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# Mycological Profile Of Pulmonary TB-HIV Co-Infected Patients With A Relationship To CD4 Count Levels In A Tertiary Care Hospital of Southern Odisha, India.

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**Conflicts of Interest:** Nil.

## Abstract

Human immunodeficiency virus (HIV) associated tuberculosis (TB) remains a major global public health challenge, with an estimated 1.4 million patients worldwide. Co-infection with HIV leads to challenges in both the diagnosis and treatment of tuberculosis. This co-infection leads to further immunosupression predisposing to many opportunistic infections including fungal infections. The range of illness varies from asymptomatic mucosal candidiasis to overwhelming disseminated infections. Data on the pattern of opportunistic pulmonary mycoses and the immunological profile of TB-HIV co-infected patients in southern Odisha are scarce, so we investigated respiratory mycoses and CD4 count among pulmonary TB-HIV coinfected patients attending the Integrated Counseling And Testing Centre of MKCGMC, Berhampur, Odisha.

### Aims and objectives

To detect the opportunistic fungal infections occurring in HIV- Pulmonary TB coinfected patients with LRTI and correlate the fungal isolates with CD4 count.

### Materials and methods

This was a prospective study conducted from March 2017 – August 2017, on 50 pulmonary TB- HIV co-infected patients who attended the Integrated Counseling And Testing Centre of MKCGMC, Berhampur, Odisha. Two separate early morning samples were collected 3 days apart from individual patients and were processed for isolation and identification of fungal species. CD4+T lymphocyte count estimation was done by BD FACS COUNTER.

### Results

From the 50 samples fungus was isolated from 36 cases(72%), the most common isolate being *Candida albicans* (26%) followed by *Aspergillus niger*(16%) and *Aspergillus flavus*(10%). Non Albicans Candida were isolated from a significant number of cases 24% in our study. Majority of yeasts were isolated from patients with CD4+T lymphocyte count less than 200 cells/mm$^3$ and moulds and mucormycetes were isolated from patients with CD4+T lymphocyte count less than 100 cells/mm$^3$.

### Conclusion

Finding from this study revealed opportunistic pulmonary mycoses arise frequently from TB- HIV co- infected

patients with lower CD4+T lymphocyte counts. A high level of clinical suspicion for fungal etiology of respiratory infections in TB-HIV co-infected patients should be kept in mind and routine management of pulmonary mycoses in TB-HIV co-infected patients should include management of the fungal isolates with a correlation to their CD4 count.

**Keywords**

TB- HIV coinfection, CD4+ T Lymphocyte count, Fungal infection, *Candida, Aspergillus, Syncephalastrum*

**Introduction**

Currently HIV infection is affecting about 40 million people world wide[1]. India has third highest number of people living with HIV and AIDS[2]. As the HIV virus affects the CD4 + T lymphocytes and gradually reduces their number which ultimately leads to opportunistic infection. Opportunistic respiratory tract infection is a major cause of morbidity and mortality[3] in HIV patients.

Again in India tuberculosis is the most important infectious disease which kills more adults. Patients with tuberculosis become more immunocompromised because of the chronic nature of the disease and further use of long term antibiotics further worsens the condition of patients.

TB- HIV co-infection remains a major global public health challenge. Many fungal species which were once considered non pathogenic can cause serious disease in immunocompromised hosts like TB – HIV co-infected patients.

*Candida spp, Aspergillus spp, Pneumocystis jirovecii, Cryptococcus neoformans* and fungi included in Mucormycetes are the most common opportunistic pathogens which causes pulmonary infection in HIV/AIDS patients[4].

Data on the pattern of respiratory mycoses and the immunological profile of TB- HIV coinfected patients in Southern Odisha are scarce and uncoordinated, so this study was carried out to investigate respiratory mycoses and CD4 + T lymphocyte count among TB- HIV coinfected patients attending the Integrated Counselling Cum Testing Centre of MKCG Medical College, Berhampur, Odisha, India.

**Aims and objectives**

To detect the opportunistic fungal infections occurring in HIV- Pulmonary TB coinfected patients with LRTI and correlate the fungal isolates with CD4 count.

**Material and Method**

**Study design**

Prospective study

The present study was conducted in Department Of Microbiology, MKCG Medical College and Hospital, Berhampur, India.

**Study population and period**

Patients attending the integrated counseling and testing centre and art centre of MKCG Medical College and Hospital, Berhampur, India for duration of 6 months from March 2017 to August 2017.

**Material / sample**

A total of about 50 early morning sputum samples were collected from known pulmonary TB- HIV coinfected patients presenting with LRTI, in a sterile wide mouth container, attending the ICTC clinic on two different occasions three days apart. The quality of expectorated sputum was evaluated by Bartlett's scoring method[5].

The CD4 count for each patient enrolled was determined by flow cytometry, using the BD FACSCounter fluorescent- activated cell sorter system (BD BIOSCIENCES, San Jose, CA, USA) as per the manufacturer's instructions[6].

**Methodology**

The sputum specimens were processed for direct microscopy using Gram staining and KOH mount[7,8].

India ink preparation was done when capsulated budding yeast cells were seen in Gram stain to identify *Cryptococcus spp.*

Specimens were inoculated in duplicate on Sabouraud's dextrose agar (SDA) with and without chloramphenicol (16µg/ml) and incubated at 25˚c and 37˚c. The cultures were examined for growth every alternate day for 21 days before declaring them as negative.

Macroscopically fungal growth was identified by rate of growth, colony morphology, texture and pigmentation in obverse and reverse of the SDA tube[9]. Microscopic identification was done by identifying the arrangement of spores and hyphae on Lacto Phenol Cotton Blue (LCB) mount. Micro slide culture was done for better identification of molds[9].

*Candida spp* were identified by germ tube test, chlamydospore and blastospore formation in corn meal agar with tween 80, pigment production in CHROMcandida agar, sugar assimilation and fermentation test.

When mucoid yeast like growth was present on SDA, Gram staining was performed. If capsulated budding yeast cells were seen on Gram staining Christensen's urea agar and Bird seed agar were inoculated for the identification of *Cryptococcus neoformans.*

**Results and discussion**

This study was carried out in the department of microbiology for a period of 6 months. A total of 50 sputum samples were analyzed for fungal growth if any. The results were analyzed as follows.

In the present study the majority of patients were in the age group of 21 to 40 years which is in consistence with the study by Jyotsna Chandwani et al[10]. Males outnumbered females with male to female ratio of 1.4 : 1. Majority of the patients were migrant labourers. About 75% of women testing positive have a husband who is a seropositive migrant labourer. And these group transmit the disease to their wives[11].

TABLE 1-AGE AND SEX DISTRIBUTION OF TB – HIV PATIENTS

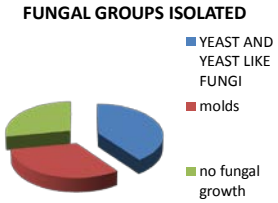
| AGE GROUP ( IN YEARS) | MALE | FEMALE | TOTAL |
|---|---|---|---|
| 0-10 | 0 | 0 | 0 |
| 11-20 | 7 | 3 | 10 |
| 21-30 | 8 | 6 | 14 |
| 31-40 | 7 | 8 | 15 |
| 41-50 | 3 | 2 | 5 |
| 51-60 | 3 | 1 | 4 |
| >60 | 1 | 1 | 2 |
| TOTAL | 29 | 21 | 50 |

of the 50 samples, fungal growth were detected in the sputum of 36 patients(72%) which is higher the findings of Kaur et al[12] who reported fungal growth in 41%.

In the present study, fungi were isolated from 72% of patients. Of which yeast and yeast like isolates accounted for 40% of the cases and molds formed the remaining 32%.

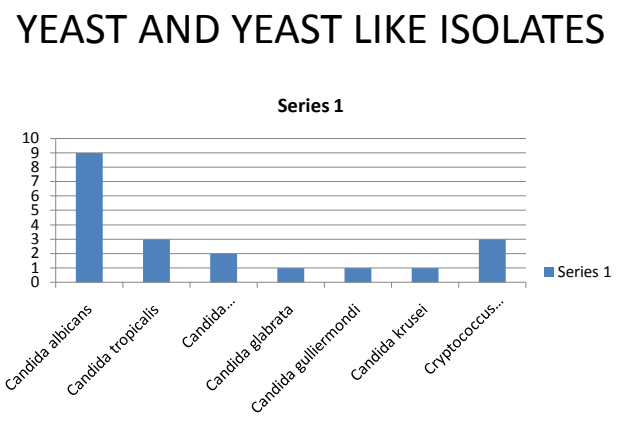
TABLE 2 FUNGAL GROUPS ISOLATED

| FUNGAL GROUPS ISOLATED | NUMBER OF CASES | PERCENTAGE |
|---|---|---|
| Yeast and yeast like fungus | 20 | 40% |
| Molds | 16 | 32% |
| No fungal growth | 14 | 28% |




Amongst the yeast and yeast like isolates *Candida albicans* was the most common isolated fungi in 26% of patients and non *albicans candida* accounted for 22% of patients with fungal isolates. Our findings are consistent with the findings of Bharathi et al who reported *Candida albicans* in 26% and non *albicans Candida* from 29% of patients[13].

In the present study 89% of Candida albicans and 100% of non *albicans Candida* were isolated from patients with CD4 count < 350 cells/mm$^3$ which is consistent with the study done by Rajeev Shah et al[14]. Lower immunity makes *Candida albicans*, the otherwise normal flora of upper respiratory tract an opportunistic pathogen. Other predisposing factors in HIV patients like neutropenia, lymphopenia, selective T cell defects , altered monocyte macrophage function and frequent lowered immunity due to low CD4 + T lymphocyte count[15].

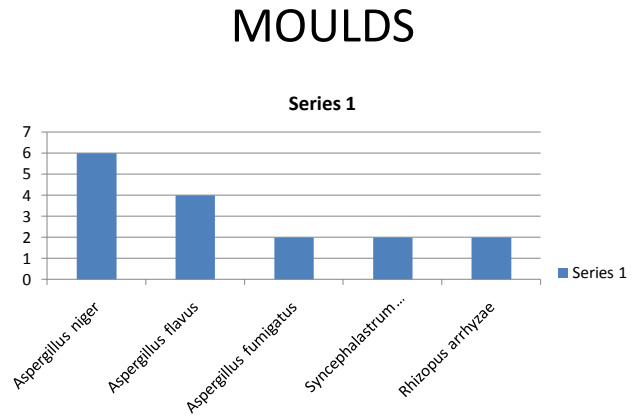



Graph 1 Yeast and Yeast Like Isolates

*Cryptococcus neoformans* was isolated from 3 samples with CD4 + T lymphocyte count < 100 cells/ mm$^3$.

In the present study 11(91%) isolates of *Aspergillus* species were from patients with CD4 + T lymphocytes < 100 cell/ mm$^3$. The most common species was *Aspergillus niger*(16%) followed by *Aspergillus flavus* (10%) and *Aspergillus fumigatus* (5.5%). In human defence against the inhaled spores of Aspergillus begins with the mucus layer and the ciliary action in the respiratory tract[15]. Macrophages and neutrophils engulf and eradicate the fungus[15]. Immunosuppression in TB- HIV coinfection leads neutrophil dysfunction and reduction in its number leading to failure in eradication of *Aspergillus.*

In the present study *Syncephalastrum racemosum* was isolated from two samples with CD4 count 86 cells/ mm$^3$ and 78 cells/ mm$^3$. To the best of our knowledge it was the first case reported from TB- HIV coinfected patient in India.




Graph 2: Moulds Isolates

| FUNGUS | ISOLATES | CD4 + T LYMPHOCYTE CELL COUNT (CELLS/ mm$^3$) | | | | |
|---|---|---|---|---|---|---|
| | | <50 | 51-100 | 101-200 | 201-350 | 351-500 |
| *C albicans* | 9 (26%) | 1 | 0 | 3 | 4 | 1 |
| *C tropicalis* | 3 (8.3%) | 0 | 0 | 3 | 0 | 0 |
| *C parapsilopsis* | 2(5.5%) | 0 | 1 | 1 | 0 | 0 |
| *C glabrata* | 1(3.1%) | 0 | 0 | 1 | 0 | 0 |
| *C gulliermondi* | 1(3.1%) | 1 | 0 | 0 | 0 | 0 |
| *C krusei* | 1(3.1%) | 0 | 0 | 1 | 0 | 0 |
| *A niger* | 6(16%) | 1 | 5 | 0 | 0 | 0 |
| *A flavus* | 4(10%) | 1 | 3 | 0 | 0 | 0 |
| *A fumigatus* | 2(5.5%) | 0 | 1 | 1 | 0 | 0 |
| *Crytococcus neoformans* | 3 (8.3%) | 1 | 2 | 0 | 0 | 0 |
| *Syncephalastrum racemosum* | 2(5.5%) | 0 | 2 | 0 | 0 | 0 |

Table 3: Distribution of Fungal Isolates in Tb – Hiv Coinfected Patients And Its Relation With Cd4 + T Lymphocyte Cell Count

**Conclusion**

Findings from this study revealed opportunistic pulmonary mycoses arise frequently from patients with lower CD4 + T Lymphocyte counts. Majority of the Yeast and yeast like isolates were mostly found in patients with CD4 + T

Lymphocyte count < 200 cells/ $mm^3$ and moulds in patients with CD4 + T Lymphocytes < 100 cells/ $mm^3$. Fungal screening should be done in all patients of TB-HIV co-infections with CD4 count < 200 cells/$mm^3$ to reduce the mortality and morbidity due to opportunistic fungal infections. Empirical antifungal treatment should be included in this category of patients.

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