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Prevalence of opportunistic fungal infection in patients with pulmonary tuberculosis in Madhya Pradesh, Central India

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Abstract

Tuberculosis kills more adults in India than any other infectious disease. Chronic nature of tubercular infection and prolonged administration of heavy doses of antibiotics not only leaves patients with an impaired or weak immunological status but also predisposes them to mycotic infection. A total of 100 pulmonary tuberculosis patients were screened, out of which 49% of total were positive for fungal infections. The most dominant pathogens were *Candida albicans* 34.69% (n=17) followed by *C. parapsilosis* 26.53% (n=13), *Aspergillus fumigatus* 12.24% (n=6), *A. niger* 10.2% (n=5), *Fusarium solani*. 6.12% (n=3), *Rhizopus oryzae*. 4.08% (n=2), *Rhodotorula mucilaginosa*. 2.04% (n=1), *Geotrichum candidum* 2.04% (n=1) and *Myridontium keratinophylum* 2.04% (n=1). The present study revealed that 65.31% infections were due to yeasts while 37.69% infecctions were due to filamentous fungi. The coexistence of fungi with tubercle bacteria adds complication to a patient's condition by adding more damaging and fatal dimensions to it.

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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 and it is on the top of the list of 22 high TB burden countries [1] Chronic nature of tubercular infection along with prolonged chemotherapy with or without corticosteroids severely affects patient's immune system predisposing it to super infection by opportunistic fungi. Due to immune deficiency or suppression in Tuberculosis patients are easily vulnerable to opportunistic fungal infections [2]. Greer and Gemoets [3] suggested a tendency for pulmonary tuberculosis to develop a more devastating course when it is associated with an invasion of parasitic fungi thus increasing the activity and virulence of tuberculosis.

Bansod and Rai [4] observed that clinical features of mycotic infection and coexisting bacterial pneumonia altered the manifestations of tuberculosis. They observed radiological features and sputum examination of tuberculosis and mycotic infections and sepsis co-existed in a large proportion of patients.

Despite the existence of a National Tuberculosis Programme since 1962, there was little impact on the TB burden till 1992. On the recommendation of an expert committee, a revised strategy to control TB was pilot tested in 1993. A full fledged programme was started in 1997 and rapidly expanded with excellent results. This Revised National Tuberculosis Control Programme (RNTCP) uses the DOTS (Directly Observed Treatment, Short course chemotherapy) strategy, which is based on results of tuberculosis research done in India (Centre TB Division, New Delhi). The "DOTS Strategy" is the globally accepted standard for diagnosis and treatment of tuberculosis.

The chronic nature of tubercular infection is further aggravated if it is accompanied by mycotic infections as these infections after remain undiagnosed and thus untreated. Thus mycotic infections add fatal dimensions to the diseased condition of the patient if superimposed with fatal infection such as pulmonary tuberculosis.

The chronic nature of tubercular infection with prolonged administration of heavy doses of antibiotics further weakens the immunological status of the patient and thus acts as a predisposing factor for secondary fungal infections.

Even today, tuberculosis remains the most important cause of sub-acute and chronic respiratory morbidity in our country which most often leaves behind a scarred pulmonary parenchyma vulnerable to fungal colonization [5].

Materials and Methods

Sample Population

In the present study 100 patients undergoing the DOTS Therapy (Directly Observed Treatment, short course chemotherapy), under the Revised National Tuberculosis Control Programme (RNTCP) during the year 2006-2007 at health care centers at Jabalpur and Mandla district of central India were selected and screened for fungal infections. The Government centers included Primary Health Care (PHC) center, Narayanganj, District Mandla (M.P.); Community Health Care (CHC) center, Babaliya, District Mandla (M.P.); Netaji Subhash Chandra Bose Medical College, Jabalpur (M.P.). The patients were selected on the basis of three different categories of the Tuberculosis disease viz. CAT I Pulmonary Tuberculosis, smear positive for Acid Fast Bacilli (AFB) (n=59), CAT II Pulmonary Tuberculosis, smear negative for AFB (n=25) and CAT III Extra pulmonary Tuberculosis (n=16) i.e. TB of any organ other than the lungs, such as the pleura (TB pleurisy), lymph nodes, intestines, genitourinary tract, skin, joints and bones, meninges of the brain etc.

Isolation of etiological agents

Early morning sputum samples and blood from vein puncture was collected aseptically and subjected to routine procedures comprising of direct microscopy using Lactophenol cotton blue mounting. Sputum samples were inoculated on Sabouraud's Dextrose Agar medium supplemented with 50mg/ml chloramphenicol.

The blood was mixed in Sabouraud's broth in the ratio of 1:10 and incubated for 24 hours at 28 ± 1 °C. About 0.1 ml of this solution was inoculated into SDA slants with chloramphenicol (50 mg/ml). The inoculated specimens were incubated at 28 ± 1 °C and observed at regular intervals up to four weeks. The visible colonies were subcultured on SDA slants. The yeast like colonies were identified using Biochemical and Physiological tests viz. Germtube production, chlamydospore formation on cornmeal agar, sugar fermentation and assimilation tests. [6,7]. Moulds were identified on the basis of their and macro and micro morphology. [8,9,10].

Results

A total of 100 patients suffering from Pulmonary Tuberculosis were undertaken for the present study. Of the 100 patients taken 49% samples were positive for opportunistic fungi and out of these 49 positive samples, 65.31% (n=32) were yeasts while 37.69% (n=17) were filamentous fungi. It was observed that of the 100 sputum samples taken for the present study 46% (n=46) were positive for fungal culture while of the 45 blood samples taken, 31.11% (n=14) were positive.

The isolates obtained in the present study were as follows: *Candida albicans* (34.69%), *C. parapsilosis* (26.53%), *Aspergillus niger* (10.2%), *A. fumigatus* (12.24%), *Fusarium solani*. (6.10%), *Rhizopus oryzae* (4.08%), *Rhodotorula mucilaginosa* (2.04%), *Geotrichum candidum* (2.04%) and *Myridontium keratinophylum* (2.04%). Some of the yeast isolates have been deposited at BCCM/IHEM – Belgian Co-ordinated Collections of Micro-organisms, Belgium; *C. albicans*: BCCM/IHEM 22854, 22855, 22856, 22857, 22858, 22859, 22860, 2262, 22863, 22864, 22866. *C. parapsilosis*: BCCM/IHEM 22883, 22884, 22861, 22865, 22865, 22886, 22887, 22888. *Geotrichum candidum*: BCCM/ IHEM 22983.

It was observed that from sputum 63.04% yeasts and 36.96% filamentous fungi were obtained while from blood 85.71% yeasts and 14.29% filamentous fungi were obtained. The identification of moulds was further confirmed by Dr. Joseph Guarro, Spain.

Table 1 shows 2 different sexes and age groups. The study included 64 males and 36 females out of which 32 males (50%) and 17 females (47.2%) were positive for fungal culture. A mean age of 38.03 years amongst male and 30.82 years amongst females were culture positive for fungal agents. The table also elaborates that amongst males maximum culture positive patients were obtained in the age group of 50-59 years (70%, n=7). None of the male patients in the age group of 10-14 years was positive for fungal infections. On the other hand amongst female patients' maximum culture positively was observed in the age groups of 10-14, 50-59, 60+ years.

Table 2 shows 31 patients belonging to CAT I, 17 patients belonging to CAT II and 1 patient belonging to CAT III were positive for fungal culture. The mean age of culture positive patients were 36.06 years in CAT I, 33.41 years in CAT II and 55 years in CAT III. Amongst patients belonging to CAT I maximum fungal infection (100%, n=2) was observed in 60 + group while in CAT II maximum fungal culture positivity (100%) was observed in 10-14 (n=1) and 50-59 (n=3), years age group. In CAT III 50% (n=1) patients belonging to the age group 50-59 years were positive for fungal culture. The most dominant species were *C. parapsilosis* (16.9%, n=10) in CAT I, followed by *C. albicans* (32%, n=8), CAT II and *A. niger* (6.3%, n=1) CAT III. Fourty nine samples were positive for culture of which 75.5% (n=37) samples were positive for both direct microscopy and culture and 24.5% samples (n=12) were positive for fungal culture and negative for direct microscopy (Table 3 -4).

It was observed that amongst CAT I 50.8% (n = 30/59), CAT II 52% (n = 13/25) and CAT III 25% (n = 4/16) cases were positive for direct microscopy. It is clearly exhibited that 13% patients had tuberculosis since less than 2 months, 52% had tuberculosis for 2-6 months, 29% had tuberculosis for 6-12 months, while the rest 6% of the total had tuberculosis disease for more than 12 months. (Table 5 - 6).

Table 7 shows a scoring of 1+ indicates 10 - 99 Acid fast Bacilli (AFB) per 100 oil immersion field when number of fields to be examined were 100, 2+ scoring indicates 1 - 10 AFB per oil immersion when number of fields to be examined were 50, 3+ Scoring indicates more than 10 AFB per 100 oil immersion field when number of fields to be examined were 20. A negative scoring indicates no AFB in oil immersion field when number of field examined were 100. This type of scoring helps to assess the load of the disease. Of the 49 positive cases of fugal culture 6.1% cases were negative for AFB, 53.1% cases showed a scoring of 1 +, 30.6% cases exhibited a scoring of 2+ and 10.2% cases exhibited a scoring of 3+. Amongst 49 fungal culture positive patients 2% (n = 1) had extra pulmonary tuberculosis and 98% (n = 48) patients had pulmonary tuberculosis (Table 8).

Discussion

The idea of a commensal relationship between fungus and tuberculosis infections of the lungs has been suggested in the past [11,12,13]. In the present study we report 49% cases of opportunistic fungal infections in tuberculosis patients. Shome *et. al.,* [14] also reported fungal infections organisms only in 18% cases. The specimens used by Shome *et. al*., [14] comprised of sputum, bronchial aspirate and bronchoscopic material. No doubt, the bronchial aspirate and bronchoscopic material are likely to be more specific for pulmonary pathology than sputum itself. This might explain the difference between our results. Similar results were reported by Chadeganipour *et. al*., [15] reported 16 patients were affected with definite tuberculosis and 11 of these cases i.e. 68.75% had pulmonary fungal infection in addition to tuberculosis. Of these 11 cases, 45.45% (n=5) were due to filamentous fungi and 54.45% (n=6) of these infections were due to yeasts.

Recently Dabo and Yusha'u [16] reported systemic mycoses in patients with suspected tuberculosis in Nigeria. and showed 10.59% patients were positive for systemic fungal infection. In the present study we report 65.31% (n=32) yeasts and 37.69% (n=17) filamentous fungi.

This is in contrary to the studies reported by Temek *et. al.,* [17] and Shadzi and Chandeganipour [18]. Temek *et. al*., [17] presented a report on pulmonary infection in immunocompromised hosts and showed that out of 17 patients with tuberculosis, five cases had (29.41%) yeast infections and two cases (11.76%) were affected with filamentous fungi. Shadzi and Chadeganipour [18] reported that 43 patients with tuberculosis as predisposing factor had 7.3% yeast infections and 0.4% aspergillosis.

The majority of isolates obtained in the study were *Candida albicans* . The frequency of *C. albicans* isolates was 34.69% (n=17) followed by *C. parapsilosis* 26.53% (n=13). The frequency of *Aspergillus* sp. obtained are as follows: *A. fumigatus* 12.24% (n=6) and *A. niger* 10.2% (n=5) i. e. *Candida* was obtained in 61.2% and *Aspergillus* in 22.45% samples. The results are supported by the study done by Khanna *et. al*., [19]. They obtained *Candida* in 26.36% cases and *Aspergillus* in 10% cases. Sixty two percent of the *Candida* species isolated from their cases comprised of *C. albicans* while the present study reveals 56.7% of *Candida* species isolated comprised of *C. albicans*.

On the contrary Schwarting and Skinner [20] examined 500 samples of sputum of patients in a tuberculosis sanatorium and isolated *Candida* from 99 (20%) samples. From among 860 cases screened by Shome *et. al*. [14], there were 155 positive cases out of which 79 cases were of candidiasis i. e. 50.97%, while only 6 cases were of aspergillosis i.e. 3.87%.

The relatively high incidence of candidiasis among tuberculosis cases is quite in line with the earlier records {Pandalai and Kurup, [21]}. The occurrence of candidiasis concomitantly with tuberculosis is of paramount interest in the treatment and management of tuberculosis patients as *C. albicans* is supposed to enhance the virulence of *Mycobacterium tuberculosis* [14].

*Aspergillu*s species are among the most frequently recovered fungi from opportunistic pulmonary infections in the immuno compromised hosts [22, 23].

Aspergillus fumigatus has been reported as the most common species isolated from respiratory tract secretions and is responsible for most cases of Aspergillosis followed by *A. flavus*, *A. niger*, *A. terreus* and *A. nidulans* [22, 23, 24] . In the present study, *A. fumigatus* (12.24%) was reported as the most common mould followed by *A. niger* (10.2%).

Bansod and Rai [4] reported 46% (203 out of 500 samples) mycotic infections in pulmonary tuberculosis patients. They observed that 68.8% males and 46.6% females were culture positive. However, in our study we found that out of the total cases 50% males and 47.2% females were reported for fungal infection.

Andleigh [25] investigated 32 cases of non tuberculous chronic pulmonary disease and 10 cases of chronic pulmonary tuberculosis not responding to the routine treatment, to find out if fungi played any role in their etiology. Out of the 32 cases of non- tuberculosis chronic pulmonary disease, 9 cases showed growth of fungi. Out of them *C. albicans* was isolated from eight cases and from one case *Actinomyces bovis* was isolated. Out of the 10 cases of pulmonary tuberculosis two were positive for *C. albicans*. The acid fast bacilli isolated from these cases were sensitive to streptomycin. They concluded that in some cases of pulmonary tuberculosis, suspected of infection with a resistant strain, the disease was kept up due to a superadded fungal infection and not due to any resistance developed by the organisms against streptomycin. Fungi as such seem to play some role in the etiology of pulmonary diseases and probably also help in keeping up the disease in tuberculosis cases [25].

The prevalent knowledge that fungi are ordinarily saprophytic and ubiquitous has, perhaps, led us into the erroneous attitude that all fungi found in the sputum are harmless contaminants, and their importance as a factor in an existing tuberculosis process is slight and of no consequence in the consideration of the subsequent course of the tuberculosis invalid who is unfortunate enough to be the host to both a tuberculous and a fungus infection [11].

The object of this study was thus to refute the impression that these co-existing fungi are harmless, saprophytic invaders, and to advance added data to support the premise that an infection of the lungs with pathogenic fungi in association with a tuberculous infection of the lungs is not harmless and coincidental, but that it definitely increases the activity and virulence of the tuberculosis process.

Longo *et. al*., [26] investigated the presence of fungi in the sputa of newly admitted patients in a tuberculosis hospital during a period of 11 months in order to determine the coexistence of fungi and tubercle bacilli in the lungs of patients with pulmonary tuberculosis. Out of the total 505 samples investigated there were 137 (28.1%) samples were found to be positive for both tubercle bacilli and fungi. The isolates recovered included *C. albicans* (n=100), *Candida* spp. (n=35), *Cryptococcus neoformans* (n=1) and *Geotrichum candidum* (n=1). In the present study also a single isolate of *G. candidum* (2.04%) was obtained.

The evidence obtained from the present study indicates that opportunistic fungal infections of compromised hosts are becoming much more common and that any organism should be considered to be a potential agent of infection in those suspected of tuberculosis who do not respond to tuberculosis therapy. This situation presents a unique challenge to laboratory personnel and clinicians. It requires that all organisms found in clinical specimens from such patients should be thoroughly identified and reported and clinicians should assess the clinical significance of such isolates and be familiar with the variety of clinical pictures that may occur with opportunistic infections in patients suspected of tuberculosis therapy.

Shome *et. al.,* [14] stated that India has the largest number of recorded tuberculosis cases, which in itself is a very big public health problem for India. The lack of proper laboratory and diagnostic facilities in India not only let pulmonary mycoses go undiagnosed but quite frequently the mycoses remains untreated as well.

The lack of any specific clinical or radiological symptoms in case of mycoses is confusing, leading to complication in the treatment. In spite of the absence of a proper reporting system of pulmonary mycoses in India it is evident that the importance of mycoses of the human broncho pulmonary system far surpasses our current estimation and is a positive hazard to the health of the people.

It can be concluded that the present study helps in establishing that far more damaging and dangerous dimensions are added to as complications to tuberculosis with mycoses. Secondary fungal infections in immunocompromised individuals need to be taken seriously by medical personnel for timely management of the disease. To acquire the proper health management of a patient, accurate and faster diagnosis of such infections is required.

The proper diagnosis of fungal isolates will help in reduction in mortality and morbidity associated with invasive fungal disease in immunocompromised patients.

Table 1: Showing frequency of culture positive patients amongst both genders in different age groups

Age Group	Male			Female		
	Culture +	Culture -	Total	Culture +	Culture -	Total
10-14	0	1 100%	1 1.56%	2 100%	0	2 5.56%
15-19	3 50%	3 50%	6 9.38%	4 66.6%	2 33.33%	6 16.67%
20-29	6 54.54%	5 45.45%	11 17.19%	3 25%	9 75%	12 33.33%
30-39	8 44.44%	10 55.56%	18 28.13%	2 28.57%	5 71.43%	7 58.33%
40-49	7 46.67%	8 53.33%	15 23.44%	3 50%	3 50%	6 16.67%
50-59	7 70%	3 30%	10 15.63%	2 100%	0	2 5.56%
60+	1 33.33%	2 66.67%	3 4.69%	1 100%	0	1 2.78%
Total	32	32	64	17	19	36
Mean ±SD	38.03 13.057	36.69 12.833	37.36 12.860	30.82 16.816	29.84 9.365	30.31 13.214

SD: Standard Deviation

Table 2: Showing culture positive patients amongst the 3 categories of tuberculosis patients and different age groups.

Age Group	CAT I			CAT II			CAT III		
	Culture +	**Culture -**	**Total**	**Culture +**	**Culture -**	**Total**	**Culture +**	**Culture -**	**Total**
10-14	1 50%	1 50%	2 3.39%	1 100%	0	1 4%	0	0	0
15-19	5 62.5%	3 37.5%	8 13.56%	2 50%	2 50%	4 16%	0	0	0
20-29	6 42.86%	8 57.14%	14 23.73%	3 60%	2 40%	5 20%	0	4 100%	4 25%
30-39	5 41.67%	7 58.33%	12 20.34%	5 62.5%	3 37.5%	8 32%	0	5 100%	5 31.25%
40-49	7 50%	7 50%	14 23.73%	3 75%	1 25%	4 16%	0	3 100%	3 18.75%
50-59	5 71.43%	2 28.57%	7 11.86%	3 100%	0	3 12%	1 50%	1 50%	2 12.5%
60+	2 100%	0	2 3.39%	0	0	0	0	2 100%	2 12.5%
Total	31	28	59	17	8	25	1	15	16
Mean ±SD	36.06 14.993	32.57 11.423	34.41 13.420	33.41 14.094	29.50 8.799	32.16 12.589	55.00	39.53 13.362	40.50 13.476

CAT I: Pulmonary Tuberculosis, Smear positive

CAT II: Pulmonary Tuberculosis, Smear negative

CAT III: Extra Pulmonary Tuberculosis

SD: Standard Deviation

Table 3: Showing frequency of isolates amongst 3 categories of tuberculosis patients.

Isolate		CAT			Total
		I	II	III	
Aspergillus fumigatus		5	1	0	6
		8.5%	4.0%	0%	6.0%
Aspergillus niger		4	0	1	5
		6.8%	0%	6.3%	5.0%
Candida albicans		9	8	0	17
		15.3%	32.0%	0%	17.0%
Candida parapsilosis		10	3	0	13
		16.9%	12.0%	0%	13.0%
Fusarium solani.		1	2	0	3
		1.7%	8.0%	0%	3.0%
Geotrichum candidum		0	1	0	1
		0%	4.0%	0%	1.0%
Myridontium Keratinophylum		1	0	0	1
		1.7%	0%	0%	1.0%
Rhodotorula mucilaginosa.		0	1	0	1
		0%	4.0%	0%	1.0%
Rhizopus oryzae.		1	1	0	2
		1.7%	4.0%	0%	2.0%
Negative		28	8	15	51
		47.5%	32.0%	93.8%	51.0%
Total		59	25	16	100

CAT I: Pulmonary Tuberculosis, Smear positive

CAT II: Pulmonary Tuberculosis, Smear negative

CAT III: Extra Pulmonary Tuberculosis

Table 4: Showing comparative analysis of direct microscopy and culture examination for fungal isolation

Direct Microscopy	Culture		Total
	+	-	
+	37	10	47
	75.5%	19.6%	47.0%
-	12	41	53
	24.5%	80.4%	53.0%
Total	49	51	100

Table 5: Showing direct microscopy results with respect to different category of tuberculosis disease.

Direct Microscopy	CAT			Total
	I	II	III	
+	30	13	4	47
	50.8%	52.0%	25.0%	47.0%
-	29	12	12	53
	49.2%	48.0%	75.0%	53.0%
Total	59	25	16	100

CAT I: Pulmonary Tuberculosis, Smear positive

CAT II: Pulmonary Tuberculosis, Smear negative

CAT III: Extra Pulmonary Tuberculosis

Table 6: Showing frequency of fungal culture with respect to duration of disease.

Duration of Disease (months)	**Culture**		**Total**
	+	-	
<2	4	9	13
	8.2%	17.6%	13.0%
2-6	28	24	52
	57.1%	47.1%	52.0%
6-12	16	13	29
	32.7%	25.5%	29.0%
>12	1	5	6
	2.0%	9.8%	6.0%
Total	49	51	100

Table 7: Showing scoring of sputum examination for *Mycobacterium tuberculosis* versus fungal culture results

Sputum examination for *Mycobacterium tuberculosis*	**Culture**		**Total**
	-	+	
-ve	15	3	18
	29.4%	6.1%	18.0%
1+	21	26	47
	41.2%	53.1%	47.0%
2+	11	15	26
	21.6%	30.6%	26.0%
3+	4	5	9
	7.8%	10.2%	9.0%
Total	51	49	100

-ve: No Acid Fast Bacilli (AFB) in oil immersion field, No. of field examined: 100

1+: 10-99 AFB per 100 oil immersion field, No. of field examined: 100

2+: 1-10 AFB per oil immersion field, No. of field examined: 50

3+: More than 10 AFB per 100 oil immersion field, No. of field examined: 20

Table 8: Showing Fungal culture results versus site of infection of tuberculosis disease

Site of infection		Culture		Total
		+	-	
EP		1	9	10
		2.0%	17.6%	10.0%
P		48	42	90
		98.0%	82.4%	90.0%
Total		49	51	100

EP: Extra pulmonary P: Pulmonary

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