 DOI: 10.18231/2455-6807.2017.0029

Association of Candida and its antifungal susceptibility pattern in patients with pulmonary tuberculosis

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Abstract

Aim: The purpose of this study was to determine the incidence of Candida species and antifungal susceptibility pattern among smear positive pulmonary tuberculosis patients in a coastal tertiary care teaching hospital.

Materials and Method: One hundred and twenty one sputum samples were found to be positive for acid fast bacilli by Ziehl-Neelsen stain. All positive sputum samples were taken into consideration and subjected to standard mycological procedures.

Results: Out of 121 patients sputum samples positive for acid fast bacilli, Candida co infection was revealed in 41 samples(33.89%). Candida albicans was the most common species isolated and accounted for 60.98% followed by C.tropicalis(21.95%), C.krusei(9.76%) and C.glabrata(7.31%). Out of 539 AFB negative sputum samples, 107(19.85%) samples yielded the growth of Candida. Candida albicans 69 (64.49%) was the most commonest species followed by Candida tropicalis 35(32.71%). Majority of C.albicans and C.tropicalis were showed resistance to azole group. Only 52% of susceptibility exhibited towards fluconazole and clotrimazole by Candida albicans. Susceptibility of C.tropicalis towards fluconazole(55%) and clotrimazole(33%) was also high. All strains of C.krusie and C.glabrata were showed susceptibility to azole group as well as amphotericin B. Few strains of C.albicans(12%) and C.tropical(12%) were shown resistance to amphotericin B.

Conclusion: Candida co infection was found in 33.8% of 121 AFB proven pulmonary tubedrculosis patients. Antifungal susceptibility testing revealed resistance to tested azole drugs as well as to amphtericin B. Hence there is a need to perform the Antifungal susceptibility testing before administering antifungals.

Keywords: Candida albicans, CHROM agar, Tuberculosis.

Introduction

Tuberculosis is one of the oldest infectious diseases. India is the country with the highest burden of TB. The World Health Organisation (WHO) TB statistics for India for 2015 give an estimated incidence figure of 2.2 million cases of TB for India out of a global incidence of 9.6 million.(1) Progress of the disease and prolonged treatment with antibiotics or immunosuppressive agents makes tuberculosis patients immunocompromised and hence susceptible to oppurtunistic infections.(2) The incidence of opportunistic fungi has dramatically increased in past few years. The rate of opportunistic fungal infections in turberculous patients is also very high.(3) Candida albicans is the most commonly isolated fungal pathogen and may cause severe secondary infections.(4) Nine to eighty percentages of pulmonary tuberculosis patients. are infected by Candida species.(2,3,5) polysaccharide fraction of C. albicans for enhance the growth as well as reduce the generation time of tubercle bacilli.(3) In recent years, there is a great concern in probing disturbed fungal respiratory flora and its importance in pulmonary tuberculosis patients.(6) Though Candida albicans persists to be the foremost species in pulmonary candidiasis, other Candida species are also being reported frequently. Few of them are associated with particular risk factors or groups of patients.(7) Antifungal susceptibility varies from species to species. Therefore, species level identification of Candida is important in treating candidiasis. Most commonly used drugs in treating candidiasis is azole group. Due to prolonged usage of these drugs, drug resistance has emerged in C.albicans and other species of Candida. Amphotericin B, a polyene fungicidal agent is being used in treating serious fungal infection. But cost and dose related side effects limit its usage.(8)

In, this region, so far no study has been done on Candida speciation from sputum samples of AFB positive. Hence, we aimed to determine the incidence of candida co infection among tuberculosis patients and their antifungal susceptibility.

Materials and Method

This is a prospective study in which a total of 660 sputum samples were collected from patients suspected of tuberculosis over a period of nine months (January 2016 to September 2016) in the department of microbiology, Vinayaka missions medical college and hospital. A total of 121 patients sputum samples were found to be positive for acid fast bacilli by Ziehl-Neelsen stain. All tuberculosis positive samples were subjected to standard mycological identification for Candida species. Direct examination was done by 10% KOH mount and Grams stain. For culture specimens were inoculated onto sabouraud's dextrose agar with chloramphenical and the SDA slants were observed daily for the presence of growth. If there was no growth for 4 weeks, they were discarded. Kahanpaas Criteria was used in evaluating growth Criteria for diagnosis of candida infection. Kahanpaa criteria includes, 1) Colonies of Candida should be not less than thirty on sabouraud's dextrose agar. 2) Sight of budding yeast

cells, pus cells along with pseudohyphae in Gram stained smears.[9]

All the samples satisfied the criteria of Khanna et al[9] were included. Further speciation of Candida was done by using CHROM agar. Inoculated plates of CHROM agar plates were incubated for 48 hours at 37°c and the colonies were identified based on the color produced by the Candida species. Light green colonies- Candida albicans, blue colonies with pink halo - Candida tropicalis, purple colonies- Candida glabrata and pink colonies- Candida krusei.

Antifungal susceptibility test was performed as per CLSI recommendations in document M44-A.[10] Following antifungal discs were used: Fluconazole, clotrimazole, itraconazole, ketaconanole, Amphotericin B .

Quality control strain: C. albicans (ATCC90028)

No other species of candida are used for quality control.

Results

A total of 121 patient's sputum samples were found to be positive for acid fast bacilli. There were 100 (82.64%) male and 21 (17.35%) female patients. In AFB positive sputum samples, association of Candida was revealed in 41 patients (33.89%).(Table 1)

Table 1: Age and sex wise distribution of Candida speciation among tuberculosis patients

Age	Male	Female	Total	Candida(n=41)
1-20	3	2	5	-
21-30	7	4	11	3(7.31%)
31-40	24	6	30	5(12.20%)
41-50	26	6	32	9(21.95%)
51-60	29	3	32	21(51.22)
61-70	11	-	11	3(7.31%)

Most common species was Candida albicans and accounted for 60.98%. Among non albicans, C.tropicalis(21.95%) was predominant followed by C.krusei(9.76%) and C.glabrata(7.31%).(Table 2)

Table 2: Distribution of various species among tuberculosis patients

Candida species	Number
C.albicans	25(60.98%)
C.tropicalis	9(21.95%)
C.krusei	4(9.76%)
C.glabrata	3(7.31%)
Total	41(100%)

Out of 539 AFB negative sputum samples, 107(19.85%) samples yielded the growth of Candida. Candida albicans 69 (64.49%) was the most commonest species followed by Candida tropicalis 35(32.71%). Candida glabrata 4(3.74%) was the least common pathogen isolated.

Antifungal susceptibility pattern showed, majority of Candida albicans and C.tropicalis strains showed resistance to commonly used azole group antifungal agents. Among azole group, itraconazole was found to be effective and its susceptibility ranged between 92%-100%. Three strains of Candida albicans were resistant to all tested antifungal agents and only one strain of Candida tropicalis showed resistance all drugs tested. Susceptibility to amphotericin B was high against both C.albicans and C.tropicalis accounted for 88%. No drug resistance was recorded in C.krusei and C.glabrata.(Table 3)

Table 3: Antifungal Susceptibility Pattern of Candida isolates

Candida species	Fluconazole	Clotrimazole	Itraconazole	Ketakonazole	AmphotericinB
C.albicans[25]	13(52%)	13(52%)	23(92%)	21(84%)	22(88%)
C.tropicalis[9]	5(55.5%)	3(33.33%)	8(88.88%)	6(66.66%)	8(88.8%)
C.krusei[4]	4(100%)	4(100%)	4(100%)	4(100%)	4(100%)
C.glabrata[3]	3(100%)	3(100%)	3(100%)	3(100%)	3(100%)

Discussion

Various studies revealed the association of candida in sputum samples of patients with pulmonary tuberculosis. This association in different studies varied from nine to eighty percent.[5] The present study revealed 33.89% candida co infection. As per Jain et al,[7] Candida co infection in pulmonary tuberculosis patients was 23.57%. In our study, Candida albicans (60.98%) was the most common species followed by Candida tropicalis (21.95%). Except in some studies, C. albicans has been reported as the predominant isolate from sputum samples of tuberculosis patients followed by C. tropicalis.[6,7] Candida krusei and Candida glabrata was accounted only for 9.76% and 7.31 respectively. As per Arunava kali et al, C.albicans was the common species isolated and accounted upto 50% followed by C.tropicalis(20%) and C.glabrata (20%). Least commonly isolated species were C. parapsilosis(6.7%) and C. krusei(3.3%).[11] As per Grower et al Candida tropicalis in 4(4%) and Candida parapsilosis in 2 (2%) in their series of 100 cases.[12] According to Baradkar et al. C. tropicalis 3.25%, Candida parapsilosis 3.25%.[5] These

variations in isolation rates are probably due to differences in local prevalence of different species due to different environmental factors, as well as various identification techniques employed.[9]

Antifungal susceptibility pattern of the Candida species have been discussed and shown to have resistance to both azoles and Amphotericin B antifungal. So the alternative antifungal regimens are essential and the function of other combinations of antifungals in treating serious Candida infections needs to be assessed.[13] In our study, drug resistance was noticed in C.albicans and C.tropicalis towards azloe group (76%) and amphotericin B(66%).

As per Kul KO et al.[14] C.albicans showed, fluconazole resistance in 2% and itraconazole resistance in 6%. According to KK Prabsobh and Udhaya , quite high level percentage (48%) of the resistance towards the antifungal drug Nystatin was observed from all the Candida species followed by Azole drug (24%) and Amphotericin B (22%).[15]

It was observed that the emergence of antifungal resistance to single drug or to multiple drugs in Candida species. Three stains of C.albicans and one strain of C.tropicalis were resistant to all antifungal agents used. It was found to be difficult to treat such secondary infections in immunocompromised patients and lead to therapeutic failure because of association with multi drug resistance. Such multi drug resistance Candida species increasing morbidity and mortality in immunocompromised patients.

Conclusion

Incidence of Candida infection among pulmonary tuberculosis was 33%. Antifungal susceptibility pattern revealed drug resistance to commonly used antifungal agents. Appropriate antifungal agent should be chosen to treat Candida infection in pulmonary tuberculosis after obtaining susceptibility results.

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How to cite this article: Kavitha Y., Moinuddin K., Anandi V., Nazeer H. Association of Candida and its antifungal susceptibility pattern in patients with pulmonary tuberculosis. International Journal of Medical Microbiology and Tropical Diseases 2017;3(3):126-128.