ORIGINAL RESEARCH PAPER

Volume-7 | Issue-5 | May-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

# OCCURRENCE OF OPPORTUNISTIC MYCOSES AMONG PATIENTS WITH SPUTUM SMEAR POSITIVE TUBERCULOSIS.

Microbiology

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## ABSTRACT

Fungal infections of lungs in tuberculosis patients should be considerd life-threatening infection. 500 suspected sputum samples were taken from DOTS centre of TMMC&RC.150 sputum samples was found positive by AFB Z.N. stain. KOH mount was done for the 150 positive samples, in which 38 were positive in direct microscopy. After culture on SDA agar total 46 samples were found positive for fungus, In which 30 were Candida and 16 were aspergillus species. Candida and Aspergillus species identification was done by inoculation on CHROM agar and by LCB mount respectively. Following Candida and Aspergillus species were found: C.albicans(36.95%), C.tropicalis(15.21%), C.glabrata(8.69%), C.parapsilosis(2.17%), C.krusei(2.17%), A.fumigatus(17.39%), A.niger(10.86%), A.flavus(6.52%). The co-infection of opportunistic fungus with tuberculosis is higher in males than females.C.albicans and A.fumigatus found in high frequency than other species.

## KEYWORDS

**Z.N. stain-** Ziehl-Neelsen stain, **AFB-** Acid Fast Bacilli, **DOTS-** Direct Observed Treatment Short-Course Therapy.

## INTRODUCTION

Tuberculosis is a major health problem worldwide[1].India is the highest tuberculosis (TB) burden country in the world, accounting for nearly one-fifth of the global incidence.The causative agent of pulmonary tuberculosis is *Mycobacterium tuberculosis*[2] .The primery site of disease involvement is Lungs but also affect the other organs.[3] Opprtunistic fungal infection can be acquired primarily or secondarily in TB, immunodeficiency patients, other chronic disease such as diabetes mellitus or malignancy, HIV and it may lead to more worst condition of primary disease[4,5].Due to widely used broad spectrum antibiotics and steriods, Fungal pulmonary infection has been emerging recently[6] The opportunistic mycoses are those fungal infections which are found among patients with pulmonary predisposing conditions. Such clinical entities are essentially disease processes produced by fungi of lesser pathogenic potential in the host whose immunological defense mechanism is weakened.Main organisms isolated most frequently from Tuberculosis patients are *Aspergillus* and *Candida spp. Cryptococcus spp*, *Fusarium spp*, *Zygomycete*, *Dematiaceous fungi* and opportunistic yeast-like fungi other most relevant aetiologic agents[7]. Transmission of fungal infection by the inhalation of spores (*Aspergillosis,Cryptococcosis,Histoplasmosis),* commensal organisms such as *Candida albicans* penetrate the mucosa[8].

After TB, modeling suggests that about 375,000 will go on to develop Chronic Pulmonary Aspergillosis each year, and with an annual 15% mortality . Therefore about 1.2 million people probably have CPA[9].

## MATERIALAND METHOD

The study was carried out from February 2017 to January 2018 at TMMC & RC, Moradabad (U.P.) India. Patients having systoms of persistent cough(> 2 weeks), loss of weight, night sweats and fever are suspected for Pulmonary TB, at least 2 sputum samples ( spot sample and early morning sample) were collected and examined by microscopy. 500 sputum samples were taken from suspected cases of tuberculosis from DOTS Center of TMMC & RC. Further proceed the sputum sample in Mycology and Mycobacteriology section of Microbiology department of TMMC & RC.

The sputum sample were collected from adults of both gender .Sputum founds positive for tuberculosis by Z.N. staining where further processed for fungus detected by 10% KOH examination, SDA culture ,inoculation on CHROM agar and LCB mount.

## RESULTS

After study of 500 suspected samples,105 positive sputum samples for tuberculosis were analyzed for fungal elements. Out of 105 samples 38 (36.19%) were positive for opportunistic fungi in direct microscopy and 67 (63.80%) samples were negative in direct microscopy for fungal elements. All samples which was positive and Negative by KOH mount were culture on SDA agar.

38 samples were found positive in culture and out of 67, 8(12.90%) samples were found positive and 59(56.19%) samples were negative in culture which was found negative in KOH mount.After culture on SDA agar and direct microscopy by KOH mount total 46(43.80%) samples were found positive in which 30(65.21%) were *Candida* species and 16(34.78%) were *Aspergillus* species. Isolated *Candida* colonies on SDA agar was again culture on CHROM agar to identification of various *Candida species* by color as a result of biochemical reactions.Out of 30 Candida species were *C.albicans (36.95%), C.tropicalis (15.21%), C.glabrata (8.69%), C.parapsilosis(2.17%),C.krusei(2.17%).* On the basis of macro and micro morphology on SDA agar and LCB mount the filamentous fungi were following *Aspergillus* species: *Aspergillus fumigatus(17.39%)Aspergillus niger(10.86%),Aspergillus flavus(6.52%).*

**Table-1. Sex wise distribution of total smear positive pulmonary tuberculosis patients**

| Sex | Positive for AFB by ZN stain | Negative for AFB by ZN stain | Total |
|---|---|---|---|
| Male | 67(63.80%) | 269(68.10%) | 336(67.2%) |
| Female | 38 (36.19%) | 126(31.89%) | 164(32.8%) |
| Total | 105 (21%) | 395(79%) | 500(100%) |

**Figure-1 Sex wise distribution of AFB positive samples**

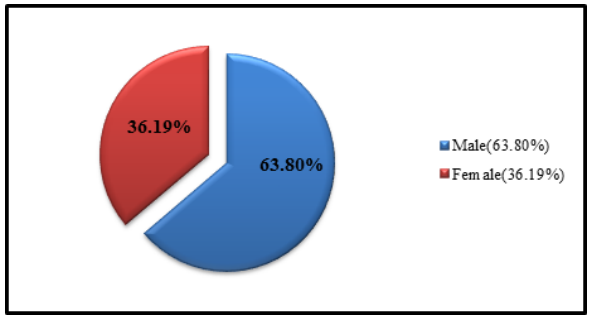


Table- 1 and Figure-1 Showing out of 105 smear positive pulmonary tuberculosis patients *67(63.80%)* were males and 38(36.19%) were female.

**Table- 2. Age and sex wise distribution of smear positive pulmonary tuberculosis patients.**

| Age group | Male | Female | Total (%) |
|---|---|---|---|
| 11-20 year | 2 | 2 | 4(3.80%) |
| 21-30 year | 11 | 5 | 16(14.28%) |
| 31-40 year | 23 | 10 | 33(19.04%) |
| 41-50 year | 18 | 12 | 30(21.90%) |
| 51-60 year | 6 | 4 | 10(26.66%) |
| 61-70 year | 5 | 3 | 8(10.47%) |
| >70 year | 2 | 2 | 4(3.80%) |
| Total | 67 | 38 | 105(100%) |

**Figure-2. Age and sex wise distribution**

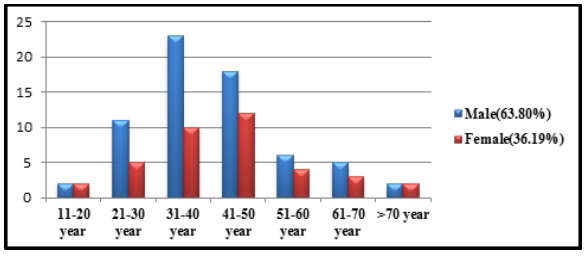


Table-2 and Figure- 2 showing age and sex wise distribution of smear positive pulmonary tuberculosis patients, the most high prevalence in the age group of 31-40 (31.42%) year.

**Table -3. Sex wise distribution of opportunistic fungal species.**

| Fungal isolates | Male | Female | Total |
|---|---|---|---|
| *Candida spp.* | 18(39.13%) | 12(26.08%) | 30(65.21) |
| *Aspergillus spp.* | 10(21.73%) | 6(13.04%) | 16(34.78%) |
| Total | 28(60.86%) | 18(39.13%) | 46(100%) |

**Figure-3. Sex wise distribution of opportunistic fungal species.**

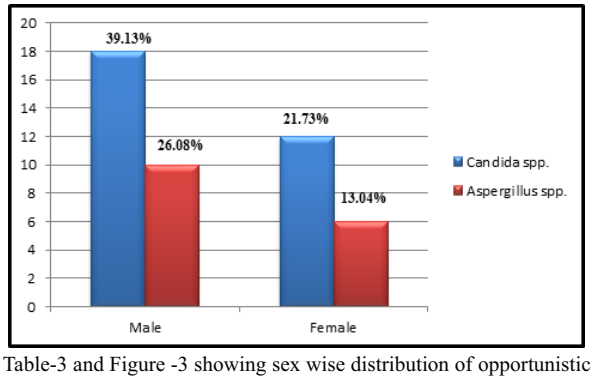


Table-3 and Figure -3 showing sex wise distribution of opportunistic fungal infection in smear positive pulmonary tuberculosis patients. High prevalence obtained in males for *Candida and Aspergillus* speceis respectively.

**Table-4. Species distribution of *Candida***

| S.No. | Candida species | Total isolates N=30 (65.21%) |
|---|---|---|
| 1 | *Candida albicans* | 17(36.95%) |
| 2 | *Candida tropicalis* | 7 (15.21%) |
| 3 | *Candida glabrata* | 4 (8.69%) |
| 4 | *Candida parapsilosis* | 1 (2.17%) |
| 5 | *Candida krusei* | 1 (2.17%) |

**Figure-4. Species distribution of *Candida***

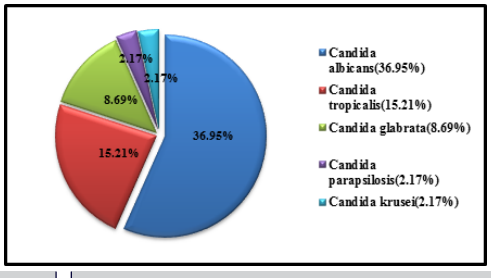


Table -4 and Figure-*4*showing isolated Candida species in pulmonary tuberculosis patients. *Candida albicans* (36.95%) shows high prevalence.

**Table-5. Age wise distribution of Candida species**

| Age group | *C.albicans* | *C.tropicalis* | *C.glabrata* | *C.parapsilosis* | *C.krusei* | Total |
|---|---|---|---|---|---|---|
| 11-20 year | 0 | 0 | 0 | 0 | 0 | **0** |
| 21-30 year | 1 | 0 | 0 | 0 | 0 | **1** |
| 31-40 year | 3 | 0 | 1 | 0 | 0 | **4** |
| 41-50 year | 5 | 2 | 1 | 0 | 1 | **9** |
| 51-60 year | 4 | 3 | 2 | 1 | 0 | **10** |
| 61-70 year | 3 | 2 | 0 | 0 | 0 | **5** |
| >70 year | 1 | 0 | 0 | 0 | 0 | **1** |
| **Total** | **17** | **7** | **4** | **1** | **1** | **30** |

**Figure-5. Age wise distribution of Candida species**

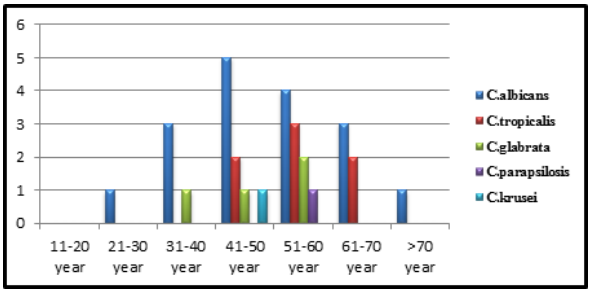


*Table-5*and *Figure-5* Showing age wise distribution of *Candida* species. More frequency of *Candida* species obtained in the age of 51-60 year age.

**Table-6.Species distribution of Aspergillus**

| S.No. | Aspergillus species | Total Isolates N=16 (34.78%) |
|---|---|---|
| 1 | *Aspergillus fumigatus* | 8(17.39%) |
| 2 | *Aspergillus niger* | 5 (10.86%) |
| 3 | *Aspergillus flavus* | 3 (6.52%) |

**Figure- 6. Species distribution of Aspergillus**

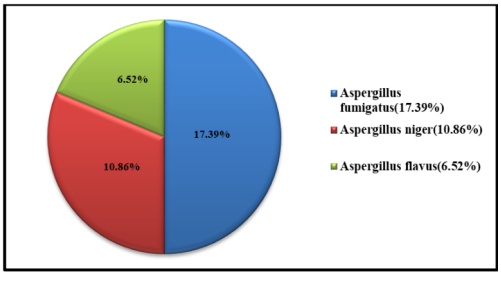


Table-5 and Figure-6 Showing species distribution of Aspergillus. The most high prevalence found in *Aspergillus fumigatus* (17.39%).

**Table-7.Age wise distribution of *Aspergillus* species**

| Age group | *Aspergillus fumigatus* | *Aspergillus niger* | *Aspergillus flavus* | Total |
|---|---|---|---|---|
| 11-20 year | 0 | 0 | 0 | **0** |
| 21-30 year | 0 | 0 | 0 | **0** |
| 31-40 year | 0 | 1 | 1 | **2** |
| 41-50 year | 2 | 1 | 0 | **3** |
| 51-60 year | 2 | 1 | 1 | **4** |
| 61-70 year | 3 | 2 | 1 | **6** |
| >70 year | 1 | 0 | 0 | **1** |
| **Total** | **8** | **5** | **3** | **16** |

**Figure-7.Age wise distribution of *Aspergillus* species**

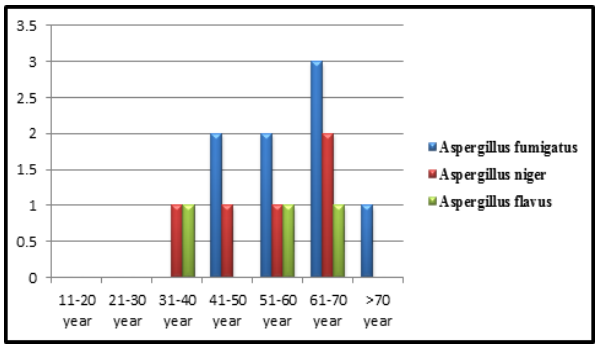


Table-7 and Figure-7Showing age wise distribution of *Aspergillus species*. High prevalence obtained in the age of 61-70 year.

## DISCUSSION

The incidence of opportunistic fungal infections has been increasing and may well increase further in the near future. *Candida* and *Aspergillus* species are the main organisms isolated most frequently from tuberculosis patients.

There are many species of Aspergillus in the environment but the most frequently recovered species depending on the geographical region are *Aspergillus fumigatus, A. flavus, A. niger or A. terreus*. *Candida* is isolated from 84-88 per cent of mucocutaneous surfaces in hospitalized patients or even healthy adults. Because of its stronger adherence capacity, *C. albicans* is found more often than other *Candida species*.

In total AFB positive samples which were 105, the number of male patients were 67 (63.80%) and the number of female patients were 38 (36.19%). Age wise distribution shows the highest incidence in 31-40 age group in which 33 patients were included. In total 28 patients, 23 were males and 10 were females. **P.Shrimali *et al*.** reported highest number of patients were from 31-40 age group, total 39 patients in which 29 were male and 10 were female patients.

In the present study 46(43.80%) patients were found positive for *Candida* and *Aspergillus* fungal infection including following species – *Candida albicans* 17 (36.95%), *Candida tropicalis* 7 (15.21%) *Candida glabrata* 4(8.69%), *Candida parapsilosis* 1(2.17%), *Candida krusei* 1(2.17%), *Aspergillus fumigatus* 8(17.39%), *Aspergillus niger* 5(10.86%), *Aspergillus flavous* 3(6.52%). **Ruchika *et al.*** also found 49% positive cases of fungal infection.

We reported total 30 (65.21%) cases of *Candida* infection in 105 positive cases of tuberculosis in which the number of *C.albicans* were 36.95%, *C.tropicalis* were 15.21%, *C.glabrata* 8.69%, *C.parapsilosis* 2.17%, and *C.krusei* 2.17. Another study carried out by **Mwaura *et al*.** was found *C.albicans* (19.2%), *C.dubliensis*(1.7%), *C.parapsilosis* (1.2%),*C.tropicalis*(1.7%).

In this study out of 105 ,16(34.78%) samples positve for *Aspergillus* including following species- *A.fumigatus* in 8(17.39%) , *A.niger* in 5(31.25%) and *A.flavus* in 3(18.75%) samples. Similar findings were reported by **Njunda *et al*.** in which 30(15%) of *Aspergillus* species comprised of 10(5%) *A.fumigatus*, 9(4.5%) *A.niger*, 6(3%) *A.flavus* and 5(2.5%) *A.terreus*. The sample size of this study is higher than our study.

## CONCLUSION

The present study shows that there is a significant co-infection of opportunistic mycoses with pulmonary tuberculosis patients.

The prevalence of opportunistic fungal infection in pulmonary tuberculosis patients cannot be ignored.

According to our study and other studies conducted worldwide, the prevalence of male patients of tuberculosis is high than female tuberculosis patients. This prevalence and incidence in male and female patients is poorly understood. Other issues such as differences in risk of exposure to infection, in health seeking behaviour and health systems response, economic consequences, and stigma associated with being known as a tuberculosis patient.

*C.albicans* is the most commmon fungal co-infection with mycobacterium tuberculosis.out of 105 smear positive cases of tuberculosis in 30 cases *Candida* species was isolated.

Aspergillosis infection occurs mainly in old age (low immunity) and preexisting lung cavity are formed in tuberculosis disease. In our study we found 16(34.78%) *Aspergillus species* out of 46(43.80%) positive samples for fungal isolation, in which most common species *Aspergillus fumigatus(17.39%)* were isolated.

so it requires the diagnosis and management of opportunistic fungal infection should be done properly. The antifungal should be started after identification of fungal species. In general, can prevent fungal infection by keeping your skin clean and dry, by using antifungal only as your physician directs, and by following a healthy lifestyle, including proper nutrition. Patients can use the mask to avoid environmental exposure because the spores and conidia of fungus freely present in the environment.

## REFERENCES

1) Ganguly D. Tuberculosis –triumphs and tragedies, J, Indian, Assoc 2000; 11; 96-8.
2) Jasmer,R.M., Nahid,P., and Hopewell, P.C., 2002, clinical pratice, Latent tuberculosis infection ., The New England Journal of Medicine, 347(23), 1860-6.
3) Mukerji jain D K. S K Agarwal and R Prasad, A study on anaerobic infections in patients of (PTB). Indian J Tuberculosis . 1989; 36: 171-5.
4) Basu S, Chakraborty D, Das S. Susceptibility of Candida species from HIV infected and Newborn Candidaemia patients to Amphotericin B. J Biol Sci 2010;10:109-13.
5) Kovacicova G, Krupova Y, Lovaszova M, Roidova A, Trupl J, Liskova A, et al. Antifungal susceptibility of 262 bloodstream yeast isolates from a mixed cancer and non-cancer patient population. J Infect Chemother 2000;6:216-21.
6) Nucci M, Marr KA. Emerging fungal diseases. Clin Infect Dis 2005;41:521-6.
7) Pfaller MA, Diekema DJ. Rare and emerging opportunistic fungal pathogens: concern for resistance beyond Candida albicans and Aspergillus fumigatus. J Clin Microbiol 2004; 42 : 4419-31.
8) Badiee P, Alborzi A, Moeini M, Haddadi P, Farshad S, Japoni A, et al.Antifungal susceptibility of the Aspergillus species by E test and CLSI reference methods. Arch Iran Med 2012; 15 : 429-32.
9) Global Action Fund for Fungal Infection (GAFFI) Improving outcomes for patients with fungal infections across the world a road map for the next decade 95/95 by 2025.