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Analysis of oral and pulmonary mycoses in suspected subjects of pulmonary tuberculosis visiting Indian healthcare center

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***Abstract*---**Background: Fungal infection of the lungs caused by endemic, opportunist, or combined fungi is pulmonary mycosis comprising a wide range of fungal diseases commonly seen in debilitated or immunocompromised subjects. Aims: To identify the pulmonary mycoses occurrence in suspected subjects of pulmonary tuberculosis (TB) by identifying and isolating various fungi causing pulmonary mycoses. Methods: 400 samples were taken from 200 suspected subjects of pulmonary TB where acid-fast staining was done to identify AFB (acid-fast bacilli), KOH, and gram stain to assess fungal element presence followed by culture on SDA to study fungal morphology. Germ-tube test used for C. Albicans identification. Results: Pulmonary mycosis was seen in 58 study subjects where candida species were the most commonly isolated fungal organism seen in 51.72% (n=30) study subjects followed by Aspergillus Niger, which was isolated in 20.68% (n=12) study subjects from their sputum. Candida albicans were most common and were isolated in 7 males where 3 were in the age of 11-20 years and 4 in 31-40 years, whereas Candida species were isolated from 23 females where in 11-20, 41-50, and 51-60 years had 3 females each, 61-70 years range

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had 5 females, and 9 females were in the age of 71-80 years. Conclusion: Pulmonary mycoses can be either primary infection or co-infection in pulmonary tuberculosis. Hence, can be mistreated and misdiagnosed which can be avoided by assessing fungi in clinically suspected pulmonary tuberculosis cases.

***Keywords*---**aspergillosis, candidiasis, fungal infection, pulmonary mycoses, pulmonary tuberculosis.

Introduction

Pulmonary mycosis is the common fungal infection of the lungs caused by either opportunistic or endemic fungi or a combination of both. In distribution, pulmonary mycoses are cosmopolitan. Fungi are highly pathogenic and commonly affect debilitated or immunocompromised subjects.[1] The subjects affected by tuberculosis are commonly immunocompromised, and hence, are at very high risk of getting the superadded fungal infections. Commonly seen opportunistic infections are of the Aspergillus and Candida species in subjects having tuberculosis. The most common fungal infection in subjects with pulmonary tuberculosis is the infection of Candida Albicans.[2]

The data from the previous studies conducted on the prevalence and incidence of pulmonary mycoses shows fragmentary results. The diagnosis of fungal infections poses a challenge and makes diagnosis difficult owing to the lack of characteristic radiological features and pathognomonic clinical syndromes associated with pulmonary mycoses.[3] Mycobacterium TB (tuberculosis) is the organism causing pulmonary tuberculosis which is an acid-fast and aerobic bacillus that affects nearly one-third of the population globally.[4]

Recently, there is a gradual decrease or stable incidence of subjects getting exposed or sick with the mycobacterium tuberculosis. In India, new cases are being increased every year as reported owing to the overpopulation and low socioeconomic status. The prevalence and incidence of fungal infections in subjects with pulmonary tuberculosis in developing countries like India remain unexplored, and hence, the data is not reliable which is concerning. In countries like India, there is an increase in subjects with either subclinical or clinical pulmonary tuberculosis infection.[5]

With the comparable features of pulmonary tuberculosis and pulmonary mycosis, it is vital to get a differential diagnosis to avoid mistreatment and misdiagnosis of pulmonary mycosis to pulmonary tuberculosis as it might expose the subjects to not needed complications of the chemotherapy, which is otherwise not required.[6] The present study aimed to identify the pulmonary mycoses occurrence in suspected subjects of pulmonary tuberculosis (TB) by identifying and isolating various fungi causing pulmonary mycoses.

Materials and Methods

The present cross-sectional study was done aimed to identify the pulmonary mycoses occurrence in suspected subjects of pulmonary tuberculosis (TB) by identifying and isolating various fungi causing pulmonary mycoses. The study was carried out after the clearance was given by the Institutional Ethical committee board. The study population was contributed by the subjects from the Outpatient Department of the Institute. The inclusion criteria for the study were subjects from both genders and with the clinical presentation of the symptoms and signs of pulmonary tuberculosis. The exclusion criteria were subjects of age <10 years, subjects with extrapulmonary diseases, and subjects who were not able to produce the sputum. The study included 400 sputum samples collected from 100 subjects who were suspected to have pulmonary tuberculosis. After explaining the detailed study design informed consent was taken from all the study subjects.

After final inclusion, from each participant, two sputum samples, spot and early morning, were collected in a sterile container. No subject was on the antifungal therapy. This was followed by the microscopic examination where acid-fast staining was done using Ziehl- Neelson stain following the guidelines by RNTCP (Revised National TB Control Programme). To see acid-fast bacilli, the stained section was assessed in the oil immersion objective. The results were graded following the AFB grading system.

KOH (potassium hydroxide) mount was done and slides were assessed under high and low power magnification of 40X and 10X respectively to see fungal element presence. Also, the presence of a fungal element, pseudohyphae, or any filament was noted. Gram staining was done in the smears from the mucopurulent part and was seen for the arrangement, shape, and size of the fungal element. The absence/presence of pseudohyphae was seen in yeast-like gram-positive/negative cells along with bacterial gram reaction.

SDA (Saboraud's dextrose agar) culture was done for all the samples followed by incubation. The samples were assessed for fungal growth every day for 1 week followed by twice a week. After incubation, macroscopic assessment of colony reversal on SDA tube, colony texture, surface, growth rate, and colony morphology were assessed. Microscopically, LPCB (lactophenol cotton blue mount) and Gram staining were assessed. The species level was evaluated using standard mycological procedures including germ tube test for Candida and LPCB, pigment production, and colony characters for Aspergillus species. Negative fungal growth was considered if no growth was seen till week 6.

Results

The present study aimed to identify the pulmonary mycoses occurrence in suspected subjects of pulmonary tuberculosis (TB) by identifying and isolating various fungi causing pulmonary mycoses. The study included 400 sputum samples collected from 100 subjects who were suspected to have pulmonary tuberculosis. The demographic characteristics of the study subjects are listed in table 1. The mean age of the study subjects was 48.6±6.84 years and the age

range were 11-86 years. There were 59% (n=118) males and 41% (n=82) females in the present study. Majority of the study subjects were in the age range of 21-30 years with 34% (n=68) subjects followed by 20% (n=40) subjects in 71-80 years, 11% (n=22) in 61-70 years, 9% (n=18) subjects in >80 years, 8% (n=16) subjects in 31-40 years, 7% (n=14) subjects in 51-60 years, 6% (n=12) subjects in 11-20 years, and 5% (n=10) subjects in 41-50 years of age respectively (Table 1).

On assessing the pathogens in the sputum samples of the 200 study participants, pulmonary mycosis was seen in 58 study subjects where candida species were the most commonly isolated fungal organism seen in 51.72% (n=30) study subjects followed by Aspergillus Niger, which was isolated in 20.68% (n=12) study subjects from their sputum, Aspergillus flavus isolated from 15.51% (n=9) sputum samples, and the least common isolate being the non-candida species isolated from 12.06% (n=7) sputum samples of the study subjects as shown in Table 2.

Concerning the age and gender-based distribution of the pulmonary mycosis in the study subjects, it was seen that Aspergillus Niger was seen in 2 males from 51-60 years and 6 males of 61-70 years, whereas, in females, it was seen in 4 females of 51-60 years of age. For Aspergillus flavus, it was isolated in 6 males of 61-70 years of age and 3 females 51-60 years of age. Non-candida Albicans species were isolated in 7 females where 3 were in the age range of 51-60 years and 4 in 61-70 years. Candida albicans were most common and were isolated in 7 males where 3 were in the age of 11-20 years and 4 in 31-40 years, whereas Candida species were isolated from 23 females where in 11-20, 41-50, and 51-60 years had 3 females each, 61-70 years range had 5 females, and 9 females were in the age of 71-80 years as depicted in Table 3.

Discussion

The present study aimed to identify the pulmonary mycoses occurrence in suspected subjects of pulmonary tuberculosis (TB) by identifying and isolating various fungi causing pulmonary mycoses. The study included 400 sputum samples collected from 100 subjects who were suspected to have pulmonary tuberculosis. The mean age of the study subjects was 48.6±6.84 years and the age range were 11-86 years. There were 59% (n=118) males and 41% (n=82) females in the present study. Majority of the study subjects were in the age range of 21-30 years with 34% (n=68) subjects followed by 20% (n=40) subjects in 71-80 years, 11% (n=22) in 61-70 years, 9% (n=18) subjects in >80 years, 8% (n=16) subjects in 31-40 years, 7% (n=14) subjects in 51-60 years, 6% (n=12) subjects in 11-20 years, and 5% (n=10) subjects in 41-50 years of age respectively. These demographics were comparable to the studies of Bansod S et al[7] in 2008 and Jha BJ et al[8] in 2006 where authors assessed sputum samples of the subjects with comparable demographic data as in the present study.

For the assessment of the pathogens in the sputum samples of the 200 study participants, pulmonary mycosis was seen in 58 study subjects where candida species were the most commonly isolated fungal organism seen in 51.72% (n=30) study subjects followed by Aspergillus Niger, which was isolated in 20.68% (n=12) study subjects from their sputum, Aspergillus flavus isolated from 15.51% (n=9)

sputum samples, and the least common isolate being the non-candida species isolated from 12.06% (n=7) sputum samples of the study subjects. These results were consistent with the findings of Babita SS et al[9] in 2016 and Njunda AL et al[10] in 2012 where authors reported the isolation of similar species from the sputum samples as in the present study in their respective studies.

For the age and gender-based distribution of the pulmonary mycosis in the study subjects, it was seen that Aspergillus Niger was seen in 2 males from 51-60 years and 6 males of 61-70 years, whereas, in females, it was seen in 4 females of 51-60 years of age. For Aspergillus flavus, it was isolated in 6 males of 61-70 years of age and 3 females 51-60 years of age. Non-candida Albicans species were isolated in 7 females where 3 were in the age range of 51-60 years and 4 in 61-70 years. Candida albicans were most common and were isolated in 7 males where 3 were in the age of 11-20 years and 4 in 31-40 years, whereas Candida species were isolated from 23 females where in 11-20, 41-50, and 51-60 years had 3 females each, 61-70 years range had 5 females, and 9 females were in the age of 71-80 years. These findings were in agreement with the studies of Buthia T et al[11] in 2015 and Kali A et al[12] in 2013 where authors reported comparable gender and age-based distribution for the isolated species in subjects with pulmonary mycosis.

Conclusion

Considering its limitations, the present study concludes that Pulmonary mycoses can be either primary infection or co-infection in pulmonary tuberculosis. Hence, can be mistreated and misdiagnosed which can be avoided by assessing fungi in clinically suspected pulmonary tuberculosis cases. However, the present study had a few limitations including a small sample size, short monitoring time, and geographical area biases. Hence, more longitudinal studies with a larger sample size and longer monitoring period will help reach a definitive conclusion.

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Tables

Characteristics	Percentage (%)	Number (n=200)
Mean age (years)	48.6±6.84	
Age range (years)	11-86	
11-20	6	12
21-30	34	68
31-40	8	16
41-50	5	10
51-60	7	14
61-70	11	22
71-80	20	40
>80	9	18
Gender		
Males	59	118
Females	41	82

Table 1: Demographic characteristics of the study subjects

Pathogens	Percentage (%)	Number (n=58)
Aspergillus Niger	20.68	12
Aspergillus Flavus	15.51	9
Non-candida Albicans	12.06	7
Candida albicans	51.72	30

Table 2: Pathogens detected in the study subjects

Age	Numb	Aspergillus	Aspergillus	Non-Candida	Candida

range (years)	er (n)	Niger		flavus		Albicans		albicans	
		Males (n)	Females (n)	Males (n)	Females (n)	Males (n)	Females (n)	Males (n)	Females (n)
11-20	12	-	-	-	-	-	-	3	3
21-30	68	-	-	-	-	-	-	-	-
31-40	16	-	-	-	-	-	-	4	-
41-50	10	-	-	-	-	-	-	-	3
51-60	14	2	4	-	3	-	3	-	3
61-70	22	6	-	6	-	-	4	-	5
71-80	40	-	-	-	-	-	-	-	9
>80	18	-	-	-	-	-	-	-	
Total	200	8	4	6	3	-	7	7	23

Table 3: Pulmonary mycosis occurrence based on age and gender in the study subjects