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Original Research Paper

Pathology

A STUDY OF COVID-19 ASSOCIATED FUNGAL CO- INFECTIONS-EXPERIENCE FROM A TERTIARY CARE HOSPITAL

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ABSTRACT COVID 19 causes wide range of fungal infections that may coexist with possible association with preexisting morbid conditions (diabetes mellitus, lung disease) or may develop as a hospital acquired infection. Some fungal infections like mucor mycosis, Aspergillus and Candida infections in COVID-19 patients will require early clinical detection and diagnosis by a comprehensive interventions (Histopathology, direct microscopic examination, culture,(1, 3)-b-D-glucan, galactomannan and PCR-based assays) to ensure effective treatments. This study deals with clinical diagnosis, risk factors, type of mycosis, Angio invasion and treatment response in post COVID patients.

Materials and methods: The present study includes Post - COVID 19 patients with associated histologically proven fungal co-infections in Alluri Sitarama Raju Academy of Medical Sciences, and hospital during the period of March 2021 to June 2021.

Results: A total of 84 patients with age range 35- 71 years were included. Most common associated disease was diabetes mellitus (47.6%). Histopathology revealed infection with Mucor, Aspergillus and candida in 85.7%, 4.7% and 9.5% patients, respectively. Sino-nasal involvement is most common, seen in all patients. The most common symptoms and signs are headache and nasal discharge seen in 85.7% and 76.1% respectively.

Conclusion: Histopathological confirmation of post COVID-19 associated fungal infections is necessary as it helps in early management with antifungal therapy and surgical debridement, which proves better outcomes and higher survival rate.

KEYWORDS : COVID-19 SARS-CoV-2 Fungal co-infection, Mucormycosis, Aspergillosis, candidiasis

INTRODUCTION:

Corona virus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), is the leading cause of an emergency pandemic worldwide [1, 2]. These COVID-19 patients, have a higher probability of suffering from fungal infections especially severely ill or immunocompromised. They were found to have immune-suppression attributed to a decrease in CD4+T and CD8+T cells [3]. Corona virus disease 2019 (COVID-19) has become a pandemic. Age, sequential organ failure, and D-dimer are the main prognostic factors of COVID-19 patients [4]. The presence of bacterial and/or fungal secondary infection or co-infection is likely another important factor affecting mortality and it has received inadequate attention. Bacterial and fungal infections are common complications of viral pneumonia, especially in critically ill patients. They lead to increased need for intensive care and increased mortality. The human airway is continuously open to the non- sterile environment where fungal spores have the potential to enter inside and produce disease. In the immunocompromised host, many fungi considered non-pathogenic can have the potential to cause serious morbidity and mortality. Data regarding the bacterial or fungal infection in viral pneumonia led by corona virus are limited. The bacterial and fungal infections in COVID-19 patients have been inadequately investigated and reported so far. [4,5,6]. Sometimes it can be difficult to distinguish bacterial or fungal infection in an existing viral pneumonia based on clinical and radiological appearance, microbiological examination followed by histopathological confirmation can add great value to diagnoses. Invasive fungal infection is a potentially fatal infection seen in immune-compromised patients with subsequent serious morbidity and mortality. These are reported usually in individuals with invasive fungal infections which include patients with cancer on chemotherapy or immunosuppressive drugs, uncontrolled diabetes, AIDS/HIV and recently, COVID-19.[7,8] This study aimed at demonstrating the fungal co-infections in relation to COVID-19 pandemic, its clinical diagnosis, risk factors, type of mycosis, angioinvasion and treatment response (thus reducing the mortality) of COVID-19 patients.

SUBJECTS AND METHODS:

This study includes 84 patients diagnosed with fungal co-infections associated with a recent COVID-19 infection at the Department of Pathology, Alluri Sitarama Raju Academy of Medical Sciences, from May 2021 to June 2021 were analyzed. All these patients had a definite diagnosis of COVID-19 by polymerase chain reaction (PCR) and computed tomography (CT) studies for post-COVID site and extension of fungal infection. Diagnosis was made with histopathology and the documentation done for invasion i.e. within sinus mucosa, sub mucosa, bone and blood vessels.

Patients with incomplete data or lost follow-up were excluded from the study. The specimen was fixed in 10% formalin for 12 hours and processed routinely, after embedding four to five micron paraffin-embedded tissue sections taken on glass slides and H&E and PAS staining was done. Other special stains are done where and when necessary. Histopathological

examination was done by two pathologists, individually, to reduce observer bias. The morphology of fungus, angioinvasion and bone invasion along with adjacent tissue changes were examined and recorded.

RESULTS:

Total 84 patients were diagnosed with fungal infection in relation to COVID-19 infection. All patients were diagnosed in the post COVID-19 infection period, with a mean duration of 18-20 days after being PCR negative, except for three patients who developed disease in active COVID-19 infection. The age ranged between 35–71 years. Patient's clinical data, and comorbidities and mean duration between negative result and fungal infection are listed in Table 1. The most common associated disease was diabetes mellitus (DM) (47.6%); asthma is associated with 28.7% patients. Associated malignancy seen in four patients (4.76%) and are on Chemotherapy **(Table 1)**. All the cases (100%) show mucosal involvement of nose and sinuses, headache and nasal discharge are the most common presenting symptoms in these patients **(Table 2).** Mucormycosis is the most common fungal infection (85.7%) in our study followed by candidiasis (9.52%). Angioinvasion is seen in 66.6% of cases of mucormycosis **(Table 3).** Histopathological evaluation of mucosal biopsies confirmed that the main causative fungi were Mucor species i.e. mucormycosis in 85.7% and Aspergillus fumigatus in four patients (4.76%) while eight patients (9.52%) were infected with candida (Table 3). Two patients have dual infection with mucor, candida and two patients had mucor and aspergillus and one patient had triple infection with mucor, aspergillus and candida. Mucosal ulceration and necrosis is seen in most of the cases, but associated squamous metaplasia is seen in only 22 cases (28.9%). Associated granulomatous inflammation seen in 29 cases (38.1%) .

DISCUSSION:

As the ongoing COVID-19 pandemic, more and more experts are aware of fungal co-infections. Morphology of fungus along with clinical data regarding fungal infections is valuable in guiding evidence-based treatment of COVID-19. Hence, it is necessary for severely ill patients to receive fungal pathogens surveillance, including (i) etiology, direct microscopy and culture; (ii) histopathology; (iii) serology with antigen and antibody, (1,3)-b-D-glucan (BDG) [9] and galactomannan (GM) are useful for suspicious patients, while bronchoalveolar lavage fluid (BALF) and tracheal aspirate (TA) sampling for culture and biomarker testing should be performed under well-protected conditions due to the risk of aerosol spread [10]; (iv) PCR-based methods.

In the present study, COVID-19 patients with diabetes mellitus are co-infected with invasive mucormycosis 47.61%, followed by asthma/COPD in 28.5% cases, most of these patients show lymphopenia, studies have found that COVID-19 patients with trauma, diabetes mellitus, glucocorticoids use, prolonged neutropenia, allogenic Hematopoietic stem cell transplantation, Solid organ transplants are more likely to develop mucormycosis [11]. Mucormycosis is usually suspected based on results of direct microscopy that mucor hyphae are non-septate or pauci-septate with a variable width of 6–16 microns and obtuse angled branching **(Fig 1).** Angioinvasion and bony invasion seen in 66.6% and 77.7% cases respectively**(Fig 1)** Adjacent granulomatous reaction is seen in 38.8% cases indicating good immune response in these patients**(Fig 2).**

In the present study Aspergillosis is seen in 4 cases and among these two patients was known diabetics and had prolonged leukopenia. Two patients had associated mucormycosis **(Fig 3)** (dual infection) and two patients had pulmonary fungal infection which was suspected in CT scan and confirmed in bronch-oalveolar lavage **(Fig 4).** Yang et al. found three (3/52, 5.8%) patients with pulmonary fungal co-infection in 52 critically ill patients[12]

Other studies have found a higher percentage of secondary pulmonary infections (8%–15%) in COVID-19 patients, but it is not clear whether it is bacterial or fungal infection **[13, 14].** The potential risk factors for these patients include prolonged leucopenia, chronic obstructive pulmonary disease. Histopathologic examination shows characteristic septate hyphae with acute angle branching of Aspergillus. Periodic acid Schiff (PAS) stain helped in highlighting the fungal elements (Fig 5). Granulomas are seen in one case (25%).

In the present study candidiasis is found in 8 cases and all are associated with mucormycosis (dual infection) **(Fig 6).** This candida is seen as pseudo hyphae with attached budding yeast forms, which are invading the tissue.

One case showed triple fungal infection with mucor, aspergillus and candida **(Fig 7)**. Single most important element for successful attenuation of this infection is early diagnosis followed by aggressive medical care, surgical debridement, and control of associated comorbid diseases. In our study all these patients were given antifungal therapy with amphotericin B followed by oral Posaconazole. Among the 84 patients, 28 cases died, the cause may be disease or due to drug associated or disease associated complications.

CONCLUSION

Early clinical diagnosis and histopathology confirmation of COVID-19 associated fungal infections are crucial for management. Patients without comorbidities or angioinvasion have better treatment outcomes. Rapid initiation of antifungal therapy, treatment of associated comorbidities and surgical debridement could affect the prognosis of these patients and improve survival rates.

FIGURES AND TABLES

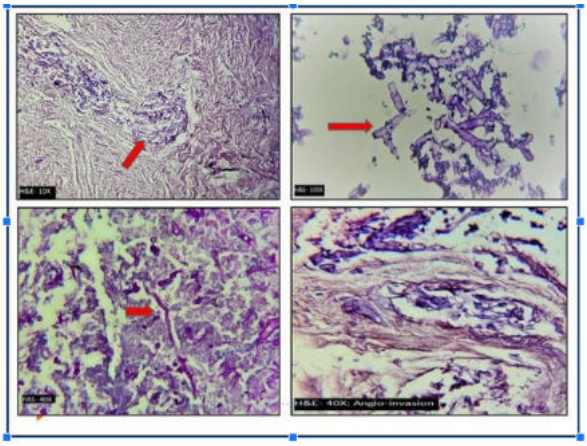

Fig 1 – H& E sections and PAS show broad, aseptate hyphae of mucor with obtuse angled branching. Angio-invasion also noted.

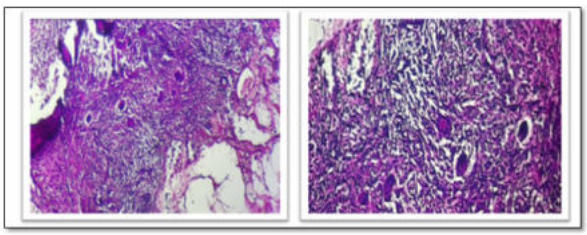

Fig 2 – H&E section show granulomas with foreign body giant cells adjacent to the the fungal hyphae

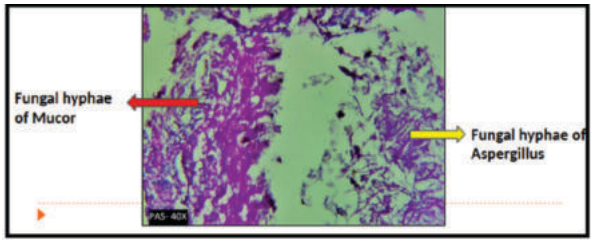

Fig 3 – PAS shows dual fungal infection with mucor and aspergillus

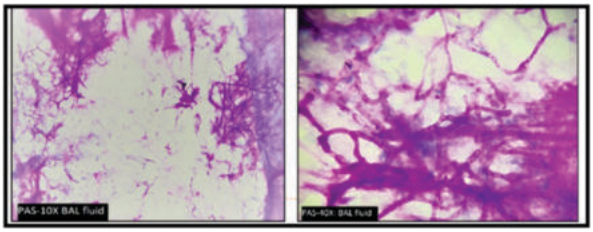

Fig 4 - PAS of BAL fluid show thin, filamentous hyphae of aspergillus with septations and acute angled branching.

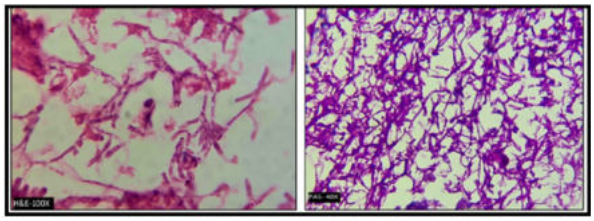

Fig 5 – H&E and PAS show thin, filamentous hyphae of aspergillus with septations and acute angled branching

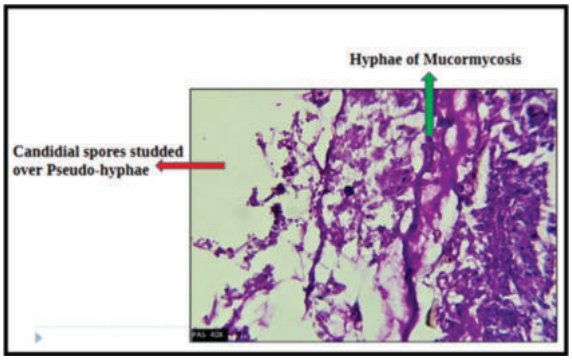

Fig 6 – PAS show dual infection with Candida and mucor

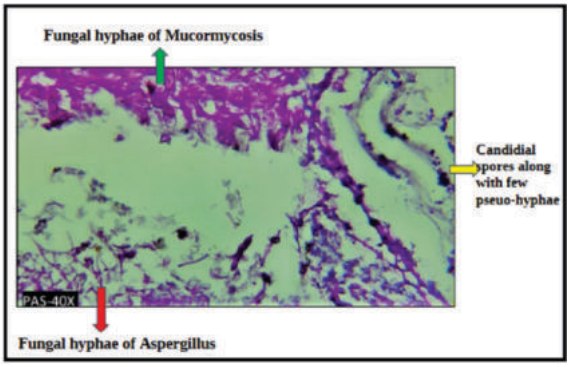

Fig 7 – PAS of nasal mucosal biopsy tissue show triple infection with Candida, Mucor and Aspergillus.

Tables –

Table-1 clinico pathological data in post COVID patients with associated fungal co infections.

Data variable	Number of cases	Frequency % positivity
1.Total number of cases	84	100%
2. Age	Age range 35-71 years	
3. Sex - male	56/84	66.6%
- female	28/84	33.3%
4. Associated comorbidities		
A. Diabetes mellitus	40/84	47.61%
B. Malignancy	04/84	4.76%
C. Asthma /COPD	24/84	28.57%
D. Chronic renal failure on dialysis	16/84	19.04%
5. Mean duration between negative PCR and fungal infection is 18-20 days		

Table 2 - clinical extension with signs and symptoms of fungal co infections in post COVID 19 patients

Variable	No of cases	Frequency
1.Clinical extension		
Nasal septum	84/84	100%
Lateral wall of the nose	84/84	100%
Sinuses	84/84	100%
Orbit	12/84	14.28%
Intracranial extension	16/84	19.04%
Palate and oral mucosa ulceration	24/84	28.57%
2. Symptoms and signs		
Headache	72/84	85.71%
Nasal discharge	64/84	76.19%
proptosis/diplopia/visual loss	12/84	14.28%
Altered sensorium	32/84	38.09%

Table 3 - Microscopic examination of 84 cases of fungal co infection infections with their histomorphological characteristics.

Histomorphology of the fungus/ type of fungus	Frequency of cases	Frequency of Angioinvasion	Frequency of Bone invasion
1. Mucormycosis	72/84(85.71%)	48/72(66.66%)	56/72(77.77%)
2. Aspergillus	04/84(4.76%)	00/04	00/04
3. Candida	08/84(9.52%)	00/08	00/08
4.Rhizopus	01/84		

Table 4 - Microscopic examination of adjacent tissue

Type of adjacent tissue change	Mucormycosis (n=72)	Aspergillosis (n=04)	Candidiasis (n=08)
Mucosal ulceration	72/72 (100%)	01/04(25%)	05/08(62.5%)
Squamous metaplasia of overlying epithelium	21/72(29.1%)	01/04 (25%)	00/08
Necrosis	70/72 (97.2%)	04/04(100%)	02/08(25%)
Granulomatous inflammation with foreign body giant cell reaction	28/72 (38.8%)	01/04(25%)	00/08

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