



## Microbiological profile of Post COVID Mucormycosis in various samples

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### Abstract

**Introduction:** Co-infections or Super infections are possible to appear in covid-19 patients and also increase in fungal super infections among post COVID-19 patients created a panic during pandemic times in India. Interpretation of fungal diagnostic report is a quite challenging task for lab doctors. We undertake this study to know the post covid mucormycosis from different clinical scenarios and its epidemiology in this region.

**Materials and Methods:** This study involves a total of 230 patients, who were diagnosed probably as post covid fungal infections. All samples were processed by 10% KOH mount for fungal elements after that subjected to culture on SDA for further confirmation.

**Results:** A total of 113 fungal pathogens were isolated from clinical samples, among them we didn't notify any polymicrobial isolation. Among various fungal pathogens, Rhizopus and Mucor fungi were isolated predominantly, it was 43 (38%) and 40 (35.4%) out of 113 pathogens respectively. These fungi were followed by 11.5% Candida species, 11.5% Aspergillus fumigatus, 1.7% Absidia species, 1.7% Penicillium marneffeii.

**Conclusion:** COVID-19 teaches us in many aspects which need to be followed stringently in health care settings such as adherence to infection control protocols, early diagnosis and management, personal hygiene and cleanliness in community.

**Keywords:** Post Covid, Mucormycosis.

### INTRODUCTION

Severe acute respiratory syndrome corona virus 2 (SARS CoV2) has synonyms such as 2019 novel corona virus (2019 - nCoV) or human coronavirus 2019 (HCoV-19 or hCoV-19), this virus causes COVID-19 disease [1]. SARS CoV-2 is a virus which causes respiratory illness and which is also responsible for COVID-19 pandemic. WHO declared COVID-19 disease as pandemic on 11 March 2020 [2].

After a few weeks of SARS CoV2 infection if the patient had health issues related to post effects of COVID-19 such patients were referred as Post

COVID-19 patients or long haulers and that condition has been called Post COVID-19 conditions.

Most likely to experience lingering COVID-19 symptoms over time in post covid-19 patients including fatigue, SOB, dyspnea, cough, chest pain, joint pain, palpitations, memory or sleep problems, anosmia, dysgeusia, fever, dizziness etc.

Co-infections or Super infections are possible to appear in covid-19 patients. Literature reviews or published articles regarding co-infections and super infections were few [3]. Researches on post covid-19 infections are underway, few studies reported about

8% of COVID-19 patients who were severely ill and those who expired had co-infections and super infections [4].

Increase in fungal super infections among post COVID-19 patients created a panic during pandemic times in India. The 2019 novel corona virus disease causes respiratory symptoms primarily, ranging from mild to severe pneumonia. Mucormycosis has a rise in India among COVID-19 patients, usual presentation as facial pain, dental pain, loss of tooth, proptosis etc., It is a rare opportunistic invasive necrosis and infarction of involved tissues. Risk factors include DM, sinusitis, previous respiratory pathologies, use of corticosteroids, cancer and stem cell transplant [5,6].

Diagnostic modalities available for fungal infections in many tertiary care hospitals. Fungal culture is a gold standard method. For screening KOH or India ink or KOH with LPCB or Calcofluor white stain helps for rapid diagnosis.

Imaging modalities such as MRI and HRCT are valuable modalities that can be used to diagnose fungal infections and to know the extension of fungus and its complication [7].

Interpretation of fungal diagnostic reports is quite a challenging task for microbiologists, pathologists and radiologists because the isolate from clinical specimen could be a commensal or pathogen or colonizer. Correlation of fungal report with clinical picture and radiological findings may help for accurate management of invasive fungal infections.

This hospital is a government tertiary care hospital and also serves as a dedicated COVID hospital since the first wave of COVID-19 pandemic. We have been diagnosing fungal infections in covid-19 patients since the starting of a second wave of covid-19 pandemic. Publishing or projecting the post covid-19 fungal infections data from this region may be helpful for clinicians, epidemiologists and state health authorities to treat, manage and prevent fungal infections in SARS CoV2 infected patients. In the view of community building we have undertaken this study. The aim of this study is to know the post covid mucormycosis from different clinical scenarios and its epidemiology in this region.

#### **Objectives:**

1. To Study the secondary fungal infections in Post COVID patients
2. To Know the Epidemiology of fungal infections in and around Vijayawada

#### **MATERIALS AND METHODS**

**Study Design:** A Retrospective observational study was conducted at Department of Microbiology, Siddhartha Medical College, Vijayawada. This study involves a total of 230 patients, who were diagnosed probably as post covid fungal infections during the study period of May 2021 to June 2021. Informed consent was taken from the study population during the study period.

#### **Inclusion Criteria:**

Fungal infection case confirmed by microbiological investigations and were correlated clinically

All ages of both sexes

Post Covid-19 infected patients

#### **Exclusion Criteria:**

Patients without any history of SARS CoV2

Patients already on antifungal medicine

**Sample Collection:** Tissue biopsy samples after FESS, pus, sputum and nasal swabs were received for microbiological processing from Department of ENT, Siddhartha Medical College, Vijayawada.

SARS CoV2 infection of the study population was confirmed by either of microbiological procedures such as RT-PCR, Truenat PCR, and Rapid antigen test of COVID-19. Tissue bits collected from post covid-19 patients were kept in normal saline and sent immediately to Microbiology Laboratory.

**Sample Processing:** All samples were processed by 10% KOH mount for fungal elements after that were subjected to culture on SDA for further confirmation. Microscopy examination was done and observed for fungal hyphal filaments and other fungal elements. Culture samples were inoculated on two sets of Sabouraud dextrose agar and incubated at 25<sup>0</sup>C and 37<sup>0</sup>C.

Fungal cultures were followed until growth occurs or till 28 days. Positive cultures of fungal growth were identified by macroscopic and microscopic characteristics.

**Data Collection:** All the data collected pertaining to patient details, covid-19 details, presenting symptoms, clinical features, microbiological details and entered into spread excel sheet.

**Statistical data:** Statistical analysis was done using graphpad software. All Descriptive variables were presented as numbers, percentages. Comparative variables were analysed by 2x2 contingency tables and the p value of <0.05 was considered as significant.

## RESULTS

Study on Post covid-19 patients clinical samples to know the epidemiology was conducted and the data analysed. Patients were hailed from various districts of Andhra Pradesh including 172 patients from Krishna district, 8 from Guntur, 42 from West

Godavari, 2 from Kadapa, 2 from Prakasam, 2 from East Godavari and remaining 2 from Nellore district out of 230 patients.

Out of 230 strongly suspected fungal infections of post covid-19 patients various clinical samples showed that majority were males i.e., 160 (70%) patients and remaining 70 (30%) were female patients.

On assessment of age distribution of these suspected fungal infections we observed majority of patients were fallen in the age group of 31-60 years. Youngest age with suspected fungal infection was 12 years and the eldest age was 77 years of age (Table 1).

**Table 1: Age Distribution of Post Covid -19 patients suspected by fungal infections**

Age in Years	No. of patients	Percentage
10-20	1	0.43
21-30	8	3.47
31-0	47	20.43
41-50	85	36.95
51-60	68	29.56
61-70	17	7.39
71-80	4	1.73

Most of the clinical samples observed were tissue bits from nasal cavities or sinuses, it was 73.4% followed by 17.8% of nasal swabs, 6.9% of sputum samples and 1.7% of pus samples from Brain abscess patients (Table 2).

**Table 2: Clinical specimens suspected of fungal infections**

Clinical Specimen	No. of patients	Percentage
Nasal Swabs	41	17.8
Tissue bits from nasal cavities or sinuses	169	73.4
Sputum	16	6.9
Pus from brain abscess	4	1.7

Out of 230 samples, KOH and culture positive were 113 (49.1%), KOH negative and culture positive were 51 (22.1%), KOH positive and culture negative were (1.3%). KOH Vs Culture statistical association shown significance of p value as <0.00001, which is considered as extremely significant and culture as a gold standard test (Table No.3 & Fig 1).

**Table 3: KOH and Culture Statistical association in diagnosing fungi**

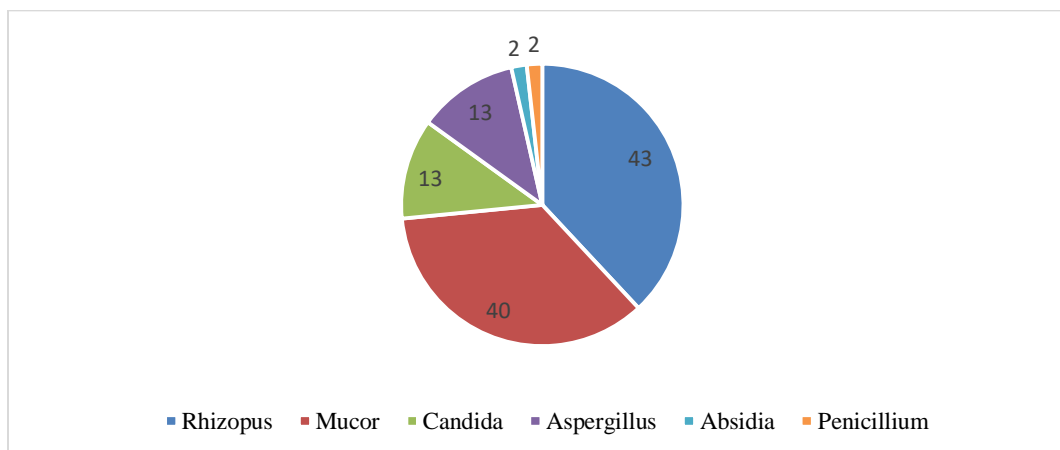
Diagnostic Methods		Culture		Total
		Positive	Negative	
KOH	Positive	113	3	116
	Negative	51	63	114
Total		164	66	230

**Fig 1: Showing Mucormycosis in KOH mount**



A total of 113 fungal pathogens were isolated from clinical samples, among them we didn't notify any polymicrobial isolation. Among various fungal pathogens, Rhizopus and Mucor fungi were isolated predominantly, it was 43 (38%) and 40 (35.4%) out of 113 pathogens respectively. These fungi were followed by 11.5% Candida species, 11.5% Aspergillus fumigatus, 1.7% Absidia, 1.7% Penicillium marneffeii (Fig2, 3, 4, 5).

**Fig 1: Depicting various fungal pathogens in Post covid-19 patients**



**Fig 2: SDA tube showing *Rhizopus arrhizus***



**Fig 3: SDA culture showing *Aspergillus flavus***



**Fig 4: LPCB of fungal culture showing *Rhizopus* with rhizoids and sporangium**



## DISCUSSION

Infections in COVID-19 could be community acquired infections and hospital acquired infections. Hospital acquired infections are those that appear after 48 hours of hospital admission; if the patients acquire infections within 48 hours of hospital admission or in incubation period during the admission comes under community acquired [8].

Many of the concomitant/coinfections in asymptomatic COVID-19 positive need to be addressed especially in patients lung pathologies or respiratory system disorders, clinicians and microbiologists are facing limitations to evaluate whether these are true pathogens or commensals or carriers.

Rhino-orbital mucormycosis involves the paranasal sinuses and orbits may extend into the cerebral parenchyma [5,6].

SARS infection was first identified in 2003 February month in China which spread to 4 other countries. WHO stated that it is a airborne virus which can transmit along the routes of international travel and is noted as the first severe and readily transmissible new disease that emerged in 21st Century. During the years of 2003-2004 SARS infectious period secondary infections due to fungal infections were noted. Few published literature were Zhang Y et al [9] found the incidence of fungal infection in SARS patients was 14.8-2.7%, Yin CH et al [10] reported the fungal incidence of 21.9-33% in severely ill patients. Li CS et al [11] stated that fungal infection was the major cause of death in SARS patients which accounts for 25-73.7% in all cases of deaths. SARS coinfections or super infections have been noticed during outbreaks.

Mucormycosis is a rare fungal condition in developed countries when compared to developing countries prevalence. The prevalence of mucormycosis projected in India may not be so high due to under reporting. Higher prevalence of mucormycosis in India is due to the presence of a large number of mucorales in community and hospitals. Incidence of Mucormycosis in India in various literature gives us an opinion about how it is encroaching into human lives. During 2015-2019, incidence of mucormycosis from Southern India has 9.5 cases per year [12]. Mucormycosis prevalence increases in patients with

underlying diseases and risk factors such as Diabetes, ICU patients, haematological malignancies, solid organ transplants [13], however, it's raising an alarm threat to immunocompetent hosts [14].

Data regarding fungal infections in viral pneumonia or covid-19 patients is limited. Deepak Garg et al [15] reviewed 8 cases of COVID-19 with mucormycosis, reported that all cases affected by mucorales after 10-14 days after hospitalization and one cases was expired which was evident by post mortem report as covid-19 associated mucormycosis.

Mucormycosis manifestations can be in various forms such as gastrointestinal mucormycosis which is more common in young children, rhinocerebral mucormycosis which can be noticed more in uncontrolled diabetes and kidney transplant patients, disseminated mucormycosis, pulmonary mucormycosis more commonly seen in cancer and organ or stem cell transplant patients and cutaneous mucormycosis. Usual mucormycosis fungi appear in patients are: Rhizopus species, Mucor species, Rhizomucor species, Syncephalastrum species, Cunninghamella Bertholletia, Apophysomyces species, and Lichtheimia species [16].

As per this study most of the post covid-19 patients suspected with fungal infections were noted in more than 40 years of age and male predominance was noted. Few case reports observations in this covid-19 pandemic supported our study stating that fungal infections were observed most commonly in elderly age group males [17-19]. Karimi-Galougahi M et al [20] found a invasive type of mucormycosis in a elderly female patient who had a past history of covid-19 treated by remdesivir, interferon alpha and systemic corticosteroid.

Among various fungal pathogens, Rhizopus and Mucor fungi were isolated predominantly, it was 43 (38%) and 40 (35.4%) out of 113 pathogens respectively. These fungi were followed by 11.5% Candida species, 11.5% Aspergillus fumigatus, 1.7% Absidia, 1.7% Penicillium marneffeii in this study. Moorthy A et al [21] conducted a multi-centric study in Bangalore, India on Covid-19 patients with uncontrolled diabetes. Patients had fungal infections including 16 mucormycosis, 1 case of aspergillosis and 1 mixed fungal infection. Six of these patients

were expired. Sen M et al [22] did a study in 2021 who observed 6 rhino-orbital mucormycosis in covid-19 patients, all those patients underwent endoscopic sinus debridement and two among them underwent orbital exenteration followed by antifungal therapy.

A Retrospective study conducted on covid-19 critically ill pneumonia patients in Wuhan, China, found there 3 out of 52 (5.8%) patients had fungal infections including *Aspergillus flavus*, *Aspergillus fumigatus* and *Candida albicans* [23]. A Study from Germany on COVID-19 associated invasive pulmonary aspergillosis was found that 5 out of 19 (26.3%) had fungal cultures positive [24].

A Chinese study [25] reported that 31 out of 1099 (2.8%) patients with suspected fungal infections were empirically treated with antifungal therapy, there was no evidence of fungal co-infection. Few studies have not reported any fungal infections among SARS CoV2 infected patients [26].

## CONCLUSION

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From this study, it has been clear that fungal pathogens do occur in SARS-CoV2 infected patients predominantly affecting males and 31-60 years age group. *Rhizopus* and *Mucor* were most common fungi isolated from various clinical samples. Majority of the samples were tissue bits from nasal cavities and sinuses which reflects that rhino-orbital mucormycosis is a significant manifestation in post covid-19 patients.

COVID-19 teaches us in many aspects which need to be followed stringently in health care settings such as adherence to infection control protocols, early diagnosis and management, personal hygiene and cleanliness in community. Many studies also supported antibiotic therapy, rationale use of reserved antibiotics, infection control recommendations, management of mild, moderate and severe categories of COVID-19 patients. Upgrading and practising expertise suggestions and recommendations helps us to stop the chain of infection.

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