and radiological characteristics of pulmonary mycosis are very similar to that of pulmonary tuberculosis thereby making the disease easily misdiagnosed and mistreated as tuberculosis. Thus, they may suffer from avoidable complications of unwarranted chemotherapy.

Fungal elements were frequently observed in sputum smear for acid fast bacilli in our laboratory. Furthermore in Sikkim, India, little or no work has been done on pulmonary mycoses, which inspire us to investigate the occurrence of pulmonary mycoses in suspected cases of

pulmonary tuberculosis attending central referral hospital, Tadong, Sikkim.

METHODS

Study design

A prospective hospital based study was conducted in the department of microbiology, Sikkim Manipal institute of medical sciences.

Study duration

The study was carried out from April 2013 to September 2014.

Sample size

Sputum samples were collected from 200 clinically suspected pulmonary tuberculosis patients.

Study subjects

Patients with signs and symptoms of pulmonary tuberculosis who visited the department of microbiology for the diagnostic microscopic examination of sputum samples for acid fast bacilli were recruited into the study.

Signed informed consent was obtained from each patient prior to specimen collection.

Specimen collection

Two sputum samples (early morning and spot) were collected into sterile wide neck universal containers as per the RNTCP guidelines, from 200 patients who were clinically suspected to have pulmonary tuberculosis.[4] None of the participants had been placed on antifungal therapy.

Specimen processing

A. Microscopic examination

1. Acid Fast Bacilli (AFB) analysis: On the same day a smear from each of the sputum sample was made and stained by Ziehl-Neelsen technique as per the RNTCP guidelines.[4] The stained slide was examined under oil immersion objective for the presence of AFB. The result was recorded as per the grading system for AFB microscopy by RNTCP.

2. Potassium hydroxide (KOH) mount: With the use of Pasteur's pipette, a large drop of 10% 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.

3. Gram stain: Smear was made from the most purulent or mucopurulent part of the sputum. Gram stained smears were used to look for the gram reaction of bacteria and fungi and also the size, shape and arrangement of fungal elements. In case of gram positive yeast like-cells, presence or absence of pseudohyphae was noted.

4. Giemsa stain: Sputum smear was made on a clean glass slide. The slide was flooded with methyl alcohol and left for 3-5 minutes for fixation. Prepared Giemsa stain was added onto the slide and left undisturbed for 45 minutes. The slide was washed thoroughly with tap water, blotted dry with absorbent paper and observed under oil immersion lens for the presence of trophozoite of *Pneumocystis jiroveci* and intracellular budding yeast cells.

5. Saline wet mount for eggs and larvae of parasites: One drop of sterile normal saline was placed on the centre of a clean glass slide and using a sterile wire loop, a small amount of sputum was transferred and mixed to make a uniform suspension. A coverslip was placed over the suspension and was examined under 10x and 40x objective for the presence of eggs and larvae of parasites.

B. Fungal culture

Irrespective of the outcome of the sputum microscopy, all samples were cultured given that the full characterization of mycotic agents is achieved through culture. The sputum samples were cultured on plain Sabouraud's Dextrose Agar (SDA) and also on SDA containing chloramphenicol and cycloheximide. Inoculation was done by seeding at the center of the tube using sterile wire loop after which the tubes were incubated aerobically at 25^{o}C for 6 weeks. The tubes were examined everyday for a week following incubation and twice a week from second week onwards. No fungal growth till 6 weeks were reported as no fungal growth. Cultural identification: After appropriate incubation, the colony morphology considering the rate of growth, surface, texture of the colony and pigmentation on the surface and reverse of the colony on SDA tubes were noted. The significant fungal isolates recovered on culture were identified to the species level, using standard mycological procedures.[5]

The *Aspergillus* species were identified with the various morphological features seen on tease mount. For identification of yeasts like colonies, the tests performed were gram's stain, India ink stain, germ tube test, Dalmau culture plate, urease test, nitrate assimilation test, sugar assimilation and fermentation tests.

C. Bacterial culture

The sputum samples were inoculated into blood agar, chocolate agar and Mac Conkey agar plates. The Blood agar and chocolate agar plates were incubated at 37°C in the presence of 5-10% carbon dioxide for 24 hours, whereas Mac Conckey agar plates were incubated at 37 for 24 hours. The isolates were identified by the standard microbiological method.[6] The known bacteria causing community acquired pneumonia and nosocomial pneumonia were considered as the causative agent of pneumonia in the given patient.

Ethical consideration

This study was conducted with the ethical approval of the research and ethics committees of the Sikkim Manipal University. Informed written consent was obtained from each study participant and all personal information about the participants was treated as confidential.

Limitation of the study

Patients with viral cause of pneumonia could not be excluded due to unavailability of the diagnostic investigations required for the diagnosis of viral pneumonia.

RESULTS

Sputum samples were collected from 200 clinically suspected pulmonary tuberculosis patients. Out of 200 patients, various types of pathogens were detected in 54 (27%) patients (Figure 1). Fourteen (7%) patients were positive only for AFB, while fungus as a primary etiological agent was detected in 16 (8%) patients. Fungus as a secondary etiological agent was detected in 4 (2%) patients [AFB with fungus in 2 (1%), AFB with fungus and bacteria in 1 (0.5%) and bacteria with fungus in 1 (0.5%) patient].

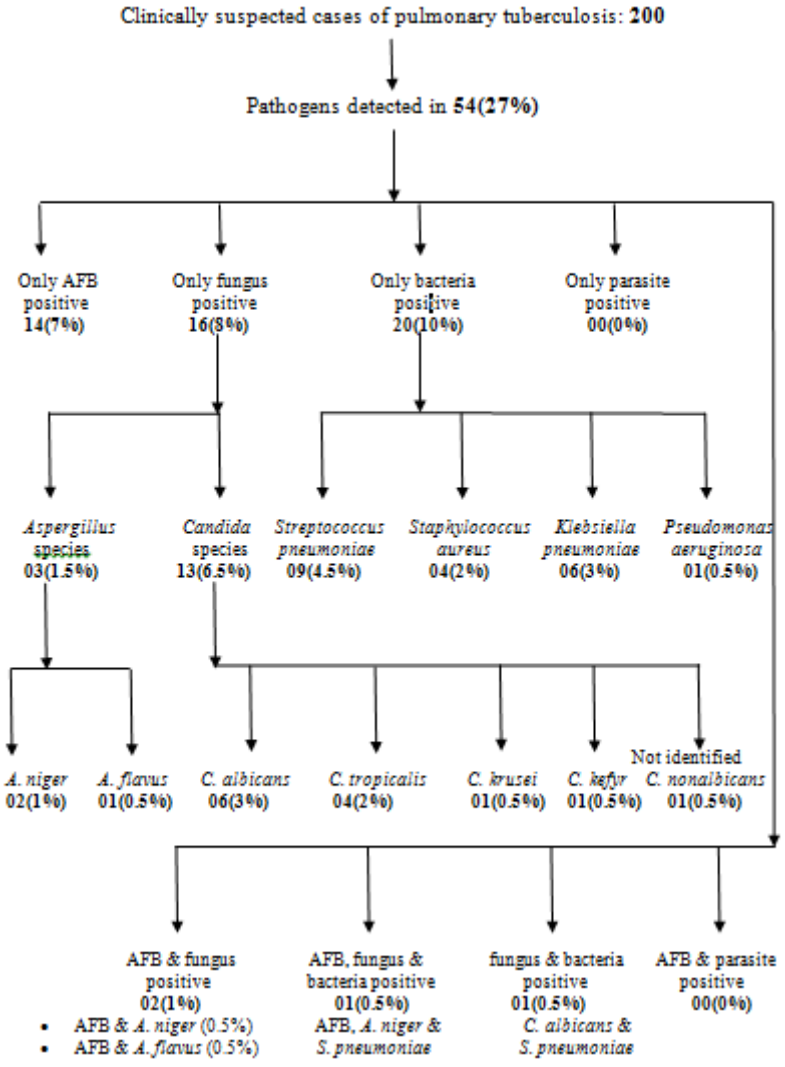

Figure 1: Organisms isolated from sputum samples.

Bacteria as the primary cause of pulmonary infection were detected in 20 (10%) patients. In none of the cases, parasites were detected.

The isolated fungi were *Aspergillus* species and *Candida* species. *Aspergillus* species was isolated in 3% of patients (n=6), whereas *Candida* species was isolated in 7% of patients (n=14). The *Aspergillus* species comprised of *Aspergillus niger* (2%) and *Aspergillus flavus* (1%). The *Candida* species isolated were *Candida albicans* (3.5%), *Candida tropicalis* (2%), *Candida krusei* (0.5%) and *Candida kefyr* (0.5%). One non albicans isolate (0.5%) could not be identified up to the species level.

The isolated bacteria were *Streptococcus pneumonia* (4.5%), *Staphylococcus aureus* (2%), *Klebsiella pneumonia* (3%) and *Pseudomonas aeruginosa* (0.5%).

Direct microscopic examination revealed the presence of septate hyphae with dichotomous branching in 3 (1.5%) patients and budding yeast cells with pseudohyphae in 14 (7%) patients. Fungal culture yielded *Aspergillus* sp. in 6 (3%) patients and *Candida* sp. in 20 (10%) patients. Examination by direct microscopy failed to detect 3 (1.5%) samples which were later found to be culture positive for *Aspergillus*.

Candida species was recovered in 20 (10%) patients by culture but in direct microscopy budding yeast cells with pseudohyphae was seen only in 14 (7%) patient, so 6 culture positive (*Candida*) with absence of pseudohyphae in direct microscopy were considered as commensals.

Age and gender distribution of patients

The ages of the participants ranged from 11-90 years, the male participants (59.5%) were more than the female participants (40.5%) (Table1).

Table 1: Age and gender distribution of patients.

Age group (years)	Male (%)	Female (%)	Total (%)
11-20	18 (9.0)	06 (3.0)	24 (12.0)
21-30	32 (16.0)	22 (11.0)	54 (27.0)
31-40	13 (6.5)	11 (5.5)	24 (12.0)
41-50	15 (7.5)	08 (4.0)	23 (11.5)
51-60	16 (8.0)	11 (5.5)	27 (13.5)
61-70	11 (5.5)	12 (6.0)	23 (11.5)
71-80	11 (5.5)	06 (3.0)	17 (8.5)
81-90	03 (1.5)	05 (2.5)	08 (4.0)
Total	119 (59.5)	81 (40.5)	200

Occurrence of pulmonary mycosis in clinically suspected pulmonary tuberculosis cases

Out of the 200 clinically suspected pulmonary tuberculosis cases, a total of 20 (10%) patients suffered from pulmonary mycoses (Table 2). Total fungal cultures isolated were 20 and among these *Candida albicans* was the most common fungus (35%) isolated (Table 3). Pulmonary mycosis was more common in female patients (6%) compared to male patients (4%), P = 0.0610 and was significantly more common (0.0126) in patients of more than 70 years of age compared to patients of less than 70 years of age (Table 4).

Table 2: Occurrence of pulmonary mycosis in clinically suspected pulmonary tuberculosis cases (n=200).

Fungal species	Total (%)
Aspergillus niger	04 (2.0)
Aspergillus flavus	02 (1.0)
Candida albicans	07 (3.5)
Candida tropicalis	04 (2.0)
Candida krusei	01 (0.5)
Candida kefyr	01 (0.5)
Candida non-albicans (not identified)	01 (0.5)
Total	20 (10.0)

Table 3: Fungus isolated by fungal culture (n=20).

Fungal species	No. of isolates	% of isolates
Aspergillus niger	04	20
Aspergillus flavus	02	10
Candida albicans	07	35
Candida tropicalis	04	20
Candida krusei	01	5
Candida kefyr	01	5
Candida non-albicans (not identified)	01	5
Total	20	100

Fungus causing primary and secondary pulmonary infection

A total of 16 (8%) patients had primary pulmonary mycosis, while 4 (2%) patients had secondary pulmonary mycosis (P = 0.0067). *Candida* species was the most common cause of primary pulmonary mycosis (P = 0.0281) (Table 5).

Occurrence of pulmonary aspergillosis and candidiasis in relation to gender and age

Aspergillus flavus (2.67%) infection was seen in the older age group (>50 years), whereas *Aspergillus niger* (3.2%) infection was seen in the younger age group (<50 years). *Aspergillus flavus* infection was seen only in female patients (1%) (Table 6), whereas for *Candida*, no such significant difference in age was noted (Table 7).

Table 4: Occurrence of pulmonary mycosis according to gender and age.

Age group (years)	*Aspergillus sp.*			*Candida sp.*			Total		
	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)
11-20 (n=24)	01 (4.2)	-	01 (4.2)	-	01 (4.2)	01 (4.2)	01 (4.2)	01 (4.2)	02 (8.3)
21-30 (n=54)	-	-	-	-	01 (1.8)	01 (1.8)	-	01 (1.8)	01 (1.8)
31-40 (n=24)	01 (4.2)	02 (8.3)	03 (12.5)	01 (4.2)	-	01 (4.2)	02 (8.3)	02 (8.3)	04 (16.7)
41-50 (n=23)	-	-	-	-	01 (4.3)	01 (4.3)	-	01 (4.3)	01 (4.3)
51-60 (n=27)	-	01 (3.7)	01 (3.7)	01 (3.7)	03 (11.1)	04 (14.8)	01 (3.7)	04 (14.8)	05 (18.5)
61-70 (n=23)	-	-	-	-	01 (4.3)	01 (4.3)	-	01 (4.3)	01 (4.3)
71-80 (n=17)	-	-	-	04 (23.5)	-	04 (23.5)	04 (23.5)	-	04 (23.5)
81-90 (n=08)	-	01 (12.5)	01 (12.5)	-	01 (12.5)	01 (12.5)	-	02 (25.0)	02 (25.0)
Total (n=200)	02 (1.0)	04 (2.0)	06 (3.0)	06 (3.0)	08 (4.0)	14 (7.0)	08 (4.0)	12 (6.0)	20 (10.0)

Table 5: Fungus causing primary and secondary pulmonary infection.

Name of the fungus	Primary pulmonary infection (%)	Secondary pulmonary infection (%)	Total (%)
Aspergillus niger	02 (1.0)	02 (1.0)	04 (2.0)
Aspergillus flavus	01 (0.5)	01 (0.5)	02 (1.0)
Total Aspergillus species	03 (1.5)	03 (1.5)	06 (3.0)
Candida albicans	06 (3.0)	01 (0.5)	07 (3.5)
Candida tropicalis	04 (2.0)	-	04 (2.0)
Candida krusei	01 (0.5)	-	01 (0.5)
Candida kefyr	01 (0.5)	-	01 (0.5)
C. non-albicans (not identified)	01 (0.5)	-	01 (0.5)
Total Candida species	13 (6.5%)	01 (0.5%)	14 (7.0%)
All total	16 (8.0)	04 (2.0)	20 (10.0)

Pulmonary mycosis in AFB positive cases in respect to age and gender

Only *Aspergillus* species (17.6%) was isolated causing secondary infection in pulmonary tuberculosis patients (Table 8). *Aspergillus niger* was isolated in 2 patients (11.8%) and *Aspergillus flavus* in 1 patient (5.9%). Pulmonary aspergillosis was more common in female (11.8%) compared to male (5.9%), P = 0.1183.

Pulmonary mycosis in AFB negative cases in respect to age and gender

Both *Aspergillus* and *Candida* species was isolated from AFB negative cases (Table 9). *Aspergillus sp.* was isolated in 1.6% whereas *Candida sp.* was isolated in 7.6% of the patients. Pulmonary mycosis was common in females (5.5%) than in males (3.8%), P = 0.1285.

Table 6: Occurrence of pulmonary aspergillosis in relation to gender and age.

Age group (years)	*Aspergillus niger*			*Aspergillus flavus*			Total		
	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)
11-20 (n=24)	01 (4.2)	-	01 (4.2)	-	-	-	01 (4.2)	-	01 (4.2)
21-30 (n=54)	-	-	-	-	-	-	-	-	00 (0.0)
31-40 (n=24)	01 (4.2)	02 (8.3)	03 (12.5)	-	-	-	01 (4.2)	02 (8.3)	03 (12.5)
41-50 (n=23)	-	-	-	-	-	-	-	-	00 (0.0)
51-60 (n=27)	-	-	-	-	01 (3.7)	01 (3.7)	-	01 (3.7)	01 (3.7)
61-70 (n=23)	-	-	-	-	-	-	-	-	00 (0.0)
71-80 (n=17)	-	-	-	-	-	-	-	-	00 (0.0)
81-90 (n=08)	-	-	-	-	01 (12.5)	01 (12.5)	-	01 (12.5)	01 (12.5)
Total (n=200)	02 (1.0)	02 (1.0)	04 (2.0)	00 (0.0)	02 (1.0)	02 (1.0)	02 (1.0)	04 (2.0)	06 (3.0)

Table 7: Occurrence of pulmonary candidiasis in relation to age and gender.

Age group (years)	*C. albicans*			*C. tropicalis*			*C. krusei*			*C. kefyr*			*C. non-albicans* (NI)			Total		
	M (%)	F (%)	T (%)	M (%)	F (%)	T (%)	M (%)	F (%)	T (%)	M (%)	F (%)	T (%)	M (%)	F (%)	T (%)	M (%)	F (%)	T (%)
11-20 (n=24)	-	-	-	-	-	-	-	01 (4.2)	01 (4.2)	-	-	-	-	-	-	-	01 (4.2)	01 (4.2)
21-30 (n=54)	-	01 (1.8)	01 (1.8)	-	-	-	-	-	-	-	-	-	-	-	-	-	01 (1.8)	01 (1.8)
31-40 (n=24)	01 (4.2)	-	01 (4.2)	-	-	-	-	-	-	-	-	-	-	-	-	01 (4.2)	-	01 (4.2)
41-50 (n=23)	-	-	-	-	01 (4.3)	01 (4.3)	-	-	-	-	-	-	-	-	-	-	01 (4.3)	01 (4.3)
51-60 (n=27)	01 (3.7)	01 (3.7)	02 (7.4)	-	01 (3.7)	01 (3.7)	-	-	-	-	01 (3.7)	01 (3.7)	-	-	-	01 (3.7)	03 (11.1)	04 (14.8)
61-70 (n=23)	-	01 (4.3)	01 (4.3)	-	-	-	-	-	-	-	-	-	-	-	-	-	01 (4.3)	01 (4.3)
71-80 (n=17)	01 (5.9)	-	01 (5.9)	02 (11.8)	-	02 (11.8)	-	-	-	-	-	-	01 (5.9)	-	01 (5.9)	04 (23.5)	-	04 (23.5)
81-90 (n=08)	-	01 (12.5)	01 (12.5)	-	-	-	-	-	-	-	-	-	-	-	-	-	01 (12.5)	01 (12.5)

M: Male; F: Female; NI: Not identified

Table 8: Pulmonary mycosis in AFB positive cases in respect to age and gender.

Age group (years)	*Aspergillus niger*			*Aspergillus flavus*			Total		
	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)
11-20 (n=03)	01 (33.3)	-	01 (33.3)	-	-	-	01 (33.3)	-	01 (33.3)
21-30 (n=07)	-	-	-	-	-	-	-	-	00 (0.0)
31-40 (n=03)	-	01 (33.3)	01 (33.3)	-	-	-	-	01 (33.3)	01 (33.3)
41-50 (n=01)	-	-	-	-	-	-	-	-	00 (0.0)
51-60 (n=01)	-	-	-	-	-	-	-	-	00 (0.0)
61-70 (n=00)	-	-	-	-	-	-	-	-	00 (0.0)
71-80 (n=00)	-	-	-	-	-	-	-	-	00 (0.0)
81-90 (n=02)	-	-	-	-	01 (50.0)	01 (50.0)	-	01 (50.0)	01 (50.0)
Total (n=17)	01 (5.9)	01 (5.9)	02 (11.8)	00 (0.0)	01 (5.9)	01 (5.9)	01 (5.9)	02 (11.8)	03 (17.6)

Table 9: Pulmonary mycosis in AFB negative cases in respect to age and gender.

Age group (years)	*Aspergillus species*			*Candida species*			Total		
	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)
11-20 (n=21)	-	-	-	-	01 (4.8)	01 (4.8)	-	01 (4.8)	01 (4.8)
21-30 (n=47)	-	-	-	-	01 (2.1)	01 (2.1)	-	01 (2.1)	01 (2.1)
31-40 (n=21)	01 (4.8)	01 (4.8)	02 (9.5)	01 (4.8)	-	01 (4.8)	02 (9.5)	01 (4.8)	03 (14.3)
41-50 (n=22)	-	-	-	-	01 (4.5)	01 (4.5)	-	01 (4.5)	01 (4.5)
51-60 (n=26)	-	01 (3.8)	01 (3.8)	01 (3.8)	03 (11.5)	04 (15.4)	01 (3.8)	04 (15.4)	05 (19.2)
61-70 (n=23)	-	-	-	-	01 (4.3)	01 (4.3)	-	01 (4.3)	01 (4.3)
71-80 (n=17)	-	-	-	04 (23.5)	-	04 (23.5)	04 (23.5)	-	04 (23.5)
81-90 (n=06)	-	-	-	-	01 (16.7)	01 (16.7)	-	01 (16.7)	01 (16.7)
Total (n=183)	01 (0.5)	02 (1.1)	03 (1.6)	06 (3.3)	08 (4.4)	14 (7.6)	07 (3.8)	10 (5.5)	17 (9.3)

Clinical and radiological associates of fungal infection

In the present study, association of pulmonary mycosis with the clinical condition, presence of specific risk factors and radiological findings of each patient were examined to characterize and survey the main risk factors associated with them. The main risk factors which were found in this study were Chronic Obstructive Pulmonary Disease (COPD), alcoholism, diabetes mellitus (DM), severe anaemia, tuberculosis and prolonged use of antibiotics. Of the 20 patients having pulmonary mycoses, 5 patients had normal chest X-ray and in 15 patients radiological abnormalities were detected. The common findings on X-ray were opacities (n=6), consolidation (n=5) and nodular lesions (n=4).

DISCUSSION

Fungal infections of lungs are important infective processes which are being encountered more and more often in today's practice. Fortunately, we only encounter a few of these pathogenic fungi. With the wide use of broad-spectrum antibiotics, immunosuppressive and chemotherapy agents as well as the increased incidence of respiratory diseases, including chronic obstructive pulmonary disease, lung cancer and tuberculosis, the chances of encountering these diseases are steadily increasing. Though treatment is difficult, nevertheless, the results are encouraging. Hence, it is all the more important today to know these diseases well so that we are able to manage them scientifically. Diseases like opportunistic fungal infection if diagnosed early can be treated effectively so as to prevent progression to fibrotic stage and reduce the number of respiratory cripples.[7]

Out of 200 clinically suspected pulmonary tuberculosis patients, pulmonary mycosis was noted in 20 patients (10%). Pulmonary mycosis was more common in female patients (6%) compared to male patients (4%) (P = 0.0610). It was significantly more common (P = 0.0126) in patients of more than 70 years of age compared to patients of less than 70 years of age. Primary pulmonary mycosis (8%) was significantly more common compared to secondary pulmonary mycosis (2%) (P = 0.0067).

The present study demonstrated that *Candida sp.* and *Aspergillus sp.* constitute the main fungi causing pulmonary mycosis and these findings are consistent with reports of Biswas et al and Khalidi et al.[8,9] We did not observed any difference in *Aspergillus sp.* being as the cause of primary or secondary pulmonary mycosis (1.5% versus 1.5%), but a huge difference was noted in *Candida sp.* being as a primary or secondary cause of pulmonary mycosis (6.5% versus 0.5%), (P = 0.0281). *A. niger* and *A. flavus* was seen to cause primary and secondary pulmonary mycosis equally. *C. albicans* was seen more commonly as a cause of primary pulmonary mycosis (6/200; 3%) compared to secondary pulmonary mycosis (1/200; 0.5%) (P = 0.6390). Nonalbicans *candida* was noted only as a cause of primary pulmonary mycosis but not as a cause of secondary pulmonary mycosis.

Though *Candida* sp. and *Aspergillus* sp. constitute the bulk of fungi reported in pulmonary mycoses, their relative proportion and species distribution have shown considerable geographical variation. The present study demonstrated that *Candida* sp. constitute most common fungus (70% of positive fungal culture) followed by *Aspergillus* sp. (30% of positive fungal culture) as causative agents of pulmonary mycosis. Our findings relating to occurrence of *Candida* and *Aspergillus* sp. is in accordance with previous reports of Biswas et al., Khalidi et al., Njunda et al. and Luo et al.[8-11]

The occurrence of *Aspergillus* sp. in the sputum of patients suspected of pulmonary tuberculosis was 3% which is much lower than the occurrence reported in a study conducted at Cameroon (15%).[10] Kurhade et al. and Shahid et al. also reported higher occurrence of *Aspergillus* in sputum samples (16.3% and 14.7% respectively).[12,13] Though *A. flavus* is considered as the common etiological agent among *Aspergillus* sp. in India, we found *A. niger* being the commonest *Aspergillus* sp. in pulmonary mycosis.[14] This wide variation in the incidence and frequency of isolation of various

Aspergillus sp. may be due to geographical difference. Infection of *Aspergillus* sp. was observed more in female compared to male patients (female-2%, male-1%; P = 0.1846). *A. flavus* infection was seen only in female patients (1%). *A. flavus* infection (2.67%) was seen in the older age group (>50 years), whereas *A. niger* infection (3.2%) was seen in the younger age group (<50 years).

The occurrence of *Candida* sp. in the sputum of patients suspected of pulmonary tuberculosis was 7%. Similar occurrence rate (6.5%) of *Candida* sp. in the lower respiratory tract was reported by Jha et al.,[15] whereas very high occurrence of *Candida* sp. (27.5%) in the sputum of patients suspected of pulmonary tuberculosis was reported by Njunda et al.[10] Similar high occurrence of *Candida* sp. (23.3%) was reported by Biswas et al and Mathavi et al. (23.3 and 19.6% respectively).[8,16]

In the present study, *Candida albicans* was the most frequent isolate (35% of fungal culture positive) followed by *Candida tropicalis* (20% of fungal culture positive). Our finding relating to the occurrence is in accordance with previous published reports by Njunda et al., Biswas et al., Jha BJ et al. and Khalidi et al.[8-10,15] No difference was observed in the infection by *Candida* sp. in males and females (P = 0.1884). Similar to other studies we also observed infection of the lungs by *Candida* sp. was highest in the age group of 71-80 years.[15,17] Pulmonary mycosis due to *Candida* sp. was significantly more (P = 0.0065) in patients of more than 70 years of age compared to patients of less than 70 years of age.

Tuberculosis infection and disease manifest with a wide spectrum of clinical conditions resulting from multisystem involvement producing varied clinical features due to opportunistic infections. Mycotic infection is an important co-infection in such patients. Although active mycosis may be an independent marker of advanced immunosuppression, it may also act as a co-factor in accelerating and amplifying the clinical course of tuberculosis. The occurrence of pulmonary tuberculosis was 8.5% in our study population, with the occurrence of only pulmonary tuberculosis in 7% of patients and the combination of pulmonary tuberculosis and pulmonary mycosis in 1.5% of patients. Only *Aspergillus* sp. (17.6% of pulmonary tuberculosis patients) was isolated causing secondary pulmonary mycosis in pulmonary tuberculosis patients. In other studies both, *Aspergillus* sp. and *Candida* sp. have been found to cause secondary infection.[7,9,16] Secondary infection with *A. niger* was isolated in 11.8% of patients and *A. flavus* in 5.9% of patients. *Aspergillus* in sputum sample was more common in female pulmonary tuberculosis patients compared to male patients (11.8% versus 5.9%: P = 0.1183).

Both *Aspergillus* and *Candida* sp. was observed to cause primary pulmonary mycosis in acid fast bacilli negative cases. *Aspergillus* sp. was isolated in 1.6% whereas *Candida* sp. was isolated in 7.6% of the acid fast bacilli negative patients. Pulmonary mycosis was more common in female compared to male in non-tuberculous patients (5.5% versus 3.8%; P = 0.1285).

For the diagnosis of pulmonary mycosis with *Aspergillus*, fungal culture was more sensitive compared to direct microscopy, which was similar to previous published report by Njunda et al. Around 1.5% of the suspected pulmonary tuberculosis patients with pulmonary Aspergillosis, which could not be diagnosed by direct microscopic examination, were later found to be positive with culture.[10] In pulmonary mycosis due to *Candida* sp., we found direct microscopy more useful compared to culture to label a patient with pulmonary candidiasis.

CONCLUSION

Pulmonary mycoses can be easily misdiagnosed and mistreated as pulmonary tuberculosis. Our study indicates that fungal etiology should also be sought in all the clinically suspected pulmonary tuberculosis patients, otherwise the case may be missed or misdiagnosed.

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