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Prevalence of *Candida* co-infection in patients with pulmonary tuberculosis

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RESEARCH

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Abstract

Background

Candida species are emerging as a potentially pathogenic fungus in patients with broncho-pulmonary diseases. The synergistic growth promoting association of *Candida* and *Mycobacterium tuberculosis* has raised increased concern for studying the various *Candida spp*. and its significance in pulmonary tuberculosis patients during current years.

Aims

This study was undertaken with the objective of discovering the prevalence of co-infection caused by different *Candida* species in patients with pulmonary tuberculosis.

Method

A total of 75 patients with pulmonary tuberculosis diagnosed by sputum Ziehl-Neelsen staining were included in the study. *Candida* co-infection was confirmed using the Kahanpaa et al. criteria. *Candida* species were identified using gram stain morphology, germ tube formation, morphology on cornmeal agar with Tween-80, sugar fermentation tests and HiCrome Candida Agar.

Results

Candida co-infection was observed in 30 (40%) of patients with pulmonary tuberculosis. *Candida albicans* was the most common isolate observed in 50% of the patients with co-infection, followed by *C. tropicalis* (20%) and *C. glabrata* (20%). *Candida* co-infection was found in 62.5% of female patients, while it was observed in only 29.4% of the male patients (P value 0.0133). Mean ± SD age of the patients with *C. glabrata* infection was 65.83 ± 3.19, while the mean ± SD age of the patients with other *Candida* infections was 43.25 ± 20.44 (P value 0.0138).

Conclusion

Many patients with pulmonary tuberculosis have co-infection with *Candida* spp. The prevalence of non-albicans *Candida* species is increasing and may be associated with inadequate response to anti-tubercular drugs. *C. glabrata* infection has a strong association with old age.

Key Words

Candida co-infection; *C. glabrata;* prevalence; tuberculosis

What this study adds:

1. *Candida* is an opportunistic fungal pathogen infecting immunocompromised hosts. Although the synergistic growth promoting association of *Candida* and *Mycobacterium tuberculosis* is well documented, sputum isolates of *Candida spp*. are usually ignored as an innocuous throat commensal.
2. A significant proportion of isolates were non-albicans *Candida* species which were also more frequent among tuberculosis patients with persistence of chest symptoms in spite of anti-tubercular treatment of two months or more.
3. This study implies the need for screening pulmonary tuberculosis patients for *Candida* co-infection, especially in case of patients with inadequate response to anti-tubercular therapy. Anti-fungal sensitivity test may be valuable in case of non-albicans *Candida* species.

Background

Candida albicans has emerged as a potentially pathogenic fungus rather than innocuous mucosal commensal in patients with broncho-pulmonary diseases. Although respiratory candidiasis secondary to pulmonary tuberculosis has been reported in the past,[1] it has gained more relevance recently due to increased use of broad spectrum antibiotics and immunosuppressive drugs[2] and possibly as a result of resurgence of tuberculosis in the background of the HIV epidemic.[3] The synergistic growth-promoting association of *Candida* and *M. tuberculosis* has also been documented experimentally.[4] There is increased concern with studying altered mycotic respiratory flora and its significance in pulmonary tuberculosis patients in current years due to this change in trends.[2] Although *Candida albicans* continues to

be the most predominant species in pulmonary candidiasis,[2,5-7] several non albicans *Candida* species are also reported in increasing frequency. Some of them are associated with particular risk factors or groups of patients.[5-9]

The present study was undertaken with an objective of discovering the prevalence of co-infection caused by different *Candida* in patients with pulmonary tuberculosis.

Method

This study was carried out in a tertiary care hospital in South India over a period of nine months (from June 2010 to March 2011). Early morning sputum samples were collected on three consecutive days from patients with suspected pulmonary tuberculosis (clinically and radiologically). Sputum samples of 382 patients from both hospital in-patients and out-patients were received within the study period and were processed routinely by gram stain, Ziehl-Neelsen stain, KOH mount, culture in blood agar and McConkey's agar. In sputum gram stain number of pus cells and epithelial cells per low power field, bacteria, presence or absence of fungal elements were noted.

Out of 382 patients, 75 patients were sputum positive for acid-fast bacilli in Ziehl-Neelsen stain and were further processed by inoculating in Sabouraud's dextrose agar. Gram stain was done from suspected yeast colonies. Of the 75 patients positive for acid-fast bacilli, only those with budding yeast cells and pseudohyphae along with pus cells in sputum gram stain and heavy growth of *Candida* with more than 30 colonies on SDA on three occasions were considered to have *Candida* co-infection, as per Kahanpaa et al. criteria to exclude respiratory or oral colonising flora.[10] *Candida* species were differentiated from other yeasts and were identified up to species level using gram stain morphology, germ tube formation, cornmeal agar with Tween-80 (for demonstration of chlamydospore, blastospores and pseudohyphae), urease test, sugar fermentation tests (glucose, sucrose, lactose and maltose) and HiCrome Candida agar. *Candida* isolates displaying pseudohyphae with clusters of spherical blastoconidia at the constriction sites and thick walled large round terminal single chlamydospores on cornmeal agar were considered as *C. albicans*. *C. parapsilosis* strains were identified by blastoconidia in single or in small groups along branched pseudohyphae which gradually become smaller (Christmas tree appearance). By contrast, the long branching abundant pseudohyphae with narrow sterile apex and ellipsoid blastoconidia of *C. tropicalis*, clusters of small, budding blastospores without pseudohyphae is very typical of *C. glabrata*.[11] Chrom agar colony morphology, sugar fermentation and germ tube test findings were also correlated with the microscopic findings for species identification.

All findings were entered in MS Excel data sheet and on completion of the study data was statistically analysed in SPSS software version 17.0 by calculation of two tailed P value using chi-square test. All P values < 0.05 were considered as statistically significant.

Results

Out of the total 382 patients with suspected tuberculosis, 75 patients (19.6%) who were positive for acid fast bacilli by Ziehl-Neelsen stain were screened for *Candida* co-infection. Among the 75 patients with pulmonary tuberculosis, *Candida* co-infection was observed in 30 (40%) patients. *Candida albicans* was the most common isolate observed in 50% of the patients with co-infection, followed by *C. tropicalis* (20%) and *C. glabrata* (20%) (Table 1). There was no significant difference in the mean age of the patients with and without *Candida* co-infection (Table 2). There was a significant female preponderance for occurrence of *Candida* co-infection. *Candida* co-infection was found in 62.5% female patients, while it was observed in only 29.4% of the male patients (P value 0.0133) (Table 2). *Candida* co-infection was observed in 45.0% of the out-patients with pulmonary tuberculosis, while it was noticed in only 20.0% of the in-patients. However, this difference was not statistically significant (Table 2).

Table 1: Distribution of various *Candida spp*. from patients with tuberculosis

Candida species	Frequency	Per cent
C.albicans	**15**	**50.0**
C. tropicalis	**6**	**20.0**
C. glabrata	**6**	**20.0**
C. parapsilosis	**2**	**6.7**
C. krusei	**1**	**3.3**
Total	**30**	**100.0**

Mean ± SD age of the patients with *C. glabrata* infection was 65.83 ± 3.19, while the mean ± SD age of the patients with other *Candida* infections was 43.25 ± 20.44 (P value 0.0138). *C. glabrata* infection was found in 40% (6/15) female patients with *Candida* co-infection, while it was not observed among the 15 male patients with co-infection (P value 0.0169).

Table 2: Comparison of the characteristics of patients with and without Candida co-infection

Characteristic	*Candida* co-infection (n = 30)	No *Candida* infection (n=45)	P value
Mean ± SD age	47.77 ± 20.43	48.80 ± 13.72	0.79
Sex Female (n=24) Male (n=51)	 15 (62.5%) 15 (29.4%)	 09 (37.5%) 36 (70.6%)	 0.01
Category In-patient (n=15) Out-patient (n=60)	 03 (20.0%) 27 (45.0%)	 12 (80.0%) 33 (55.0%)	 0.14

Among the 30 patient with *Candida* co-infection, 19 (63%) had had pulmonary symptoms in spite of anti-tubercular treatment of two months or more. Although, *Candida albicans* was the most common sputum isolate, in this group of patient non-albicans *Candida* species were more prevalent (12 out of 19 cases).

Discussion

Tuberculosis is well recognised for its wide range of clinical spectrum, chronicity and sequlae. Respiratory fungal infections are one of the emerging conditions complicating pulmonary tuberculosis. Though several authors have documented *Candida* species as the most common fungal agent isolated from sputum of pulmonary tuberculosis patients, its significance has always been a matter of controversy due to the fact that up to 32.5% healthy people carry *Candida* in their throat. This can contaminate the sputum sample during collection.[12] To eliminate this problem different approaches have been used. Bronchoscopy samples have lesser chance of becoming contaminated with upper respiratory flora and are preferred to sputum.[13] Yet in developing countries such as India bronchoscopy is not always feasible or practical. Some studies have detected better correlation with transbronchial biopsies.[13] Jain et al. have compared and subtracted the growth of *Candida spp.* on plain SDA from mouth rinsed water as a control to that of sputum sample.[5] Cases which showed response to specific anti-fungal agents were also considered as *Candida* infection.[14] But most commonly this evaluation is done by criteria suggested by Kahanpaa et al.[10] According to this criteria three or more repeated isolations of *Candida* more than 30 colonies on SDA with pseudomycelial forms in sputum microscopy is more suggestive of infection than colonisation. In this study we have also followed this criteria to detect *Candida* infection. However, this is not applicable for *C. glabrata* which has only yeast forms and some studies failed to find the significance of pseudohyphal forms in respiratory samples.

The prevalence of *Candida* co-infection of lung ranges between 15-32% in different studies.[5, 15-17] With some rare exceptions,[15] *C. albicans* has been reported to be the most predominant isolate from sputum of tuberculosis patients followed by *C. tropicalis*.[2, 5, 16, 17] Wide variation ranging from 45–92% was seen in the prevalence of *C. albicans* in several Indian studies.[2,5,16,17] In the present study we detected 50%, 20%, 20%, 6.7% and 3.3% prevalence of *C. albicans, C. tropicalis, C. glabrata, C. parapsilosis,* and *C. krusei* respectively. This result is in keeping with other similar studies.[2,5,16] Jain et al. reported *C. tropicalis* (9.1%), *C. pseudotropicalis* 6.06%, and *C. krusei* 6.06%.[5] Baradkar et al. detected *C. tropicalis* 3.25%, *Candida parapsilosis* 3.25%.[16] Latha et al. documented *Candida tropicalis* (19.95%), *Candida glabrata* (16.54%), *C. parapsilopsis* (13.14%) and *C. krusei* (5.10%).[2] These variations in percentages are mainly attributed to differences in local prevalence of different species due to different environmental conditions, as well as to the various detection methods employed.[5,17] Respiratory specimens like BAL, bronchial wash which precludes oropharyngeal contamination may also affect the result.[17] According to Hidalgo et al. colonisation rates of *Candida* species are equal in males and females.[18] However, we observed that *Candida* co-infection was significantly higher among female patients compared to male patients (P value 0.0133). The relatively high colonisation rates in women could have been responsible for increased risk of *Candida* co-infection among female patients with pulmonary tuberculosis. We found no statistically significant difference in *Candida* co-infection between in-patients and out-patients. However, patients with *C. glabrata* infection were predominantly female (P value 0.0168) as well as older (mean age + SD 65.83 ± 3.19 years) compared to patients with other *Candida spp.* (mean age + SD 25 ± 20.44 years) (P value 0.0138). Although old age is a known risk factor for *C. glabrata,*[7] there is no clear evidence to suggest higher susceptibility in females for pulmonary infection by *C. glabrata* than other *Candida* species. It is notorious for acquisition of azole resistance (especially fluconazole) as well as cross resistance. It is emerging as a successful nosocomial pathogen in presence of risk factors like immunocompromised or old debilitated host, prolonged hospitalisation, antibiotic use, fluconazole use, hospital acquired exposures from infected patients, hands of healthcare workers and environment in contact with hands.[7]

A significant increase in the number of cases of HIV infection has been reported in tuberculosis patients in recent years. It is often described as a HIV, tuberculosis co-epidemic.[19] However, testing for HIV infection in tuberculosis patients has not emerged as a routine practice in resource limited and developing countries like India. Only 22 (29.3%) out of 75 patients were tested for HIV and found negative. Apart from weight loss and chronic cough, none had symptoms suggestive of HIV infection.

All patients were started on anti-tubercular drugs (ATD) on diagnosis. Among them, 67 new cases received category I regimen of Revised National Tuberculosis Control Programme (RNTCP) of India, comprising of isoniazid, rifampicin, pyrazinamide and ethambutol for two months (intensive phase) followed by isoniazid and rifampicin for four months (continuation phase).[20] On follow-up sputum microscopy for acid fast bacilli and mycobacterial culture, no cases remained positive at the end of fifth month indicating treatment failure associated with resistance to ATD. There are insufficient published data comparing treatment and their outcomes in pulmonary tuberculosis versus tuberculosis and *Candida* co-infection. Although, azole resistance is not uncommon among non-albicans *Candida* species, oral or parenteral azoles are frequently used for pulmonary fungal infections and are preferred to intravenous amphotericin B.[21] In a clinical trial, itraconazole showed significant efficacy in patients with *Candida* and tuberculosis co-infection.[22] The first course resulted in complete or partial inhibition of growth of *Candida* in 68% of patients and after repeated course 84% of patients recovered completely. Only in 2% of cases, it progressed to disseminated pulmonary tuberculosis in association with azole dependent fungal strain of *C. glabrata*.

In this study we found a shifting pattern of epidemiology of *Candida* species from commensal to emerging pathogen. Non-albicans *Candida* species were isolated in increasing numbers from patients with symptomatic lung disease. There is a significant female preponderance for occurrence of *Candida* co-infection. *C. glabrata* was found to be strongly associated with old age and female sex. However, due to a small study population the results may not be representative and require further confirmation.

Conclusion

We found increasing prevalence of non-albicans *Candida* species in pulmonary tuberculosis patients in our hospital. The majority of these patients had persistence of pulmonary symptoms even after anti-tubercular treatment. Owing to the inherent anti-fungal resistance, non-albicans *Candida* species are often not amenable to anti-fungal treatment. Therefore, screening of pulmonary tuberculosis patient for *Candida* co-infection should be routinely practiced along with anti-fungal sensitivity testing for non-albicans *Candida* isolates.

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PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.