

Prevalence and characterization of opportunistic candidal infections among patients with pulmonary tuberculosis

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Abstract

Background: Although *Candida albicans* remains the most common cause of human candidiasis, the frequency of infection attributed to other members of the genus is also increasing. Hence, the present study was carried out to know the prevalence of opportunistic candidal infection in tuberculosis, and if positive, the species of *Candida* that is most commonly associated.

Materials and Methods: The present study comprised sixty pulmonary tuberculosis patients who were divided into (1) fresh or untreated group, (2A) chronic or treated group having no complications and (2B) having complications, comprising twenty patients each, respectively. The collected sputum samples were initially stained with Ziehl–Neelsen stain for confirmation of presence of tubercle *Bacilli*. Primary isolation was done on Sabouraud Dextrose Agar (SDA). The candidal colonies were confirmed microscopically for the presence of pseudohyphae. Further speciation of the positive candidal samples was carried out using ChromAgar.

Result: The total fungal prevalence among 60 patients with pulmonary tuberculosis on SDA was 33 (55%) *Candida* and 3 (5%) *Aspergillus*. The prevalence of different candidal species on ChromAgar showed *C. albicans* as the predominant one, followed by *Candida tropicalis* and *Candida krusei*. Freshly diagnosed or untreated group was less commonly associated with pulmonary mycoses than chronic or treated group. The prevalence of *Candida* had increased with treatment, duration and age, and it was more in males than females.

Conclusion: The present study confirms the phenomenon of opportunistic candidal infections in pulmonary tuberculosis patients. Rapid and reliable identification of *Candida* species is essential as they differ in their virulence and sensitivity to antifungal drugs.

Key Words: Antifungal drugs, *Candida*, ChromAgar, opportunistic infections, pulmonary tuberculosis

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INTRODUCTION

Tuberculosis has recently emerged as a major health concern. Each year, approximately two million people worldwide die of tuberculosis and nine million become infected.^[1] The average

prevalence of all forms of tuberculosis in India is estimated to be 5.05 per thousand; prevalence of smear-positive cases is 2.27 per thousand and average annual incidence of smear-positive cases is 84 per 100,000 annually.^[2] The prevalence of tuberculosis continues to increase because of the increased

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number of patients infected with human immunodeficiency virus, bacterial resistance to medications, increased international travel and immigration from countries with high prevalence and growing numbers of the homeless and drug abusers.^[1] The prevalence of opportunistic mycoses has dramatically increased during the past few years. These opportunistic fungi are potential pathogen in the immunocompromised patients, patients with some preexisting disease and patients with a long history of antibiotics. 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 antituberculous drugs of nonspecific action, which promote the growth and reproduction of the fungus flora and in turn aggravate the course of underlying process in the lung tissues.^[3]

Different fungi pathogens are involved in pulmonary tuberculosis such as *Aspergillus*, *Histoplasma* and *Cryptococcus* depending upon the geographical distribution and genetic makeup, but *Candida albicans* is the most common yeast isolated from tuberculous patients. It is responsible for causing severe secondary infections in such patients. *C. albicans* is a normal inhabitant of the respiratory tract. It is said to be recovered in sputum in over 50% of patients with pulmonary tuberculosis, about 25% of patients in hospital with other conditions and over 10% of healthy individuals. Of all the predisposing factors, candidiasis is mostly associated with wide spectrum antibacterial therapy in patients with chronic bronchopulmonary diseases.^[4]

Although *C. albicans* remains the most common cause of human candidiasis, the frequency of infection attributed to other members of the genus is also increasing. This is primarily due to the increase in the number of at-risk individuals, particularly those with impaired immunity, such as tuberculosis, transplant recipients, cancer patients receiving chemotherapy and human immunodeficiency virus-infected patients. The genus *Candida* comprises about 200 species, of which twenty have been associated with pathology in humans.^[5]

The conventional methods of yeast identification, which mainly consist of assimilation and fermentation characteristics, are reported to be cumbersome and beyond the expertise range available in local laboratories. Numerous isolation media are available in the market that can identify pathogens within 4–72 h, depending upon the system. One such medium is ChromAgar which serves as a medium for detection and identification of major *Candida* species with accuracy, reduces the time of identification and its characterization from poly fungal specimens.^[6]

Hence, this study was carried out to know the prevalence of opportunistic candidal infection among patients with

pulmonary tuberculosis, and if positive, the species of *Candida* that is most commonly associated.

MATERIALS AND METHODS

The present study comprised sixty diagnosed patients of pulmonary tuberculosis. They were divided into two study groups as follows:

- Group 1: Fresh or untreated group comprising twenty pulmonary tuberculosis patients who had taken none or <1 month of antitubercular treatment
- Group 2: Chronic or treated group who had taken antitubercular treatment for more than 1 month. They were further divided into:
 - Group 2A: Comprised twenty pulmonary tuberculosis patients undergoing antitubercular treatment and having no complications
 - Group 2B: Comprised twenty pulmonary tuberculosis patients having complications such as nonsubiding fever, marked cough and persistence of other symptoms in spite of taking antitubercular treatment.

Exclusion criteria

HIV-positive patients, patients with other immunocompromised conditions and undergoing any form of antifungal therapy were excluded from the study.

Methodology

All the patients were informed regarding the purpose of the study and signed informed consent was obtained. Ethical clearance was obtained from the concerned authority. Patients were instructed to rinse mouth with an antiseptic mouthwash chlorhexidine 2% to avoid contamination from the oral cavity. Approximately 5 ml of early morning specimen of deep productive cough was collected in a sterile wide mouth jar. Once the sample was collected, the container was transferred immediately to the laboratory for further study. The collected sputum samples were initially stained with Ziehl–Neelsen stain for confirmation of tubercle *Bacilli*. Once the pink acid-fast *Bacilli* was confirmed, the sputum sample was inoculated on Sabouraud Dextrose Agar (SDA) media (HiMedia; India) by streaking method using inoculating loops. The loop was placed at the base of the McCartney bottle and was drawn up the SDA surface while moving it from side to side and incubated at 25°C for 48 h. The smooth, creamy, white pasty colonies on SDA representative of *Candida* [Figure 1a] and rough, greenish brown-pigmented colonies representative of *Aspergillus* were observed [Figure 1b].

Further, very small inoculum from an isolated fungal colony was picked up with a sterile inoculating loop and was placed on a clean glass slide. A drop of lactophenol cotton blue stain (HiMedia; India) was suspended over this; the colony

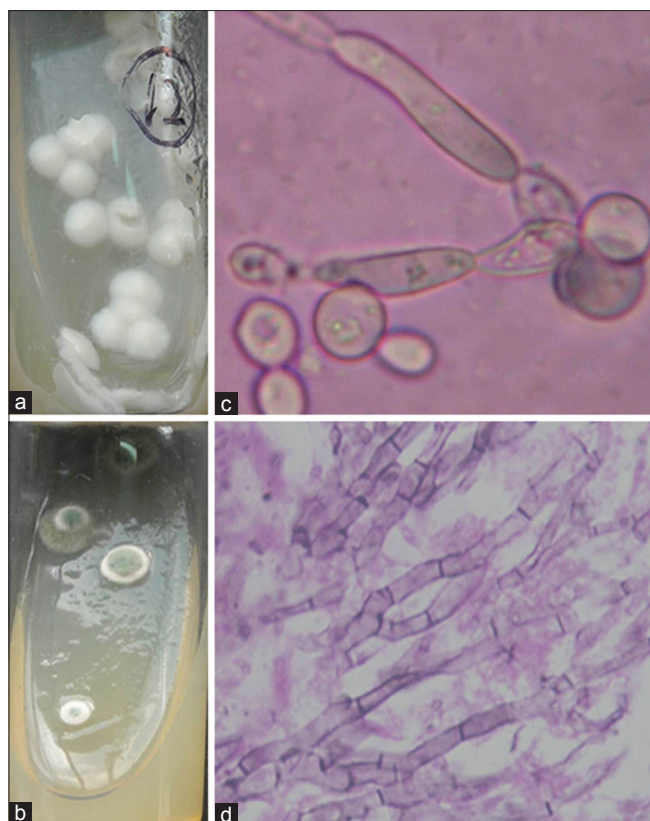


Figure 1: (a) The typical smooth creamy white pasty colonies on Sabouraud Dextrose Agar are suggestive of *Candida* and (b) rough, greenish brown colonies were characterized by *Aspergillus* (b). The colonies were observed microscopically using lactophenol cotton blue stain and (c) *Candida* was confirmed by the presence of pseudohyphae characterized by chain of elongated “buds” or cells that remain attached to the mother cells and (d) non-candidal colony by the presence of true septate hyphae

was emulsified properly and was covered with cover slip. This was first examined under a low power objective to locate the group of cells, the presence of pseudohyphae, characterized by a chain of elongated “buds” or cells that remain attached to the “mother” cell and constrict at the points of attachment to each other, confirmed it as *Candida* [Figure 1c and d]. The samples which were positive for pseudohyphae formation were then inoculated on ChromAgar for speciation.

The ChromAgar medium (HiMedia; India) was freshly prepared according to the manufacturer’s instructions and dispensed in Petri dishes after being allowed to cool slightly. With a sterile inoculating loop, the sample was streaked across the surface of ChromAgar plate. The four-quadrant streak method was used which is accomplished by streaking and rotating the plate in four sections, one-quarter at time, slightly overlapping the original streak area. The fourth quadrant contained the greatest dilution of microorganisms and provided isolated colonies for further testing. ChromAgar was incubated at 37°C and was examined after 48 h of inoculation. The morphology and color of the growth were

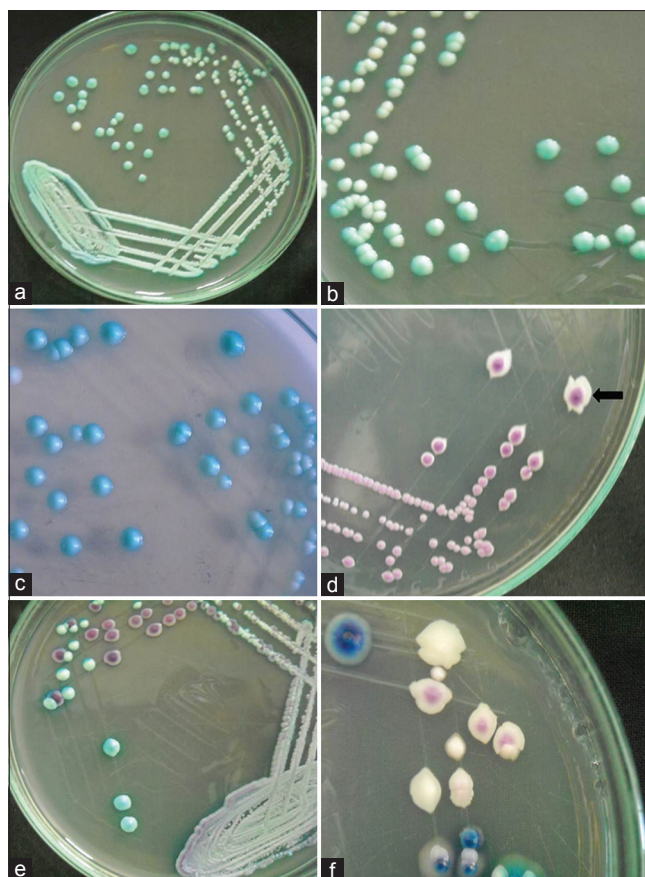


Figure 2: (a and b) The green colonies of *Candida* on ChromAgar are representative of *Candida albicans*, (c) the blue isolated colonies of *Candida* were of *Candida tropicalis*, (d) pink large spreading colonies with pale edge were of *Candida krusei*. (e) Mixed culture comprising both green and pink large spreading colonies representative of *Candida albicans* and *Candida krusei*, along with (f) mixed culture comprising blue and pink large spreading colonies representative of *Candida tropicalis* and *Candida krusei*

recorded and species of *Candida* was identified on the basis of color [(Figure 2a-d), [Table 1]].

Statistical analysis

The data thus obtained were statistically analyzed with Chi-square test using SPSS software version 14, IBM Modeler.

RESULTS

Out of total sixty samples, 36 (60%) showed positive and 24 (40%) showed negative fungal prevalence on SDA medium. The positive and negative fungal prevalence in Group 1 were 9 (45%) and 11 (55%), in Group 2A were 8 (40%) and 12 (60%) and in Group 2B were 19 (95%) and 1 (5%), respectively [Table 2].

Along with creamy white pasty colonies representative of *Candida*, other fungal growths with rough, greenish brown-pigmentation were also observed which were representative of *Aspergillus*. Thus, out of 36 positive fungal prevalence, 33 (91.6%) were representative of *Candida* and 3

(8.4%) were of *Aspergillus*. The positive fungal prevalence for *Candida* and *Aspergillus* in Group 1 was 8 (24.24%) and 1 (33.3%), in 2A was 8 (24.24%) and 0 (00%) and in 2B was 17 (51.51%) and 2 (66.6%), respectively [Table 3].

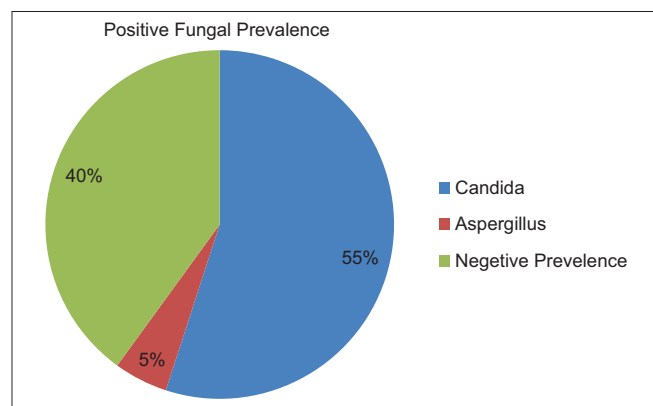
Thus, the total fungal prevalence in SDA among sixty patients with pulmonary tuberculosis included of 33 (55%) *Candida* and 3 (5%) *Aspergillus* species [Graph 1].

The further speciation of *Candida* when done on ChromAgar yielded three different species, i.e. *C. albicans*, *Candida tropicalis* and *Candida krusei*. In Groups 1 and 2A, all cases were representative of *C. albicans* with equal prevalence of 8 (40%). In Group 2B, 12 (60%) were *C. albicans*, 2 (10%) were *C. tropicalis* and 1 (5%) was *C. krusei*. Along with this, two mixed cultures were obtained comprising 1 (5%) with *C. albicans* and *C. krusei* [Figure 2e] and 1 (5%) with *C. tropicalis* and *C. krusei* [Figure 2f and Graph 2]. The prevalence of three different candidal species was statistically significant with a *P* value of 0.007, 0.003 and 0.003 for *C. albicans*, *C. tropicalis* and *C. krusei*, respectively [Table 4].

The prevalence of *Candida* with duration of treatment, i.e., between 0–1 month, 1–3 months and more than 3 months, was 8 (24.25%), 11 (33.3%) and 14 (42.42%), respectively, but was statistically not significant [Table 5]. The prevalence of *Candida* in different age groups, i.e. 20–40 years, 40–60 years and 60–80 years, was 5 (15.16%), 10 (30.30%) and 18 (54.54%), respectively. This result was statistically significant with *P* = 0.02 [Table 6]. The prevalence of *Candida* in males and females was 20 (60.7%) and 13 (39.3%), respectively, with nonsignificant *P* = 0.221 [Table 7].

DISCUSSION

Pulmonary tuberculosis is essentially a chronic destructive disease of the lungs. Caseation, necrosis and fibrosis lead to the formation of cavities with bronchiectatic dilations. These



Graph 1: Positive fungal prevalence on Sabouraud Dextrose Agar among total patients with pulmonary tuberculosis

Table 1: The colonial characteristics of different candida albicans species on ChromAgar medium

Candida species	Color and Colony characteristics
<i>C. albicans</i>	Green (Figure 2a & b)
<i>C. tropicalis</i>	Steel Blue (Figure 2c)
<i>C. krusei</i>	Pink with a pale edge (Figure 2d)
<i>C. parapsilosis</i>	Cream colored
<i>C. glabrata</i>	Purple
<i>C. lusitaniae</i>	Pink
<i>C. nivariensis</i>	Cream to white

Table 2: Total fungal prevalence on Sabouraud’s Dextrose Agar medium among patients with pulmonary tuberculosis

Study Group	Total	Fungal Prevalence			
		Positive		Negative	
		No.	%	No.	%
1	20	9	45	11	55
2A	20	8	40	12	60
2B	20	19	95	01	5
Total	60	36	60	24	40

Table 3: Positive fungal prevalence on Sabouraud’s Dextrose Agar among patients with pulmonary tuberculosis

Study Group	Positive Fungal Prevalence	Candida		Aspergillus	
		No.	%	No.	%
1	9	8	24.24	1	33.3
2A	8	8	24.24	0	00
2B	19	17	51.51	2	66.6
Total	36	33	91.6	3	8.4

Table 4: Intergroup comparison for the prevalence of different Candidal species

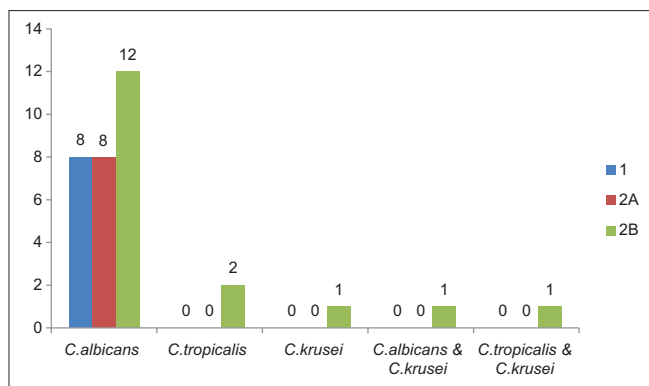
Candidal Species	1	2A	2B	Total Patients	P value
Albicans	8	8	13	29	0.007 (Significant)
Tropicalis	0	0	3	3	0.003 (Significant)
Krusei	0	0	3	3	0.003 (Significant)

Table 5: Comparison for the prevalence of Candida with duration of treatment

Duration of Treatment (Months)	Total no. of Patients	Positive Candidal Prevalence	Percentage	P Value
0-1	20	8	24.25	0.0556 (Not Significant)
1-3	23	11	33.33	
>3	17	14	42.42	
Total	60	33	100	

Table 6: Comparison for the prevalence of Candida in different age groups

Age Groups (years)	Total Patients	Positive Candidal Prevalence	Percentage	P value
20-40	20	5	15.16	0.02 (Significant)
40-60	17	10	30.30	
60-80	23	18	54.54	
Total	60	33	100	



Graph 2: The prevalence of different candidal species on ChromAgar

Table 7: Comparison for the prevalence of *Candida* in relation to gender

Total Candidal Prevalence	Males		Females		P value
	No.	%	No.	%	
33	20	60.7	13	39.3	0.221 (Not Significant)

destroyed areas of the lung will no doubt continue to remain in the body even after the tubercle *Bacilli* has been totally eliminated. These cavities form an ideal culture plate for the tubercle *Bacilli* and many other organisms including the fungi by providing plenty of oxygen along with necrotic tissue material. The fungal organisms tend to settle in the cavities and destroyed dilated bronchi, as a rule, after the tubercle *Bacilli* has disappeared from these areas.^[7]

Moreover, the prolonged antitubercular therapy in tuberculosis, which may last for well over 2 years with or without corticosteroids by itself, becomes a potent predisposing factor for the onset of superinfection by the fungal organisms. Therefore, it is not surprising to come across frequent references in the literature regarding the association of mycological superinfection with active pulmonary tuberculosis.^[7] The role of *Candida* species as secondary invader of lungs, kidneys and other organs of patients having some preexisting diseases such as tuberculosis and cancer has also been documented.^[8] There is a considerable variation of 9–80% in incidence reported in the literature on the occurrence of *Candida* species in sputum of patients with pulmonary tuberculosis. Besides, a syntrophic relationship between *C. albicans* and *Mycobacterium tuberculosis* has also been reported in a number of studies where tubercle *Bacilli* was found to enable *C. albicans* to grow leading to aggravation of tuberculosis and persistence of symptoms.^[9] Thus, occurrence of candidiasis concomitantly with tuberculosis is of paramount interest in the treatment and management of tuberculous patients as *C. albicans* is supposed to enhance the virulence of *M. tuberculosis*. It is considered worthwhile to emphasize here that patients under prolonged antitubercular therapy may also be given a protective covering dose of antifungal agents.^[10,11]

The genus *Candida* comprises about 200 species, of which close to twenty has been associated with pathology in humans or animals. Although *C. albicans* is the most common one, infections attributed to other members of the genus is also increasing.^[5] Changing *Candida* epidemiology and availability of newer antifungal drugs with different spectra means that physicians can no longer make therapeutic decisions based on broad identification of fungi as yeast and mold.^[6] Hence, identification of *Candida* is important up to the species level because of the susceptibility of different species to different drugs.

As the traditional methods are tedious and time-consuming to perform, in recent years, differential media have been developed that allow identification of certain *Candida* species based on colony appearance and color following primary culture. One such medium is ChromAgar. This medium is species-specific, allowing the organisms to be identified to the species level by their color and colonial characteristics in 48 h. Moreover, it is 100% sensitive and specific for *Candida*.^[12] The advantage of such medium is that the presence of multiple *Candida* species in a single infection can be determined which can be important in selecting subsequent treatment options.^[6,12]

In the present study, the total fungal prevalence on SDA among patients with pulmonary tuberculosis was 60% which was in accordance with the results of the studies done by Latha *et al.*^[13] and Jain *et al.*^[14] However, it was higher than the results of the studies done by Bansod S *et al.*,^[5] Khanna *et al.*,^[7] Shome *et al.*,^[10] Riddell and Clayton^[15] and Cermeno *et al.*^[16] This variation in the prevalence can be attributed to the change in the geographical distribution, genetic makeup of the patients, sample size and type of culture media used.

In the present study, *Candida* was the predominant fungus with a prevalence of 55% followed by 5% *Aspergillus*. The prevalence of candida was in accordance with Latha *et al.*,^[13] and it was higher than the similar studies coated in the literature, Naz and Tariq,^[3] Khanna *et al.*,^[7] Baradkar *et al.*,^[9] Shome *et al.*^[10] and Riddell and Clayton.^[15] However, the results of the present study were not in accordance with several investigators such as Bansod S *et al.*^[5] and Jain *et al.*,^[14] who reported *Aspergillus* as the predominant fungus and Cermeno *et al.*^[16] also reported *Coccidioides* and *Histoplasma* as the predominant one. According to the literature review by Randhawa^[17] of the well-known endemic mycoses, coccidioidomycosis, paracoccidioidomycosis and histoplasmosis duboisii (African histoplasmosis) have not been reported so far from India or elsewhere in Asia. Hence, this change in the trend of prevalence can be attributed to exogenously acquired infection, geographical distribution, occupation of the patient, most commonly among agricultural

workers, gardeners and individuals feeding poultry and other birds.^[15]

The prevalence of different Candidal species on ChromAgar, in the present study, showed *C. albicans* as the predominant one, followed by *C. tropicalis* and *C. krusei*. These results were in accordance with Khanna et al.,^[7] Baradkar et al.,^[9] Shome et al.^[10] and Riddell and Clayton.^[15] However, the results were in contrast with Naz and Tariq,^[3] who reported *C. tropicalis* as the predominant one, since their study included mostly HIV-positive patients. The literature also supports that *C. tropicalis* has an apparently strong affinity and greater capacity than *C. albicans* to invade the deep tissues of immunocompromised host.^[3,18]

Besides, in the present study, two mixed cultures were also obtained which were in accordance with the results of a study done by Jain et al.^[14] The presence of mixed culture indicates the need for prompt detection of associated yeast species which may aid in rapid and appropriate treatment decisions. In the light of differences in susceptibility of yeast species to antifungal agents as most of the non-*C. albicans*, *Candida* usually exhibits a reduced susceptibility to the common antifungal agents, especially fluconazole.^[13,19]

In the present study, the freshly diagnosed or untreated group was less associated with pulmonary mycoses than chronic or treated group and this was in accordance to the study done by Khanna et al.^[7] and Jain et al.^[14] However, when intercomparison was done within the treated group, the prevalence of *Candida* was maximum in patients with complications such as nonsubsiding fever, marked cough and persistence of other symptoms. These results were in accordance with the study done by Naz and Tariq.^[3] This could be for the reason that antibiotics and corticosteroids prescribed in tuberculosis patients predispose to fungal superinfections, and thus, the prevalence of different *Candida* species increases with the chronicity of the disease.^[14]

Intercomparison for the prevalence of *Candida* with the duration of treatment revealed an increase in the prevalence but was statistically not significant. The results were similar to the study done by Jain et al.^[14] but showed significant values and this may be attributed to variation in the sample size.

Intercomparison for the prevalence of *Candida* in different age groups revealed a statistically significant increase with maximum prevalence in 60–80 years, which was not in accordance with Khanna et al.^[7] and Shome et al.,^[10] where the maximum affected age group was 20–40 years. This variation could be pertaining to many factors, the most important of which appears to be the host defense mechanism being severely

impaired in elderly age group and thus reducing the patient's resistance to superinfections.^[10,15,20]

As far as the prevalence of *Candida* in relation to gender is concerned, it is evident from literature that the colonization with *Candida* species occurs in equal number among both males and females.^[21] However, in the present study, the candidal prevalence was more in males as compared to females although the results were not statistically significant and this was in accordance with studies done by Naz and Tariq^[3] and Shome et al.^[10] The increased prevalence in males can be attributed to their increased exposure to external environment and habit of using some addictive substances.^[3]

CONCLUSION

Hence, from this study, it is apparent that the association of pulmonary tuberculosis with endemic systemic mycoses is not unusual in our environment; therefore, it should be precisely examined using different methods. Pulmonary mycoses relatively by themselves are not grossly damaging; however, when superimposed in conditions such as tuberculosis, their impact on the morbidity and mortality pattern renders them vitally important. Recent past bears evidence to the fact that mycoses represents a greater health burden and challenge than is realized by people or their health officials. It should be remarked the need to be aware of this possibility to set the appropriate strategies to prevention, diagnosis and therapy for these mycoses in tuberculous patients. ChromAgar serves as a medium for detection and identification of major candidal species with accuracy, reduces the time for identification, is cost-effective and gives a presumptive identification in a short span of time.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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