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COVID-19 Associated Fungal infections in Guntur, Andhra Pradesh: A Descriptive Study

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Keywords

Post-COVID-19

Mucormycosis

Risk factors

Fungal species

Copyright

Abstract

Background: Since the onset of the COVID-19 pandemic's second wave in India, there has been an unprecedented rise of life-threatening cases of rhinocerebral mucormycosis (RCM) by various fungi like *Mucor*, *Aspergillus*, *Candida*, and others in post-COVID-19 patients, especially in presence of immunosuppressive conditions due to their invasive potential.

Aim: To assess the incidence of COVID-19 mucormycosis on routine culture methods and various risk factors associated with the second and third wave of COVID-19.

Material and Methods: The descriptive cross-sectional study was conducted from April 1st, 2021 to February 28th 2022, in the department of Microbiology, NRI medical college, and General Hospital, Guntur, Andhra Pradesh, India. The present study included 240 specimens from suspected post-COVID mucormycosis cases. The statistical analysis of data was done using MS Excel 2010.

Results: Out of 240 specimens, 192 (80%) fungal isolates were isolated. Most of the cases are seen in males and the main risk factor was diabetes mellitus (89%). Most of the species isolated were *Mucor* species followed by *Aspergillus* species, *Candida* species, *Rhizopus* species, and *Absidia* species. Antifungal sensitivity of *Candida* species showed good susceptibility to azole discs.

Conclusion: The study concluded that if infected, early diagnosis with culture, strategies to maintain a glycaemic index, and cautious use of corticosteroids, anti-fungal treatment, and surgical intervention should be sought for management, for good prognosis and less fulminant disease course can be achieved in post-COVID rhinocerebral mucormycosis cases.

1 Introduction

A wide range of opportunistic bacterial and fungal infections associated with coronavirus disease 2019 (COVID-19) are caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Order Mucorales which include *Rhizopus*, *Mucor*, *Rhizomucor*, *Cunninghamella*, and *Absidia* are the causative fungi of this angioinvasive disease [2]. In India, its prevalence is assessed to be 140 per million population which is approximately 80 times greater than in developed countries. The mucormycosis incidence rate varies globally from 0.005 to 1.7 per million population [3]. Due to the bizarre fact of the rapidity of dissemination of mucormycosis, even a delay of 12 hours in diagnosis could be fatal. Historically, 50% of mucormycosis cases have been diagnosed only in the post-mortem autopsy series [4]. Mucormycosis was first described by Paultauf in 1885 also called as phycomycosis or zygomycosis [5]. Later, the term mucormycosis was coined by Baker in 1957 an American pathologist for an aggressive infection caused by *Rhizopus* [6]. Zygomycosis is an invasive fungal infection caused by a number of fungi belonging to the class Zygomycetes, which is subdivided into two orders, the Mucorales and the Entomophthorales, which are associated with chronic cutaneous and subcutaneous infections that are almost exclusively limited to the tropics and rarely disseminate to internal organs [7]. Mucormycosis is an uncommon but fatal fungal infection caused by fungus of the order Mucorales that usually affects immunocompromised patients [8]. The most common species isolated was *Candida albicans* (44%) and among non-*albicans Candida* (NAC), *C. auris* (23.2%) and some rare cases of infection by *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* were also reported by some recent studies during COVID-19 period [9]. In the

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current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, fungal co-infections like *Aspergillus* and *Candida* species, among COVID-19 patients in intensive care units (ICUs) were reported [10].

In India and globally, a variety of other complications may emerge in several COVID-19 cases like hypoxia, hyperglycemia in diabetes cases, diabetic ketoacidosis, increased ferritin levels, steroid-induced hyperglycemia, and prolonged hospitalization due to mechanical ventilation including hospital-acquired secondary infections [11]. In India, the most common underlying disease in mucormycosis is uncontrolled diabetes mellitus, in contrast to patients with hematologic malignancies and solid organ transplant recipients in developed countries [12]. India has a very high prevalence rate of type 2 diabetes mellitus. Both COVID-19 infection and diabetes mellitus act as independent risk factors for mucormycosis. Following the rise of COVID-19 associated mucormycosis, the Government of India directive has named “Mucormycosis” a notifiable disease in many states of India on May 10th, 2021 [3].

The present study was done to assess the incidence of COVID-19 associated fungal infections on routine culture methods and various risk factors associated with the second and third wave of COVID-19.

2 Material and Methods

A descriptive cross-sectional study was conducted from the second wave of the pandemic, April 1st, 2021 to the third-wave, February 28th, 2022. A total of 240 specimens, including biopsy of necrotized tissues from paranasal sinuses, followed by hard palate, nasal cavity, sputum, orbital tissue, and brain abscess were obtained from patients admitted in IP departments such as ENT, Ophthalmology, ICU, and COVID-19 ward for the detection of fungal isolates in a Microbiology laboratory at NRI Medical College and General Hospital, Guntur, Andhra Pradesh. Institutional ethics committee approval was taken. The demographic details like age, gender, and clinical diagnosis (including previous positive COVID-19 reports) were recorded from medical records.

The specimens were brought to the laboratory in sterile containers and swabs. The specimens were processed by using a set of preliminary tests like wet mount (KOH Preparation), Gram stain, and culture on SDA and incubated at 25°C for 2 days to 3 weeks. Cultures were identified by the colony characters and confirmed by lactophenol cotton blue mount and Gram’s stain. Speciation of *Candida* species was done by HiChrom *Candida* agar medium (HiMedia, Mumbai, India) and antifungal susceptibility testing of the *Candida* isolates was assessed by agar diffusion method using Clinical Laboratory Standards Institute Guidelines [13]. The antifungal agents used for the disc diffusion method are amphotericin B (100 units), fluconazole (10 µg), voriconazole (1 µg), ketoconazole (10 µg), and itraconazole (10 µg). The diameters of the zones of inhibition obtained were compared with the standard zones interpretive breakpoints published by CLSI M44-A guidelines [13] with antifungal discs.

Statistical analysis (Mean, median, and percentages of continuous variables) was done using MS Excel 2010.

3 Results and Discussion

During the study period, data were recorded on patients profile such as age, sex, site of isolation, underlying risk factors along with different *Zygomucor* species, *Candida* isolated and their antifungal susceptibility pattern. Out of 240 specimens, a total of 192 (80%), fungal isolates were isolated in the present study. No specimen was received during the third wave. The highest incidence was seen in the age group of 51-60 years (26%), and the least incidence was seen in the age group of 11-20 years (1.5%). The majority of patients are males (Table-1). Male: Female ratio is 3: 1. The mean age is 51.17. In this study, most of the specimens were from paranasal sinuses (80%), followed by hard palate (8%), nasal cavity (5%), sputum (4%), orbital tissue (2%), and brain abscess (1%).

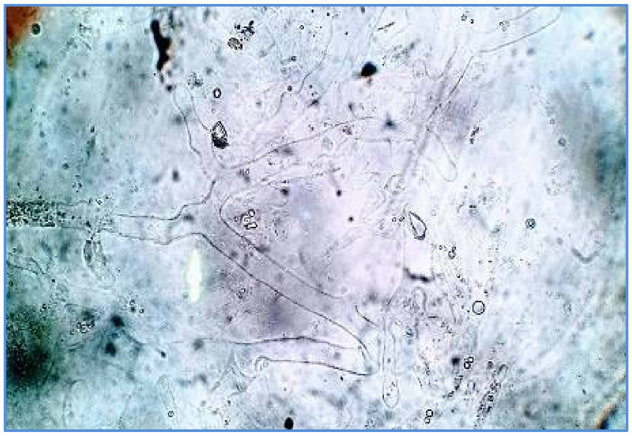
In the present study, patients with COVID-19, diabetes mellitus (n = 171; 89%) was found to be the major risk factor followed by, prolonged hospital stay with steroid therapy (n = 135; 70%), age (n = 112; 58.3%), oxygen support (n = 100; 52%), and other comorbidities like increased antibiotic use (n = 69; 36%), hypertension (n = 88; 46%), coronary artery disease (n = 78; 40%), chronic kidney diseases (n = 38; 20%), and organ transplant (n = 2; 1%).

Table 1: Age and Gender wise Distribution of COVID Associated Rhino orbital cerebral mucormycosis.

Age (years)	Female (%)	Male (%)	Total (%)
11-20	2 (1.3%)	1 (2%)	3 (1.5%)
21-30	4 (2.7%)	1 (2%)	5 (2.6%)
31-40	27 (18.7%)	14 (29.1%)	41(21.3%)
41-50	30 (21%)	9 (18.7%)	39 (20.3%)
51-60	40 (28%)	10 (20.8%)	50 (26%)
61-70	29 (20.1%)	9 (18.7%)	38 (19.7%)
71-80	12 (8.3%)	4 (8.3%)	16 (8.3%)
TOTAL	**48 (25%)**	**144 (75%)**	**192 (100%)**

The microbiological analysis of tissue specimens showed fungal hyphae treated with 10% potassium hydroxide (Fig-1) and Gram stain shows Gram-positive budding yeast cells with pseudohyphae. Culture on SDA was incubated at 25°C, and *Zygomycota* species were observed to grow rapidly in 1–2 days, especially in samples of post steroidal usage and diabetes mellitus. Fungal growth was identified on SDA. Then lactophenol cotton blue mount is used to confirm the colony morphology, septation in hyphae, vesicles, conidia, sporangia, and sporangiophores (Fig-2).

Figure 1: KOH Mount showing broad aseptate hyaline hyphae with wide-angle branching.



In the present study, out of 192 culture-positive fungal isolates only 84.3% of fungal hyphae are reported on KOH Mount (Table-2). Most of the species isolated are *Mucor* species (56.7%) followed by *Aspergillus* species (18.6%), *Candida* species (9.9%), *Rhizopus* species (9.3%), and *Absidia* species (5.2%). Most of the *Candida* species isolated on HiChrom agar are non-*albicans Candida* (NAC) i.e., *C. tropicalis*, *C. krusei*, *C. kefyr*, *C. albicans*, and *C. dubliniensis*. Antifungal sensitivity of *Candida* species showed good sensitivity to the azole group of drugs, as shown in Table-3, which is being rampantly being used in post-COVID mucormycosis cases.

During the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, a higher incidence of fungal co-infections was reported among COVID-19 patients in intensive care units (ICUs) [10]. During the second wave of COVID-19, 192 fungal species were isolated out of 240 specimens received in the Microbiology laboratory and no specimen was received after the onset of the third wave. The study findings showed that the majority of patients were male and their mean age was 51.7 years. These demographic details of patients were similar to those of a previous study which studied a population of 82 patients of which two-thirds were male and aged between 31–60 years [14], and a similar study reported mean age of 54.4 years and male preponderance [15].

Figure 2: LPCB mount showing broad aseptate hyaline hyphae with rhizoids and sporangiophores arise and then end with sporangium filled with numerous sporangiospores.

In the present study, diabetes mellitus was the main risk factor seen in 89% of patients, which is consistent with previous studies indicating that diabetes mellitus is a predisposing factor in 17–88% of cases globally and in India [11,15]. Some case series described diabetes as a main risk factor in 90% of cases of which 52% had the uncontrolled disease [16,17].

Table 2: Distribution of different fungal species isolated from COVID-associated mucormycosis specimens.

Fungal Species	Total-n (%)	KOH Mount Positive-n (%)	Culture Positive-n (%)
***Mucor* spp.**	109 (56.7%)	90 (82.5%)	109 (100%)
Aspergillus flavus	22 (11.4%)	16 (73%)	22 (100%)
***Candida* spp.**	19 (9.9%)	19 (100%)	19 (100%)
***Rhizopus* spp.**	18 (9.3%)	15 (83.3%)	18 (100%)
***Absidia* spp.**	10 (5.2%)	10 (100%)	10 (100%)
Aspergillus niger	08 (4.1%)	06 (75%)	8 (100%)
Aspergillus fumigatus	06 (3.1%)	06 (100%)	6 (100%)
Total Species	**192**	**162 (84.3%)**	**192 (100%)**

Table 3: Distribution of Candida species and their Antifungal sensitivity.

Species of Candida isolated (n=19)	Amphotericin B (100 Units)		Ketoconazole (1 μg)		Itraconazole (10 μg)		Fluconazole (10 μg)		Voriconazole (1 μg)	
	S	R	S	R	S	R	S	R	S	R
***C. tropicalis* (12)**	5 (41.6%)	7 (58.4%)	9 (75%)	3 (25%)	9 (75%)	3 (25%)	10 (83.3%)	2 (16.7%)	8 (66.6%)	4 (33.4%)
***C. albicans* (04)**	3 (75%)	1 (25%)	3 (75%)	1 (25%)	4 (100%)	0	4 (100%)	0	4 (100%)	0
***C. dubliniensis* (1)**	0	1 (100%)	0	1 (100%)	1 (100%)	0	1 (100%)	0	0	1 (100%)
***C. krusei* (01)**	1 (100%)	0	1 (100%)	0	1 (100%)	0	1 (100%)	0	1 (100%)	0
***C. kefyr* (01)**	1 (100%)	0	1 (100%)	0	1 (100%)	0	1 (100%)	0	1 (100%)	0
Total	10 (52.6%)	9 (47.3%)	14 (73.6%)	5 (26.3%)	16 (84.2%)	3 (15.7%)	17 (89.4%)	2 (10.5%)	14 (73.6%)	5 (26.3%)

S-Sensitive; R-Resistant

During the second wave most of the patients enrolled in this study received corticosteroid therapy (70%), which on prolonged use lead to opportunistic mucormycosis cases. Our findings are consistent with a recent systematic review of mucormycosis cases in India and worldwide, which found that corticosteroids were used in 76.3% of cases, which resulted in 30% of fatal opportunistic mucormycosis cases [18,19]. Other factors like unhygienic oxygen therapy 52%, indiscriminate antibiotic use 36%, and COVID-19 itself may have contributed to the crisis.

In developing countries including India, diagnoses of mucormycosis-causing pathogens are often identified by phenotypic characteristics such as growth rate, colony morphology, and reproductive structures [20]. Rhinocerebral mucormycosis is an angioinvasive disease-causing tissue infection [21]. In this study, the most common species isolated were *Mucor* species (56.7%), *Aspergillus flavus* (11.4%), *A. niger* (4.1%), *A. fumigatus* (3.1%), *Candida* species (9.9%), *Rhizopus* sp. (9.3%) and *Absidia* (5.2%). In the COVID-19 pandemic, a study reported *Mucor* and *Rhizopus* species as the main causative agent of this disease [22]. Similarly in another study, invasive *Aspergillus* species is the main causative agent for mucormycosis [23], while in our study it was only 18.6%.

Among 19 *Candida* species isolated, only 4 (21%) are *C. albicans* and most of them are non-*albicans Candida* species (79%) such as *C. tropicalis* (n = 12; 63%), one (5.2%) each of *C. dubliniensis*, *C. kefyr*, and *C. krusei* are the most prevalent fungal isolates. These findings of our study are in agreement with a study carried out by Jayant et al. [24]. Antifungal sensitivity pattern of *Candida* species showed good sensitivity to azole drugs i.e.; 73-89% and sensitivity to amphotericin B was 52.6% in most of the *Candida* species. This finding is contrary to previous studies [25].

4 Conclusion

India had grappled with the COVID-19 pandemic and also reported increasing instances of COVID-19 associated rhinocerebral mucormycosis in the second wave. But in the third wave, strategies to maintain glycaemic index and cautious use of corticosteroids and broad-spectrum antibiotics are monitored in mild COVID cases resulting in no cases of mucormycosis. If infected, early diagnosis with culture, strategies to maintain a glycaemic index, judicious use of corticosteroids, anti-fungal treatment, and surgical intervention should be sought for management, as a good prognosis and less fulminant disease course can be achieved in cases of post-COVID mucormycosis.

Conflict of interest

The authors declare no conflict of interest.

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