

MYCOLOGICAL PROFILE IN POST COVID-19 PATIENTS WITH SINUSITIS IN A TERTIARY CARE CENTRE

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Abstract

Background: Covid-19 is a viral infection caused by SARS-CoV-2. Invasive fungal sinusitis is one of the life threatening complications occurring in Post Covid-19 patients. Rapid progression of the disease without early diagnosis and treatment leads to decreased survival rate (20% to 70%). This study mainly focused on the isolation of fungal agents causing Invasive fungal sinusitis to reduce the morbidity and mortality. **Objective:** To identify the fungal pathogens in post COVID 19 patients with invasive fungal sinusitis. **Materials and Methods:** This cross sectional study was conducted for a period of six months from May 2021 to December 2021 in Govt Kilpauk Medical College, Chennai, Tamilnadu, India. This study was done in 30 post covid 19 patients with sinusitis. The collected samples were immediately sent to the laboratory. Fungal culture was done as per standard guidelines. **Result:** Majority of the patients had Diabetes Mellitus (80%). Among the Post covid-19 patients who had invasive fungal sinusitis, half of the patients had Diabetes Mellitus (50%) and half of them were stayed in the hospital more than 14 days (50%). Oxygen dependence was observed in (57%) and steroid intake was found in 14% of them. Orbital & nasal extension was seen in (33%), Paranasal sinus extension was seen in (26%) and intra cranial extension was observed in (3%) of the patients. And also, intracranial extension was seen in the patient who had invasive mucormycosis. Majority of the patients had *Mucor* infection (36.6%) and *Aspergillus flavus* infection was identified in 20%. **Conclusion:** *Mucor* and *Aspergillus* were the most common fungal pathogens causing Invasive fungal sinusitis in post covid-19 patients. Diabetes mellitus was the predominant comorbidity. Patients without any comorbidities also had invasive fungal infection was an important aspect of this study. Patients with *Mucor* infection had intra cranial extension. A high suspicious of invasive fungal sinusitis should be kept in mind when post Covid patients who are getting steroids and under oxygen dependence.

INTRODUCTION

Covid-19 infection caused by SARS-CoV-2, has been an emergency global health event due to its human to human transmission. It is mainly transmitted through infected secretions or respiratory droplets from the patients. In March 2020, the World Health Organization declared the Covid19 outbreak as a pandemic.^[1] The first case was identified in Wuhan, China in December 2019. Overexpression of

inflammatory cytokines and impaired cell mediated immunity leads to secondary fungal infections.^[2]

Covid-19 causes lymphopenia, endothelial damage, endothelitis and reduction in CD4+ and CD8+ T cells and thus predispose to secondary fungal infections.^[3] It also damages the lung parenchyma and produces large bilateral alveolo-interstitial lesions which lead to secondary infections.^[3]

Acute invasive fungal sinusitis is the most common secondary infection occurs in post covid-19 patients. It usually occurs in immunocompromised patients

having diabetes mellitus, hematologic malignancies, solid organ or bone marrow transplantation, chemotherapy induced neutropenia and advanced AIDS.^[2]

Invasive fungal sinusitis can present as Sinonasal disease (sinonasal tissue invasion), Sinopalatal disease (progression to the palate), rhino orbital disease (progression to orbits) or rhino-orbital-cerebral disease (CNS involvement). Rapid progression of the disease without early diagnosis and treatment leads to decreased survival rate (20% to 70%).^[2]

Noha Ahmed et al studied fungal pathogens causing invasive fungal sinusitis in 36 post covid 19 patients. Majority of the patients had Mucor infection (77%), and Aspergillus infection was observed in 30.6% of them. Sinonasal, orbital, cerebral and palatine involvement was observed in 100%,80.6%,27.8% and 33.3% of the patients respectively.^[4]

In Vivek Dokania et al study, among 21 patients Mucor infection identified in 95.24% and Aspergillus infection found in 14.29%. Diabetes mellitus (90.48%) was the most common comorbidity among them.^[5] This study mainly focused on the isolation of fungal agents causing invasive fungal sinusitis to reduce the morbidity and mortality.

MATERIALS AND METHODS

This cross sectional study was conducted for a period of six months from May 2021 to December 2021. The study was approved by Institutional Ethics Committee, Govt Kilpauk Medical College, Chennai, Tamilnadu, India. Informed written consent was obtained from the patients. This study was done in 30 patients presenting with signs and symptoms of sinusitis with the history of laboratory confirmed covid 19 infection less than 4 weeks duration and with CT findings of sinusitis. Tissue biopsies, nasal scrapings and nasal secretions were collected from the patients.

Inclusion Criteria

Patients with signs and symptoms of sinusitis with the history of laboratory confirmed covid-19 infection less than 4 weeks duration and with CT findings of fungal sinusitis were included in this study.

Exclusion Criteria

Patients not willing to participate in the study, patients with precovid immunocompromised conditions like malignancy, HIV, organ transplantation and patients with past history of precovid fungal sinusitis were excluded from this study.

Procedure

Under aseptic precautions, the tissue biopsies and nasal scrapings were collected from the patients. The collected samples were immediately sent to the laboratory in sterile leak proof container. Fungal culture was done as per standard guidelines.

Sample Processing

Direct microscopic examination.

Potassium hydroxide mount (KOH mount).

The tissues were teased in sterile petri dish and 20% KOH was added onto the teased specimen on the slide. The slides were kept in room temperature for overnight to enhance the dissolution of tissue material and to visualize the fungal hyphae clearly. Then it was examined under low power and high power in the light microscope for the presence of fungal elements.^[6]

Fungal Culture

The specimens were inoculated on two tubes of Sabouraud's dextrose agar slants with chloramphenicol and with or without cycloheximide. Then the slants were incubated at 25°C and 37°C for four weeks.^[6]

Interpretation of Fungal Culture

The slants were inspected daily in first week and twice weekly for the next three weeks for growth. The temperature requirement, growth rate and colony characteristics of the fungi were noted.^[6]

Lactophenol Cotton Blue Mount

A small drop of Lactophenol cotton blue stain reagent was placed on a clean and dry slide. A small portion of growth midway between the colony center and edge was taken and carefully teased using a pair of dissecting needles to make a thin preparation. A coverslip was placed on the preparation and observed under the light microscope in low and high power. Identification done based on the colour, type of branching, septation and thickness of hyphae.^[7]

Slide Culture Technique

1cm square blocks from corn meal agar or potato dextrose agar were cut aseptically. The agar blocks were transferred on to a sterile slide. Very small amount of colony was placed over the four sides of the agar block. With sterile forceps, a sterile coverslip was placed on the inoculated agar block. 1-1.5 ml of sterile water was added to the petri dish to produce a humid atmosphere. Then the slide culture petri dish was incubated in dark. When there was a growth observed, the coverslip was taken from the agar block and placed over a drop of LPCB on a sterile glass slide. Then it was focused in light microscope for identification.^[7]

RESULTS

Males were predominantly affected (63%). Patients between 50-60 years predominantly had Post covid fungal sinusitis than other age group. Biopsies taken from 53% of the patients, Nasal scrapings were collected from 23% and Nasal secretions were collected from 23% of patients.

Majority of the patients had Diabetes Mellitus (80%), few of them had Systemic hypertension(20%) and Ischemic Heart Disease(13%). 20% of patients without any comorbidities had Fungal infections [Table 1].

Nasal discharge seen in all patients (100%), Proptosis and vision loss found in 36% and facial pain seen in 26% [Table 2].

Among the Post covid-19 patients who had invasive fungal sinusitis, half of the patients had Diabetes Mellitus(50%) and half of them were stayed in the hospital more than 14 days (50%). Oxygen dependence was observed in (57%) and steroid intake was found in 14% of them [Table 3].

Orbital & nasal extension was seen in (33%), Paranasal sinus extension was seen in (26%) and intra cranial extension was observed in (3%) of the

patients. And also, intracranial extension was seen in the patient who had invasive mucormycosis. [Table 4]

Majority of the patients had Mucor infection (36.6%) and Aspergillus flavus infection was identified in 20% [Table 5].

All proven cases were undergone for endoscopic debridement. They were given Inj.Liposomal amphotericin B along with oral Posaconazole 300 mg for 3-6 weeks. Symptoms improved in 13 patients (43.3%) and 8 patients (26.6%) completely cured.

Table 1: Distribution of Comorbidities in Post Covid-19 Patients (n=30)

Comorbidities	No of patients	Percentage
Diabetes mellitus	24	80
Hypertension	6	20
Ischemic Heart disease	4	13.3
Without comorbidities	6	20

Table 2: Symptoms in Post Covid-19 Patients (n=30)

Symptoms	No of patients	Percentage
Proptosis	11	36.6
Vision loss	11	36.6
Nasal discharge	30	100
Facial pain	8	26.6

Table 3: Risk Factors & Fungal Infection (n=30)

Risk factors	No of patients with fungal infections	Percentage
Diabetes Mellitus(24)	12	50
Oxygen dependent(21)	12	57
Duration of hospital stay>=14 days(22)	11	50
Steroid intake(25)	12	48

Table 4: Severity of Infection (n=30)

Severity	Fungal growth	No of Patients	Percentage
Nasal extension (stage I)	No growth	10	33.3
Para nasal sinus extension(stage II)	Aspergillus flavus	8	26.6
Orbital extension (stage III)	Mucor	10	33.3
Intra cranial extension (stage IV)	Mucor	1	3.3

Table 5: Distribution of Fungal Pathogens among the Patients (n=30)

Fungal isolates	No of patients	Percentage (%)
Mucor	12	36.6
Aspergillus flavus	6	20
No growth	12	36.6

DISCUSSION

Covid-19 is a viral infection caused by SARS-CoV-2. Impaired cell mediated immunity and over expression of inflammatory cytokines in Covid-19 patients lead to secondary fungal infections. Invasive fungal sinusitis is one of the life threatening complications occurring in Post Covid-19 patients. The high risk groups are patients with hematologic malignancies, iatrogenic immunosuppression, acquired immunodeficiency syndrome, uncontrolled diabetes mellitus and those who have undergone organ transplantation.^[2] The present study was done to identify the fungal pathogens and to start prophylactic antifungal therapy to reduce the mortality.

In the present study, Males were predominantly affected(63%). This may be due to the following reasons. Females have higher number of CD4+ T-Cells, increased B cell production of immunoglobulins and increased cytotoxic activity of CD8+T-Cells compared to males. Females also produce more type I interferon than males. Oestradiol augments T-Cell responses and increases the neutrophils, monocyte/macrophage cytokine production in females. Oestradiol also influences ACE2 expression. But in males, testosterone suppresses the immune system.^[8]

Patients between 50-60 years predominantly had Post covid fungal sinusitis than other age group. People in this age group have increased comorbid conditions like hypertension, diabetes, cardiovascular diseases

and obesity which make them more vulnerable for secondary fungal infections.^[9]

In the present study, Biopsies taken from 53% of the patients, Nasal scrapings were collected from 23% and Nasal secretions were collected from 23% of patients. This indicates that majority of the patients underwent FESS.

In our study, majority of the patients had Diabetes Mellitus(80%), few of them had systemic hypertension(20%) and Ischemic Heart Disease (13%). Post covid-19 patients with diabetes Mellitus predominantly had fungal infections than other comorbidities. Several factors responsible for that like older age, proinflammatory, hypercoagulable state, glucose dysregulation and altered immune system.^[10] Similarly, In Vivek Dokania et al study, among 21 patients, Diabetes mellitus(90.48%) was the major underlying disorder.^[5]

In the present study, 20% of patients without any comorbidities had Fungal infections. This is an important aspect to be considered in this study. In contrast, in Vivek et al study, 2 patients (9.55%) without any comorbidities had fungal sinusitis.^[5]

In the present study, Nasal discharge seen in all patients (100%), proptosis and vision loss found in 36% and facial pain seen in 26%. Proptosis occur due to expansile sinusitis, bony orbital erosion. Vision loss occurs due to optic neuritis or retinal artery occlusion or direct compression of the bony optic canal.^[11] In Noha Ahmed El-Kholy et al study, among 36 patients facial pain was observed in 75%, ophthalmoplegia in 66.7% and visual loss in 63.9%. In Vivek Dokania et al study, facial pain was seen in 85.71% and periorbital swelling in 61.90%.

In the present study among the Post covid patients who had invasive fungal sinusitis, half of the patients had Diabetes Mellitus(50%) and half of them were stayed in the hospital more than 14 days(50%). Oxygen dependence was observed in (57%) and steroid intake was found in 14% of them. Prolonged intake of corticosteroids, leads to uncontrolled hyperglycemia and diabetic ketoacidosis in diabetic patients. It also reduces the phagocytic activity of WBC which will make them to get secondary fungal infections.^[3] In severe covid-19 patients, extensive inflammation and injury to the lung tissues occur. This leads to acute respiratory distress syndrome (ARDS). Mechanical ventilation is required for such patients making them vulnerable to get secondary fungal infections.^[3]

In the present study, the patients had orbital & nasal extension(33%), Paranasal sinus extension(26%) and intra cranial extension(3%). And also, intracranial extension was seen in the patient who had invasive mucormycosis. In contrast, in Sandeep Shetty et al study, among the 13 patients, all of them had sino-nasal involvement, none of them had orbital and cerebral involvement. In Noha Ahmed El-Kholy et al study, sinonasal, orbital, cerebral and palatine involvement was observed in 100%, 80.6%, 27.8% and 33.3% of the patients respectively.

Paranasal sinus infection enters the orbit via nasolacrimal duct and medial orbit. Intracranial spread occurs through the cribriform plate or via orbital apex and orbital vessels.^[12]

In our study, majority of the patients had Mucor infection (36.6%) and Asperillus flavus infection was identified in 20%. Similarly, in Noha Ahmed El-Kholy et al observed Mucor in 77.8% Aspergillus species in 30.6%. In contrast, in Sandeep Shetty et al study Aspergillus infection was observed in 77.77% and Mucor infection in 11.11%.

High glucose, free iron, low pH and decreased phagocytic activity of WBCs are the predisposing factors for mucormycosis.^[3] It also elicit the release of danger associated molecular patterns (DAMPs). These DAMPs proteins also enhance SARS cov2 infection causes inflammation and extensive epithelial tissue damage invasive pulmonary aspergillosis among the post covid-19 patients.^[11]

In our study, all proven cases were undergone for endoscopic debridement. They were given Inj.Liposomal amphotericin B along with oral posaconazole 300 mg for 3-6 weeks. Symptoms improved in 13 patients (43.3%) and 8 patients (26.6%) completely cured.

CONCLUSION

Mucor and Aspergillus were the most common fungal pathogens causing Invasive fungal sinusitis in post covid-19 patients. Diabetes mellitus was the predominant comorbidity. Patients without any comorbidities also had invasive fungal infection was an important aspect of this study. Patients with Mucor infection had intra cranial extension. A high suspicion of invasive fungal sinusitis should be kept in mind when post covid patients who are getting steroids and under oxygen dependence. Early diagnosis of Invasive fungal sinusitis in post covid 19 patients will reduce the morbidity and mortality. Survival rate can also be increased by immediate surgical intervention and starting appropriate antifungal therapy.

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