



Pulmonary Medicine

A STUDY OF FUNGAL INFECTIONS IN CHRONIC PULMONARY TUBERCULOSIS PATIENTS.

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ABSTRACT

Background: Tuberculosis kills more adults in India than any other infectious disease. Chronic nature of tubercular infection with extensive use of broad spectrum antibiotics, and irregular use of Anti tubercular drugs not only leads to impaired immunological status but also predisposes them to mycotic infection. As these infections are associated with high rate of morbidity and mortality, there is an acute need to study epidemiology and optimal management of opportunistic fungal pathogens in Tuberculosis patients.

Objective: This study was carried with the main objectives: (1) to find out the occurrence of pulmonary mycoses in diagnosed Pulmonary Tuberculosis cases (2) to find out the various fungi causing pulmonary mycoses and profile of risk factors associated with these cases.

Methodology: This is a prospective observational study carried out over a period of one year comprising of 101 patients attending Pulmonary Medicine Department, Siddhartha Medical College, Vijayawada from June 2016 to May 2017 with Tuberculosis who satisfied inclusion criteria like age above 18 years, taking antitubercular drugs, sputum smear negative Tuberculosis patients and associated with comorbidities like Diabetes mellitus. Descriptive and inferential statistical analysis has been carried out in this study. Identification of organisms from sputum samples were done by classical microbial staining and culture methods.

Results: Out of 101 patients, 23 patients (22.7%) have fungal culture positive. Out of 23 patients, 18 patients (17.82%) had growth of *Candida albicans*, *Candida non albicans* in 1 patient (0.99%), *Aspergillus fumigatus* in 3 patients (2.97%), *Aspergillus niger* in 1 patient (0.99%).

Conclusion: Tuberculosis infection and disease manifest with a wide spectrum of clinical conditions resulting from multisystem involvement producing varied clinical features due to opportunistic infections. This 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.

KEYWORDS : Pulmonary tuberculosis, Mycotic infection, Opportunistic fungi

INTRODUCTION

Tuberculosis (TB) is always a major public health problem in India. Every year approximately 18 lakh people develop TB in India out of which 4 lakh people die from it. India accounts for one fifth of global incidence of TB.^[1]

Fungal infections of lungs are important infective processes which are being encountered more and more often in today's practice. Fungal infections have emerged as a worldwide health care problem owing to the extensive use of broad spectrum antibiotics, long term use of immunosuppressive agents, increasing use of hyperalimentation and indwelling devices and the increasing population of terminally ill debilitated and immunocompromised patients like AIDS, Diabetes, cancer etc.^[2] With reference to Pulmonary Tuberculosis, study of Fungal diseases attained importance, because not only prolonged use of antibiotics along with ATT is required to treat this disease but also Tuberculosis leaves some residual lesions which form a nidus to fungal super infection and the prevalence of mycotic involvement in Pulmonary Tuberculosis is well documented.^[3] The opportunistic fungi are potential pathogen in the immune-compromised patients, patients with some preexisting disease and patients with as long history of antibiotics.^[4] The rate of opportunistic fungal infections in tuberculous patients is also very high. The reasons for increased prevalence are lowering of immune system due to Tuberculosis and the use of anti tuberculous drugs of non specific action which promote the growth and reproduction of the fungus flora and in turn aggravate the course of underlying process in the lung tissues.^[5]

Among the fungal pathogens, *Candida albicans* is common yeast isolated from tuberculous patients and it is responsible for causing severe secondary infections in such patients. Diabetes mellitus was found to be the most common risk factor associated with the isolation of *Candida albicans*. *Aspergillus* species represents the second most common fungal pathogen in the hospital settings. In patients who have

underlying pulmonary disease, especially previous cavity disease as seen in tuberculosis, a fungal ball or aspergilloma may form.^[6] Other fungal diseases like Cryptococcosis (Torulosis), Blastomycosis, Histoplasmosis, coccidioidomycosis, Mucormycosis are uncommon in India.

There is an increasing awareness amongst Clinicians and Microbiologists pertaining to importance of infection caused by opportunistic fungi. As these infections are associated with high rate of morbidity and mortality, there is an acute need to study epidemiology and optimal management of the opportunistic fungal pathogen especially in tuberculosis patients. The present study was done with the purpose of characterizing the fungal pathogens associated with chronic pulmonary tuberculosis patients and correlating between the individual fungal pathogens and the profile of risk factors, radiological presentations and clinical presentations in these patients.

MATERIALS AND METHODS**STUDY DESIGN**

This prospective observational study was carried out over a period of one year from June 2016 to May 2017 in the Department of Pulmonary medicine, Siddhartha medical college, Vijayawada. This study comprised of 101 patients who were randomly selected for present study amongst the diagnosed Pulmonary tuberculosis patients. They were divided into 2 groups.

1. Fresh or untreated group comprising who had taken none or less than one month anti-tuberculosis treatment with no complications.
2. Chronic or treated group cases who had anti-tuberculosis treatment for more than one month with no complications.

Methods:

All the selected patients were hospitalized. The patients were subjected to a meticulous history, clinical, radiological, and bacteriological and

biochemical examination at the time of admission. The following investigations were done in these patients like CBP, Total and Differential blood counts, Blood sugar, Complete urine examination, Sputum for AFB D/S for 2 consecutive days, Sputum for C/S of pathogenic organisms.

Sputum for fungal organisms by smear, Sputum culture for fungus, Sputum for eosinophils, X-ray Chest PA view and HRCT.

MICROBIOLOGICAL METHODS :

Patients are instructed to rinse their mouth with clean water and cough up sputum from lower airways. Early morning sputum is collected in sterile glass containers. A portion of it is used for Z-N staining for acid fast bacilli and haemotoxylene and eosin staining for eosinophils. Rest of it is taken to microbiology laboratory, Siddhartha Medical College, Vijayawada without delay where sputum is used for direct microscopic examination with 10% KOH and for inoculation into fungal culture media.

Direct Microscope Examination:

A clean glass slide is taken and a small quantity of sputum is transferred on to slide with aseptic precaution, to which a drop of 10% KOH is added. A clean cover slip is gently put taking care to avoid air bubbles. The slide is then passed through the flame of bunsen burner two or three times to eliminate whatever air bubbles remaining and to achieve clearing. By this most of the debris and host cells are converted into a translucent homogenous mass, rendering the unaffected fungal elements clearly visible. The preparation is examined under microscope with high power lens for any fungal elements.

Culture for Fungal organisms:

Culture Medium is Sabouraud’s glucose agar with chloramphenicol is selective medium used for primary isolation of fungi.

Culture of the organism:

A clean platinum loop heated till it becomes red and allowed to cool to room temperature. A loopful of sputum is inoculated on sabouraud’s glucose agar with chloramphenicol in duplicate with all aseptic precautions. One is incubated at 37°C and other is kept at room temperature for two weeks before discarding the culture as negative for various fungi.

Identification of candida albicans is based on its ability to form germ tubes within two hours when incubated in human serum at 37°C (Reynolds-Braude Phenomenon).

For filamentous fungi slide was prepared from the growth and examined with lactophenol blue for detailed microscopic morphology for the identification of the species. If there was no growth for 4 weeks, they were discarded.

Criteria for diagnosis secondary mycoses in the present study were as follows:

1. Heavy growth of fungus in culture repeatedly for at least three times.
2. Fungus positivity by direct smear examination.

INCLUSION AND EXCLUSION CRITERIA :

Patients with age above 18 years ,Pumonary tuberculosis patients using Antitubercular drugs and patients who were willing and cooperative included in this study. Patients with age less than 18 years, active tuberculosis (Sputum positive), Pneumonia, HIV Positive patients were excluded.

STUDY PARAMETERS(OUTCOME MEASURES) BEING MONITORED

1. Duration of the Disease
2. Duration of the previous treatment
3. Extent of the Disease
4. Severity of the disease
5. Associated Diseases like: Diabetes, COPD, Bronchial Asthma/ Allergy, and Bronchiectasis were recorded.

STATISTICAL METHODS

Descriptive and inferential statistical analysis were carried out in the present study. Results on categorical measurements were presented in Number (%). The following assumptions were made on data: Chi-square/ Fischer Exact test was used to find the significance of study parameters on categorical scale between two or more groups. Significance was assessed at 5 % level of significance. Statistical software namely SPSS V19 was used for analysis of the data.

RESULTS

Out of 101 patients, 23 patients (22.7%) have fungal culture positive. Most of the fungal infections occur in the age group of 31-64, 20 patients (19.8%) as shown in (table 1). Most of the fungal infections are more common in males 20 patients (19.8%) than in females 3 patients (2.97%) as shown in (figure 1). As shown in (table 2), in cavity < 4cm fungal culture positivity is 6 (19%) and in cavity > 4cm fungal culture positivity is 14 patients (37%). Total patients of fungal positivity in cavitary disease is 20 patients (56%). Irregular longer duration of treatment of Tuberculosis more than 1 year predisposes fungal infection (11.88%), less common in fresh patients i.e., treatment for 1 month duration (0.99%). Longer duration of illness i.e., tuberculosis more than 5 years (11.88%) when compared to shorter duration upto 1 year (0.99%) is more prone to fungal infections. As shown in (figure 2), more common fungal infections of lungs are Candida species 19 patients (18.81%) followed by Aspergillus species 4 patients (3.98%). Out of Candida species, Candida albicans 18 patients (17.82%), growth of which is shown in figure 3 is more than Candida non albicans 1 case (0.99%). In Aspergillus species, Aspergillus fumigatus , as in (figure 4) , the most common fungal infection 3 patients (2.97%) followed by Aspergillus niger 1 patient (0.99%), the growth of which is shown in (figure 5). As shown in (table 3), longer duration of illness exceeding 6 years (11.89%) are more prone to fungal infections. All Diabetic patients of our study were having fungal infections. As shown in (table 4), fungal infections are more common in tuberculosis with Diabetes mellitus 12 patients when compared to tuberculosis with Bronchiectasis (3 patients), Bronchial asthma (1 case) and TB with COPD (1 patient). Patients who are taking DOTS (Intermittent), residual cavity lesions at the end of treatment (78%) more prone to fungal infections who were on Non-DOTS therapy (23%). Fungal infection is more common with irregularly taken TB patients (78%) than in regularly taken TB patients (23%).

AGE	NUMBER OF PATIENTS	PERCENTAGE
20-25	1	0.98%
26-30	2	1.81%
31-44	4	3.96%
45-54	10	9.9%
55-64	6	5.94%

Table 1 . Age distribution of patients with fungal infection in chronic



Figure 1. Sex distribution of fungal infections in Chronic Tuberculosis patients

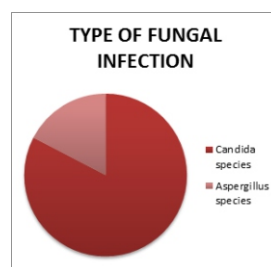


Figure 2. Type of fungal infections in Chronic Tuberculosis patients



Figure 3. Growth of Candida albicans

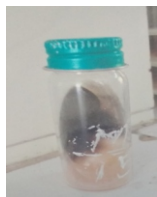


Figure 4. Growth of Aspergillus niger



Figure 5. Growth of Aspergillus fumigatus

S.No.	Radiological extent of Pulmonary Disease	No. of patients	Positive fungal culture	Negative Fungal culture
1.	Minimal lesion	20	1.5%	19.95%
2.	Moderately advanced			
	a) Cavitory	30	6.19%	24.82%
	b) Non cavitory	13	2.15%	11.85%
3.	For advanced cavitory disease	38	14.37%	24.63%
	TOTAL	101	23.23%	78.78%

Table 2. Presence of fungal infection depending upon radiological extent of Pulmonary Tuberculosis

S.No.	Duration of tuberculosis	No. of patients	Fungal positivity	Positivity percentage
1	Upto 1 year	39	1	0.99%
2	1 – 3 years	25	2	1.99%
3	3 – 6 years	20	8	7.92%
4	6– 10 years	17	12	11.88%

Table 3. Presence of fungal infections depending upon duration of Tuberculous illness.

S.No.	No. of patients	Disease	Fungal culture positive
1.	12	Tuberculosis with Diabetes mellitus	12
2.	45	Tuberculosis without Diabetes mellitus	4
3.	16	Bronchiectasis	3
4.	15	Chronic lung cavity with secondary infection	2
5.	10	Chronic bronchial Asthma	1
6.	3	COPD	1
TOTAL	101		23

Table 4. Diseases associated with fungal infections in this study

DISCUSSION

This prospective study was carried out in the Department of Pulmonary Medicine, Siddhartha medical College, Vijayawada over a period of one year i.e. from June 2016 to May 2017.

Fungal infections are the important co-infection in chronic tuberculosis patients. The prevalence of opportunistic mycoses has dramatically increased during the past few years, the etiological agents of which are otherwise incapable of causing disease in healthy individuals. These opportunistic fungi are potential pathogens in the immunocompromised patients, patients with some pre-existing disease and patients with long history of antibiotics and corticosteroids.^[7]

The immune system normally clears fungal spores, colonization and does not usually cause any significant problems. In tuberculosis the rate of opportunistic fungal infections is very high and well documented.^[8]

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. Invasive systemic mycosis is also amenable to multimodal therapy.^[3]

Mycotic infection is an important co-infection in TB patients. Their clinical and radiological manifestations depend on the levels of immune suppression.^[9] Although active mycosis may be an independent marker of advanced immune suppression, it may also act as a co-factor in accelerating and amplifying the clinical course of Tuberculosis disease.^[10]

In Tuberculosis patients with relatively intact immune system, the radiographic findings of fungal infections are similar to those met with in non-tuberculosis with predominantly upper lobe lung lesions, cavitations and fibrosis. A typical manifestations, like lower-lung field infiltrations, non-cavitory or lower lobe cavitory disease, pleural effusion findings suggestive of primary, mycotic infection in tuberculosis are met with when the immune system gets features and sputum examination of Tuberculosis and mycotic infections and sepsis co-existed in a large proportion of patients. Miliary Tuberculosis nodules were difficult to distinguish from these of Mycotic infection on many occasions.^[11]

These are examined clinically and subjected to routine investigations like x-ray, Direct smear examination for fungus and AFB, fasting blood sugar, sputum for fungal culture.

Out of 101 patients, 23 patients (22.7%) have fungal culture positive. This was strongly proved by study of Shome et al and Bansod et al who reported 18% and 40%.^[3,12]

In our study of 101 patients the peak incidence of fungal infections occurs in age group of 31-64 years. Whereas in the study of Bansod et al., the increased prevalence of fungal infections are common in age group of 35-44 years.^[3]

Among the 101 patients studied 19.8% (20 patients) were males and 2.97% (3 patients) were females. The male to female ratio is 6.5:1 approximately. This indicates approximately one female case for every seven males. Our study shows the same result as study conducted by grower & junnorkor and shome et al, and Khanna et al found male distribution is more.^[12,13,14] In the study of Bansod et al., also male distribution is more. The increased prevalence among males is because of their increased exposure to environmental open patients due to their movement due to job and presence of risk factors like smoking, alcoholism and irregular anti-T.B. treatment leaving residual lesions in the lungs.^[3]

In present study among 101 patients history presented mainly with cough with or without expectoration in 47%, Breathlessness in 21%, Haemoptysis in 13%, wheeze in 10%, loss of weight and appetite 5%, chestpain in 1% and fever in 4% of patients.

Among the 101 patients studied fungal infections of lung are more common in the cavitory lesions of the lungs (56%). In cavity <4cm fungal culture positivity is 6 patients (19%) and in cavity >4cm fungal culture positivity more 14 patients (37%). Total patients of fungal positivity in cavity disease 20 patients (56%). Whereas in the study of

S.K. Jain et.al., Candida species were found in higher percentage of patients (26.02%) in the cavitory group than that of noncavitory group (20.89%), but the difference was not statistically significant. The positivity was higher in the treated group both in the cavitory and noncavitory type of lesion than in the fresh group.^[15]

In present study among 101 patients fungal isolation more common with irregular longer duration of treatment which predisposes fungal infection less common in fresh patients 0.99% (1 patient). With irregular ATT of duration more than one year fungal infection 11.88% (12 patients). Whereas in the study SK Jain et.al., out of 140 patients, fungal positivity is 82% who had taken ATT treatment 1 year to 3 years, 40% for 1 month to 1 year and 37.5% up to 1 month.^[15]

Among 101 patients, the type of fungal infection more common in lungs are candida species. 18.81% (19 patients). Next common fungal infection is aspergillus species 3.98% (4 patients). Among candida species, more common fungi infection is Candida albicans 17.82% (18 patients) and next is candida non-albicans 0.99% (1 patient). This correlates with In the present study majority of the isolates were candida albicans 44.4% which correlates with the study of Khanna et. al and Jain et.al. where they isolated Candida albicans in 22.73% patients and 18.57% patients respectively.^[14,15]

Candida albicans was the most common isolate followed by candida non-albicans. This is in accordance with studies done by Kali et.al., SK Jain et.al., and BK Khanna et.al.^[14,15,16]

Among 101 patients, next common fungal infection is aspergillus species 3.98% (4 patients). In aspergillus species, Aspergillus fumigatus is most common fungal infection 2.97% (3 patients) and next common is aspergillus niger 0.99% (1 patient). This is similar when compared to studies done by Khalidi etal, Biswas et.al., Luo etal, Bansod etal.^[2,3,17,18]

In our study of 101 patients, 12 patients are diabetics. All diabetic patients of chronic TB are positive for fungal culture 100%. Whereas in the study of V.K.JHA etal fungal infection in diabetic is 80%. In the study of khanna et al 83.30% of diabetics and in study of S.K.Jain et al 100% diabetics are fungal culture positivity.^[14,15,19]

In my study among 101 patients, fungal infections in Tuberculosis patients associated with Bronchiectasis patients in 2.97% (3 patients), chronic lung cavity with secondary infection. 1.98% (2 patients), chronic bronchial asthma. 0.99% (1 patient), long standing COPD 0.99% (1 patient). In our study of 101 patients, Fungal infections more common in 16 patients (15.84%) who were taken intermittent DOTS therapy whereas 7 patients (6.93%) were taken daily regimen.

In India still now tuberculosis is the biggest problem. Fungus disease is also important because many patients of tuberculosis may be associated with fungus disease of lungs as also fungus disease is increasing.

A wide clinico radiological spectrum of mycotic infections among tuberculosis patients was, therefore, in consonance with their varying immune status. With the conventional sputum positivity tuberculin test and culture characteristic studies of mycotic infection providing an adequate diagnostic help familiarity with the clinico-radiological spectrum of tuberculosis and co-existing opportunistic infections is absolutely necessary for increasing the cure rate of tuberculosis patients.

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