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Study of respiratory Tract Fungal co-infection in patients with pulmonary tuberculosis

Amit Kumar¹, Manisha Khandiat², Moumita Sardar³

¹PhD Scholar, Department of Microbiology, SGT Medical College Gurugram, Haryana, India ²Professor and HOD, Department of Microbiology, SGT Medical College Gurugram, Haryana, India ³Professor, Department of Microbiology, SGT Medical College Gurugram, Haryana, India.

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	Abstract		
KEYWORDS	Background:	Fungal pulmonary infection has been	n emerging recently due to widely used
pulmonary	broad-spectrum	n antibiotics and steroids. Due to	immune deficiency or suppression in
tuberculosis,	tuberculosis (T	B), patients are easily vulnerable to	opportunistic fungal infections. The aim
Aspergillus species	of the present	study was to isolate fungi from sput	tum samples of pulmonary tuberculosis
, Califina species,	patients which	may help in the correct diagnosis of t	these patients.
Dextrose Agar.	Materials and	Methods: A total of 152 pulmonary	y tuberculosis patients were included in
VITEK-2	the study. Sput	tum samples were collected and sub	jected to Gram staining, KOH mount,
	India ink ,	cultured on Sabouraud's Dextrose	Agar, subculture of candida SDA to
	CHROM agar,	, Urease test and final identification of	of candida were subjected to VITEK-2.
	Result : in th	he present study, out of 150 spu	itum samples positive for pulmonary
	tuberculosis, 5	52 (35.33%) were positive for fungal	l infections of which 44, 1 and 7 were
	Candida, Cryp	tococcus neoformans and Aspergillus	s species respectively.

Introduction :

Pulmonary fungal infections is increasing since last few decades due to the extensive use of broad-spectrum antibiotics, long-term use of immunosuppressive agents, and in patient with co-morbidity with other disease such as pulmonary tuberculosis (TB), diabetes mellitus or malignancy .^[1] There are some other factors with tuberculosis like dysfunctions in macrophages, monocytes and T cells as well as chemotaxis that are responsible for developing of opportunistic fungal infections.^[2-5] Many fungi are able to synthesize trehalose dimycolate (TDM) like Mycobacterium tuberculosis which increases the virulence of Mycobacterium tuberculosis. Trehalose is metabolized by various fungi for energy. [6] Aspergillus niger, A. fumigatus, Histoplasma capsulatum, Cryptococcus neoformans, Candida albicans and candida non albicans were reported to cause infections in tuberculosis patients. ^[7]Among the fungal pathogens, Candida albicans is a common fungus isolated from tuberculosis patients and it is responsible for causing severe secondary infections in tuberculosis patients. ^[8]Many studies confirmed the effect of polysaccharide fraction of Candida albicans enhance the growth as well as reduction the generation time of tubercle

bacilli.The prevalence of pulmonary tuberculosis coinfection with Candida was reported to be 40% in south India.^[9]According to WHO, in 2007, annually, Chronic pulmonary Aspergillosis associated with pulmonary tuberculosis was estimated at 11 420 in the European Region, 20 615 in the Eastern Mediterranean Region and 12 610 in the Region of the Americas, minimum estimates were 98 551 for the African Region, 83 815 for the Western Pacific Region and 145 372 for the South-East Asia Region^{.[10]}Cryptococcus neoformans can infect people with intact immune systems at a rate of 0.2 cases per million populations per year. Infection is acquired by inhalation of the infectious propagule from the environment. In people with a normal immune system, the pulmonary form of cryptococcoses may be asymptomatic, but with impaired immune systems, the Cryptococcus spp. may disseminate to the meninges causing life-threatening meningoencephalitis.^[11]

MATERIAL AND METHODS :

this study included 150 sputum samples positive for pulmonary tuberculosis by ZN stain for isolation and identification of fungal species in tertiary health from

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25.5.2022 to 29.3.23 after approval of ethical committee . Early morning sputum was collected in sterile, dry, wide-necked, leak-proof container and send to laboratory with triple layer packing. Before collection of sample patients were instructed to produce cough in open air space in order to spread infection to others. Patients taking any form of anti fungal therapy and Patients with extra pulmonary tuberculosis were not included in this study. all samples were processed for Direct microscopy (KOH mount to determine yeast cell and hyphae, gram stain to determine yeast with pseudohyphae, germ tube to differentiate between candida albicans and c non albicans , india ink to determine capsule of cryptococcus species,), culture on SDA at 25°c and 37°c, CHROM agar used to identify candida species from mixed culture, slide culture ,and LPCB to study morphological feature of mould and urease test to identify cryptococcus neoformans. Identification of all yeast were subjected to VITEK-2 automated system.

Result: in the present study , out of 150 sputum samples positive for pulmonary tuberculosis , 52 (35.33%) were positive for fungal infections of which 44 , 1 and 7 were candida , cryptococcus neoformans

and aspergillus species respectively.[table 1, figure 1]. Out of 52 fungal isolates , 34 and 18 were male and female respectively and p value (0.0264) were significant. [Table 2, figure 2] Out of 44 candida species, 28 and 16 were male and female respectively. Male were found more infected with candida species and aspergillus species than female. One cryptococcus neoformans were isolated from male patient reactive with HIV. [Table 3, figure 3]. Out of 44 candida species identified by CHROM agar, 22, 16, 5 and 1 were candida albicans ,Candida tropicalis , Candida krusei and Candida Paropsilosis respectively. [Table4] In comparison with identification of candida species with CHROM agar and VITEK-2, 1 candida paropsilosis were identified as Candida kafyr by VITEK-2 and remaining species were same identified by both method (CHROM agar and VITEK-2). [Table 5] Out of 150 PTB patients, 24, 88 and 38 patients were belonged to < 18, 19-45 and 46-60 years age group respectively of which 16,24 and 4 candida species and 2, 4 and 1 Aspergillus species were isolated from 46-60 years, 19-45 and <18 years of age group respectively .one cryptococcus neoformans were isolated from male patient co-morbidity with HIV from 19-45 years age group. [Table 6].

TABLE 1: Isolation of fungal species from clinical samples $(n = /150)$						
TOTAL NUMBER	NUMBER OF	NUMBER OF	NUMBER OF	NUMBER OF		
OF SPECIMENS	FUNGAL	CANDIDA	CRYPTOCOCCUS	ASPERGILLUS		
	ISOLATES (%)	ISOLATES	NEOFORMANS	SPECIES		
		(%)	ISOLATES (%)	ISOLATES (%)		
150	52 (34.66)	44 (29.33)	1(0.66)	7 (4.66)		

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Table 2 : Distribution of Fungal isolates by gender of the patients.							
Table 2 : Distribution of fungal isolates by gender of the patients.							
NUMBER OF FUNGAL ISOLATES	MALE PATIENTS No (%)	FEMALE PATIENTS No (%)	[#] p value				
52	34(65)	18 (36)	0.0264				



Figure 2 : Distribution of Fungal isolates by gender of the patients

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TABLE 3 :Distribution of Candida , Aspergillus and Cryptococcus by gender of the patients						
Name of fungal isolates	NUMBER OF FUNGAL ISOLATES	NUMBER OF MALE (%)	NUMBER OF FEMALE (%)	P VALUE		
CANDIDA SPECIES	44	28 (63)	16 (36)	0.2891		
ASPERGILLUS SPECIES	7	4 (57)	3 (42)	0.0015		
CRYPTOCOCCUS SPECIES	1	1	0	0.4777		





	canada species by criticon agai
species	Number $(n/44 = \%)$

0

1.1.

Candida species	Number $(n/44 = \%)$
Candida albicans	22(50%)
Candida tropicalis	16 (36%)
Candida krusei	05 (11%)
Candida Paropsilosis	01 (2)

TABLE 5:Comparison of identification of candida with CHROM agar and VITEK-2						
NUMBER OF CANDIDA SPECIES BY CHROM	NUMBER OF CANDIDA SPECIE BY VITEK-2					
Candida albicans 22	Candida albicans 22					
Candida tropicalis16	Candida tropicalis 16					
Candida krusei 5	Candida krusei 05					

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Candida Paropsilosis 1

Candida kafyr 01

TABLE 6: DISTRIBUTION OF CANDIDA , ASPERGILLUS AMONG PTB PATIENTS BY AGE GROUP

Age group	Number	of	PTB	Number of candida	Number of	Number of
	patients			species	Aspergillus species	cryptococcus species
<18 years	24			4	1	
19-45 years	88			24	4	1
46-60 years	38			16	2	

TABLE 7 DISTRIBUTION OF ASPERGILLUS SPECIES IN PTB PATIENTS						
TOTAL NUMBER OF SPECIMENS	NUMBER OF ASPERGILLUS	NUMBER OF ASPERGILLUS	NUMBER OF ASPERGILLUS	TOTAL		
	NIGER (%)	FUMIGATUS(%)	FLAVUS			
150	5 (3)	1 (0.6)	1(0.6)	7 (4.6%)		

Discussion:

In our study, about 34% of pulmonary tuberculosis patients were co-infected with fungal agents That was compared with the study conducted by Khanna et al and Bansal et al (12) where 36.36 % and 39.4% of pulmonary tuberculosis patients were coinfected with fungal agents. But the present study was in contrast to the study conducted by Shome et al (13) where coinfection of fungal agent was seen only in 18% cases of pulmonary tuberculosis. Possibility of difference could be due to the difference in the samples collected. Sputum was collected in our study but bronchial aspirates and bronchoscopic materials were collected in the study by Shome et al (9) which are far better than sputum. The present study shows 29% Candida spp were isolated from pulmonary tuberculosis patients. This is similar to study done by VP Baradkar et al (14) which shows a prevalence of 26% co-infection with Candida species. But the study done by Sehar Afshan Naz and Perween Tariq et al (15)showed 15.2% of coinfection with Candida species was documented. this is low when compared to the study of. Candida is part of normal microbial flora of healthy individuals But when host resistance is low it can cause life threatening infection. Among the Candida spp, C. albicans was the commonest organism causing secondary infection in our study. C. albicans constituted 50% of the total Candida isolates, which correlates with the study conducted by Kali A et al (15) which also demonstrates C.albicans to be the commonest species causing

secondary infection comprising 50% of the total Candida isolates . Aspergillus species was isolated from 4% of cases in our study which correlated study done by by Khanna et al (12) where Aspergillus species was isolated from 10% and also with Anna N. Njula et al (17) in which the isolation rate of Aspergillus species was 15%. our study showed C. albicans, C. tropicalis, and C. krusei were found 100% accuracy between identification by CHROM agar and VITEK-2 automated system, that is similar to study done by Bauters T.G , Horvath L.L and Yucesoy M et. Al. [18-19].

Conclusion:

The prevalence of opportunistic fungal infection in pulmonary tuberculosis patients cannot be underestimated. These secondary fungal infections are associated with persistence of lung symptoms inspite of successful completion of antituberculous drug therapy. Hence adequate measures need to be taken for the early identification and treatment of these opportunistic infections, which are associated with high rates of morbidity and mortality.

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