e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(8); 1362-1367

Original Research Article

Exotic Fungi in Treated Cases of Pulmonary Tuberculosis and Treated Cases of COVID-19 with History of Pulmonary Tuberculosis: A Hidden Epidemic within Pandemic

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Received: 30-06-2023 / Revised: 31-07-2023 / Accepted: 26-08-2023
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Conflict of interest: Nil

Abstract:

Background: The occurrence of opportunistic mycoses among individuals undergoing treatment for tuberculosis and COVID-19 in India is notably elevated. This increased prevalence can be attributed to compromised immune systems and the administration of anti-tubercular therapy, which fosters the proliferation of fungal microorganisms. Consequently, this exacerbates the underlying health condition, potentially resulting in fatal outcomes.

Methods: Post-treated cases of pulmonary tuberculosis and post-treated cases of COVID-19 with a history of pulmonary tuberculosis having respiratory symptoms visiting our teaching hospital. After getting consent from the patient, a detailed history was collected from patients by administering a structured questionnaire. The present study group is divided into Tuberculosis group (Group 1) and (Group 2) Covid 19 & TB group.

Results: The total number of isolates was 19 (14 in group 1 and 5 in group 2). The most common type of isolate was *Aspergillus fumigatus*, with 5 isolates (3 in Group 1 and 2 in Group 2). The second most common type of isolate was *Candida albicans*, with 3 isolates (all in Group 1). *Aspergillus fumigatus* was more common in Group 2 than in Group 1. *Candida albicans* was more common in Group 1 than in Group 2. *Aspergillus niger* was found only in group 2.

Conclusion: There is a high prevalence of fungal infections in treated cases of pulmonary tuberculosis and treated cases of COVID-19 with a history of Pulmonary Tuberculosis. *Candida albicans* and *Aspergillus fumigates* species are the common causative agents. These secondary fungal infections are associated with the persistence of respiratory symptoms, despite successful completion of anti-tubercular drug therapy and anti-Covid treatment.

Keywords: Covid-19, Tuberculosis, Fungal infections, *Aspergillosis, Candidiasis*.

Introduction

India, the second most populous country in the world, continues to grapple with both COVID-19 and Tuberculosis (TB). TB, which has a high incidence rate in the country, relies on statistics primarily provided by the World Health Organization (WHO), indicating an incidence rate of 193 cases per 100,000 people, accounting for approximately 2.64 million cases in 2019. [1] The gravity of these infections is compounded when opportunistic mycoses are present, as they often go undiagnosed and untreated owing to the absence of specific clinical or radiological symptoms. The prevalence of opportunistic mycoses among treated tuberculosis and COVID-19 cases in India is very high. [2] The reason for the increased prevalence of these infections is the insufficiency of the immune system and the use of anti-tubercular therapy, which promotes the growth and reproduction of fungal flora, which in turn aggravates the underlying pathology leading to fatal consequences. [3] A wide range of fungal co-infections has been observed in patients and survivors of COVID-19. Fungal pathogens like *Candida, Aspergillus*, and *Mucorales* are burdening the lives of COVID-19 patients and the survivors in the form of Yellow/Green, White, and Black Fungi. [4] Hence studies say that non-pathological fungi should not be dismissed as contaminants, as they can quell immunocompromised hosts. [4] In recent studies, immunosuppressive therapy has shown promising results in the control of cytokine storm syndrome (CSS) in severe cases of COVID-19. However, it is well documented that immunosuppressive agents (e.g., corticosteroids and cytokine blockers) increase

the risk of opportunistic fungal infections. In contrast, several opportunistic fungal infections were reported in COVID-19 patients, including *Aspergillus spp*., *Candida spp., Cryptococcus neoformans*, *Pneumocystis jiroveci (carinii),* and *mucormycosis,* have been reported. [5] Post-TB Complications may lead to persistent pulmonary damage in patients whose infection has been considered clinically cured. Chronic impairment of lung function, bronchiectasis, *aspergilloma,* and chronic pulmonary aspergillosis (CPA) has been associated with TB. Fungal infections of the lungs are slow, making their presence felt in India. [6]

Hence, an urgent need for proper diagnosis of opportunistic mycoses in post-treated cases of pulmonary tuberculosis and post-treated cases of COVID-19 with a history of pulmonary tuberculosis is required to avoid fatal complications and for effective treatment. Although tuberculosis (TB) and COVID-19 are both respiratory diseases transmitted through the air, COVID-19 can be more aggressive than TB. COVID-19 can cause extensive damage to lung tissue, leading to the formation of cavities similar to those seen in chronic TB. [7]

These cavities may not be functional and can become infected by fungi. These fungal infections can manifest as pneumonia or AFB-negative TB. Studies have shown that the most common opportunistic fungal infections in pulmonary TB cases are caused by *Candida albicans*, *Aspergillus fumigatus*, and *Aspergillus flavus*. [8] In one study, 15.4% of patients with TB were co-infected with *aspergillosis*, with *A. fumigatus* being the most common species. Another study found that 52.9% of COVID-19-positive patients were infected with *Aspergillus fumigatus*. [9] To date, there have been no studies on fungal coinfections in COVID-19 and pulmonary TB cases in this region. This study was conducted to assess the identification of fungi associated with post-treated cases of pulmonary tuberculosis and post-treated cases of COVID-19 with a history of pulmonary tuberculosis.

Material and Methods

The present study is a cross-sectional study and was conducted in the Department of Microbiology J N Medical College KAHER Belagavi and KLES Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre Belagavi, Karnataka. Institutional Ethical Clearance was taken for this study and also from District Health authorities. A convenient sampling method was used for the identification of patients in the study. The average incidence of cases of interest at KLEH is approximately 120 to 140 cases per annum Average monthly caseload is about 12 cases. The number of cases in said study period is 12 x 2 = 24 cases. Adjusting for an annual increase in caseload and incidence is 11 cases (also normalizing for standard deviations in reporting) Total Sample Size worked out to be 35 cases.

Inclusion criteria:

1. Post-treated cases of pulmonary tuberculosis and post-treated cases of COVID-19 with a history of pulmonary tuberculosis with complaints of respiratory symptoms attending our teaching hospital.

Exclusion criteria:

1. Children (Age < 14 years).
2. Patients having extrapulmonary tuberculosis.

Study Population: Post-treated cases of pulmonary tuberculosis and post-treated cases of COVID-19 with a history of pulmonary tuberculosis having respiratory symptoms visiting our teaching hospital. After getting consent from the patient, a detailed history was collected from patients by administering a structured questionnaire. The present study group is divided into Tuberculosis group (Group 1) and (Group 2) Covid 19 & TB group.

Sample analysis: Early morning sputum samples were collected from post-treated cases of pulmonary tuberculosis and post-treated cases of COVID-19 with a history of pulmonary tuberculosis having respiratory symptoms visiting our teaching hospital. The samples were processed in biosafety cabinet-II adhering to universal safety precautions in the Microbiology department of our college. Samples were subjected to KOH mount to detect the fungal elements and Gram's staining was performed to see for any budding yeast cells.

All samples were cultured on Sabouraud's dextrose agar and Sabouraud dextrose agar with antibiotics. All yeast isolated as pure culture were categorized to species level by using Hichrome agar. The Molds isolated were subjected to Lacto Phenol Cotton Blue mount and reported according to their distinct morphology.

Statistical data analysis: Data was collected was uploaded to an MS Excel spreadsheet and analyzed using SPSS version 21 on Windows format. Continuous variables were represented as mean, standard deviations, and percentages. Categorical variables were analyzed by chi-square test and a p-value of (<0.05) was considered as significant. Analysis was done using descriptive statistical parameters.

Results

A total of 35 patients with respiratory symptoms who were treated for Pulmonary Tuberculosis and treated cases of COVID-19 with a history of pulmonary tuberculosis were included in the present study. The age of the youngest patient was a 22-year-old woman and the oldest was a 67-year male. The mean age of the patients in this study was 40 ±

10 years. Of the 35 patients, 62.85% were males and 37.14% were females. The percentage of males was higher than that of females in all age groups except 20-30. The percentage of males increased with age from 20-30 to 41-50 years and then decreases from 41-50 to >61 years. The percentage of females decreased with age from 20-30 to 41-50, and then increases from 41-50 to >61 years, as depicted in Table 1.

Table 1: Age-wise distribution of cases included in the study

Age Group	Male (%)	Female (%)
20 – 30	04 (11.42%)	03 (8.57%)
31 – 40	06 (17.14%)	02 (5.71%)
41 – 50	07 (20%)	05 (14.28%)
51 – 60	03 (8.57%)	01 (2.85%)
> 61	02 (5.71%)	02 (5.71%)
Total	22 (62.85%)	13 (37.14 %)

Table 2 shows the association between TB and COVID-19 in the two groups of patients. Group 1 included patients with TB, and Group 2 included patients with COVID-19 and TB. The table shows the number of males and females in each group as well as the percentage of males and females in each group. The total number of patients in the table is 35 (22 males and 13 females). Group 1 included 22 patients (14 males and 8 females), and Group 2 included 13 patients (8 males and 5 females). The percentage of males in Group 1 was 63.63%, and the percentage of females in Group 1 was 36.36%. The percentage of males in Group 2 was 61.54%, and the percentage of females in Group 2 was 38.46%. The percentage of males was higher than that of females in both groups. The percentage of males was higher in group 1 than in group 2. The percentage of females was higher in group 2 than in group 1. There was a higher percentage of males with TB than females, and there was a higher percentage of females with COVID-19 and TB than males depicted (Table 2).

Table 2: showing the distribution of cases in Group I and Group II

Tuberculosis (Group I)		Covid 19 and Tuberculosis (Group 2)	
Male	Female	Male	Female
14(40%)	8(22.86%)	8(22.86%)	5(14.28%)

The co-morbid conditions are hypertension, diabetes mellitus, smoking/tobacco chewing, and alcoholism which were found to be associated with patients.

Out of a total of 35 cases, 33 patients had one comorbid condition. 4 patients had hypertension, 15 patients had diabetes mellitus, 14 patients smoked/chewed tobacco, and 10 patients were alcoholics. 2 patients did not have any comorbidities. Hypertension was the least common co-morbid condition, followed by diabetes mellitus, smoking/tobacco chewing, and alcoholism.

Table 3: Showing the distribution of fungal isolates in both the groups

Isolates	Group 1 (%)	Group 2 (%)
C albicans	03 (21.42%)	01 (20%)
C.tropicalis	02 (14.28%)	00 (00%)
Aspegilus fumigatus	05 (35.71%)	01 (20%)
Aspergillus flavus	03 (21.42%)	01 (20%)
Aspergillus niger	01 (7.14 %)	02 (40%)
Total	14 (73.68%)	05 (26.31%)

Table 3 shows the number of isolates of each type of fungus in each group as well as the percentage of isolates of each type of fungus in each group. The total number of isolates was 19 (14 in group 1 and 5 in group 2). The most common type of isolate was *Aspergillus fumigatus*, with 5 isolates (3 in Group 1 and 2 in Group 2). The second most common type of isolate was *Candida albicans*, with 3 isolates (all in Group 1). *Aspergillus fumigatus* was more common in Group 2 than in Group 1. *Candida albicans* was more common in Group 1 than in Group 2. *Aspergillus niger* was found only in group 2.

This table suggests that Aspergillus fumigatus is more likely to co-occur with COVID-19 than *Candida albicans*.

Discussion

Since the onset of the coronavirus disease 2019 (COVID-19), attributed to SARS-CoV-2 in early 2020, the global tally has surpassed 500 million documented cases and exceeded 6 million fatalities worldwide. [10] A significant issue that arises in severely afflicted COVID-19 patients is superinfection, frequently stemming from bacteria,

fungi, or other viruses. [11-13] Invasive Fungal Infections (IFIs), particularly invasive pulmonary *aspergillosis* (IPA), manifest as a consequence of viral respiratory infections such as influenza, a condition referred to as influenza-associated pulmonary *aspergillosis* (IAPA). [14-16] similarly, the incidence of fungal infections that complicate COVID-19 has increased. The mechanisms underlying secondary fungal infections in COVID-19 remain poorly understood. Much like IAPA, it is believed that SARS-CoV-2 infection triggers the release of danger-associated molecular patterns (DAMPs), initiating a hyper inflammatory cascade and cytokine storm that ultimately leads to acute respiratory distress syndrome (ARDS). This disruption affects the integrity of the lung epithelial barrier, coupled with concurrent immune dysregulation, compromised host defences, and impaired mucociliary clearance, all of which facilitate fungal invasion and contribute to disease pathogenesis. [17]

The relation between COVID-19 and *aspergillosis* is known as CAPA (COVID-19-associated pulmonary *aspergillosis* (CAPA). Initially, COVID-19-associated pulmonary *aspergillosis* (CAPA) was reported to have a high incidence, comparable to that of IAPA, and it was linked to unfavorable outcomes and extended hospital stays. Nevertheless, the actual occurrence, associated risk factors, optimal diagnostic methods, preventive measures, and treatment strategies for fungal infections associated with COVID-19 are still evolving despite the wealth of data from numerous observational studies and recent consensus guidelines. [18] The present study found that *Aspergillus fumigatus* was the most common species among the Tb group and A. niger in the Covid-19 & TB group. It has been reported that 14–30% of hospitalized patients diagnosed with COVID-19 develop severe respiratory failure requiring intensive care admission. Invasive *aspergillosis* appears in a range from 11 to 21 days after the onset of COVID-19 and affects up to 30% of intubated patients. [19-21] some authors have reported a mortality rate ranging from 15 to 30%, with lower survival in patients with (CAPA), compared to those without CAPA. [22-24] the same trend was previously observed for influenza-related *aspergillosis*. [25] Prolonged use of corticosteroids is considered a risk factor for invasive fungal diseases. [26] The criteria to classify patients with CAPA vary from those with risk factors for invasive pulmonary *aspergillosis* (IPA), as considered by the EORTC, [27], or those with other factors such as diabetes, obesity, or hypertension.

Treatment with interleukin inhibitors or tocilizumab (monoclonal antibody), which was used as therapy in COVID patients [28, 29] could also potentially increase the risk of other fungal infections, such those, among others, caused by *Candida spp*., *Histoplasma spp*., or *Pneumocystis jirovecii* [30] Candidemia has been reported in 2.5–6.9% of COVID-19 patients in the ICU, mainly catheter-related infections and often with unfavorable outcomes. [31, 32] Considering the significant damage to pulmonary tissues and the immunosuppressive effects associated with SARS-CoV-2 infection and its treatment, it is crucial to take into account the potential involvement of fungal pathogens when evaluating suspected superinfections in COVID-19 patients. Various fungal infections, including *aspergillosis, candidiasis*, non-*Aspergillus* mold infections, endemic fungi, and PJP, have already been documented, and additional data on this topic will likely continue to emerge in future studies.

Conclusion

There is a high prevalence of fungal infections in treated cases of pulmonary tuberculosis and treated cases of COVID-19 with a history of Pulmonary Tuberculosis. *Candida albicans* and *Aspergillus* fumigates species are the common causative agents. These secondary fungal infections are associated with the persistence of respiratory symptoms, despite successful completion of anti-tubercular drug therapy and anti-Covid treatment. 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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