

Study Of Mucormycosis In Covid And Post Covid Patients From A Tertiary Care Hospital In Central India.

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Abstract

Introduction: The pandemic corona virus disease 2019(covid19) continues to be a significant problem worldwide. The opportunistic fungal infections particularly mucormycosis in the shadow of poorly controlled diabetes mellitus and inadvertent use of corticosteroids are emerging as matter of concern in covid19 patients. As there are very few studies available on the recent epidemic of mucormycosis, the study on epidemiology and mycological aspect of mucormycosis in patients infected with covid19 infection was undertaken. **Objective:** To isolate and identify different fungi causing mucormycosis in covid and post covid patients and to study the risk factors associated with post-covid mucormycosis. **Material and Methods:** Relevant clinical samples from cases of mucormycosis were received in mycology section of department of microbiology were processed by standard conventional methods. **Results:** Out of total 634 samples received, majority samples were from males with male: female ratio of 2.2:1. Various risk factors associated with covid19 infections and mucormycosis were diabetes mellitus in 72%, inadvertent use of corticosteroids in 68%, hypertension in 62% and respiratory illnesses in 45% cases. Out of total 634 samples, 154(24.29%) were culture positive. Of these, *Mucor* was grown in 29.87% cases followed by *Aspergillus* in 23.37% and *Candida* in 25.97% cases. We also isolated *Rhizopus spp.* (10.38%), *Schizophyllum spp.* (6.49%) and *Fusarium spp.* (3.89%). **Conclusion:** This study helped us to know about various fungi and risk factors associated with mucormycosis in covid and post-covid patients. The timely diagnosis and high index of suspicion is required to manage and treat mucormycosis in post-covid patients.

Key words: Covid19, Post-covid, Mucormycosis, Risk factors

Introduction

Coronavirus disease 2019(Covid19) caused by SARS-Cov2 has been associated with wide range of opportunistic bacterial and fungal infections [1]. Recently, several cases of mucormycosis in people with covid19 have been increasingly reported worldwide particularly from India. The primary reason that appears to be facilitating mucorales spores to germinate in people with covid19 in an ideal environment of low oxygen, high glucose, steroid induced hyperglycemia, diabetic ketoacidosis, high ferritin level and decreased phagocytic activity of white blood cells due to immunosuppression due to SARS-Cov2 coupled with several other risk factors. Mucormycosis is an angioinvasive disease caused by mold fungi of the class Zygomycetes [2]. The most common mode of transmission occurs through the inhalation of fungal spores. Mucormycosis usually develops 10-14 days after hospitalization [3]. Globally the prevalence of mucormycosis varied from 0.005 to 1.7 per million population, while its prevalence is nearly 80 times higher (0.14 per 1000) in India compared to developing countries, in a recent estimate of year 2019-2020 [4,5]. Hence covid associated mucormycosis should be considered among the differential diagnosis of coinfections in patients with covid19. A prompt diagnosis and treatment should be established because of the angioinvasive character and rapid disease progression leading to high mortality [6,7]. Hence the present study was conducted to isolate and identify different fungi causing mucormycosis in covid and post-covid patients and to study the risk factors associated with mucormycosis.

Material and methods

This is a single centre observational study conducted from May, 2021 to August, 2021 in Department of Microbiology of a tertiary care hospital in central India. A total of 634 samples of patients suspected to have mucormycosis were included in this study. Nasal scrapping, nasal swab, sputum, nasopharyngeal swab, maxillary and frontal sinus swab, necrotic tissue, palatal swab, abscess pus, buccal mucosa, debrided tissue, orbital sample, oral swab, nasal discharge, ear swab, etc. were processed in the mycology section. All the specimens were subjected to 20% potassium hydroxide (KOH) and examined for the presence of fungal hyphae and spores. Two Sabouraud's Dextrose Agar (SDA) slopes with and without cycloheximide and chloramphenicol were used for culture of specimens and incubated at temperatures of 25°C and 37°C for 3 weeks for isolation of moulds and yeasts. Fungal growth was identified based on colony morphology, pigmentation, growth rate, microscopy using Lactophenol cotton blue mount (LPCB), slide culture on corn meal agar as per conventional techniques [8].

Results

In our study, out of 634 samples received from suspected mucormycosis, males outnumbered females with the male to female ratio of 2.25:1. The commonest age group affected was 41-50 years followed by 51-60 years. (Table 1) In our study, 72% cases of mucormycosis were diabetic, 68% patients were on corticosteroids, 62% patients were with hypertension and 45% patients with other respiratory illnesses. (Table 2) Out of 634 samples, 167 (26.34%) were positive by direct microscopy (KOH mount) and 154 (24.29%) were culture positive. (Table 3) The most common isolate was *Mucor spp.* 46 (29.87%) followed by *Candida spp.* 40 (25.97%),

Aspergillus spp. 36 (23.37%) and *Rhizopus spp.* 16 (10.38%). We also isolated *Schizophyllum spp.* 10 (6.49%) and *Fusarium spp.* 6 (3.89%). (Table 4) We isolated *Aspergillus spp.* in 23.37% cases, of which *A. flavus* were 38.88% and *A. fumigatus* were 30.55%. Other species of *Aspergillus* like *A. terreus* (8.3%), *A. nidulans* (5.55%), *A. glaucus* (2.77%) were also isolated in our study. (Chart1) In our study, *Candida spp.* were isolated in 25.97% cases of which *C. albicans* were 60%, *C. glabrata* were 15%, *C. tropicalis* were 15%, *C. parapsilosis* were 5% and *C. krusei* were 5% cases. (Chart2)

Table No. 1 Age and Gender wise distribution of Mucormycosis cases (n=634)

Age	Male	Female	Total No of cases
21-30	20	12	32
31-40	58	30	88
41-50	150	68	218
51-60	136	61	197
61-70	60	21	81
>70	15	03	18
Total	439	195	634

Table No. 2. Risk factors associated with cases of mucormycosis (n=634)

Risk Factor	No. of patients	%
Diabetes mellitus	456	72
Corticosteroids	431	68
Hypertension	330	62
Respiratory illnesses(COPD,Asthma)	286	45

Table No. 3 Results of microscopy and culture in cases of Mucormycosis (n=634)

	KOH +	KOH-	Total
Culture +	77	77	154
Culture -	90	390	480
Total	167	467	634

Table No. 4 Fungal species isolated from cases of mucormycosis (n=154)

Fungi	Total	Percentage (%)
<i>Mucor spp.</i>	46	29.87
<i>Aspergillus spp.</i>	36	23.37
<i>Candida spp.</i>	40	25.97
<i>Rhizopus spp.</i>	16	10.38
<i>Schizophyllum spp.</i>	10	6.49
<i>Fusarium</i>	06	3.89

Chart 1 ; Distribution of Aspergillus species isolated from cases of mucormycosis (n=46)

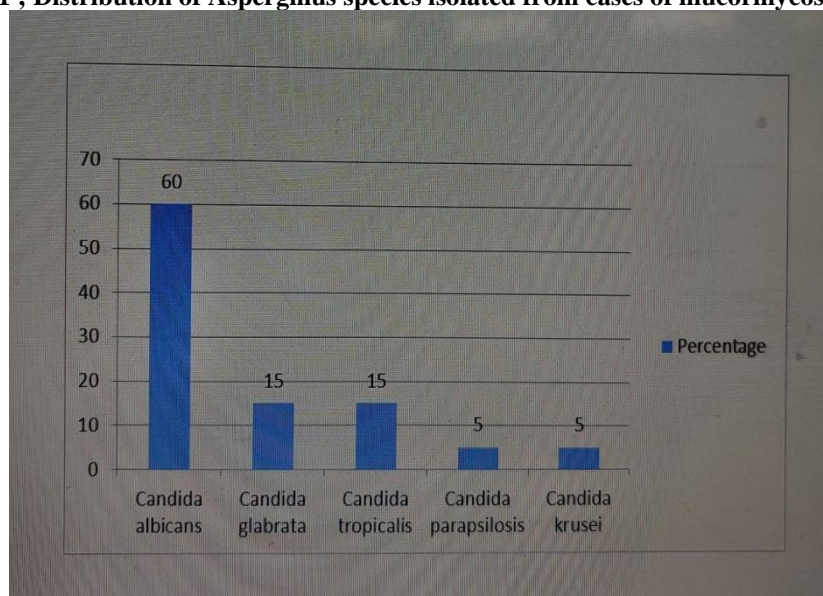


Chart 2 ; Candida species isolated from cases of mucormycosis (n=40)

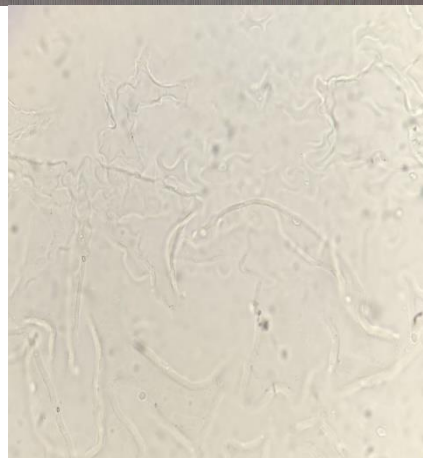
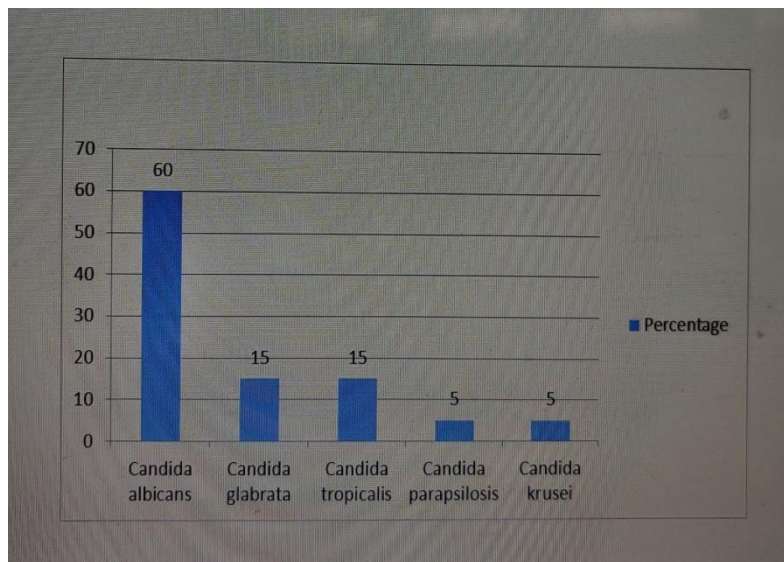


Fig 1: Nonseptate hyphae on direct Microscopy

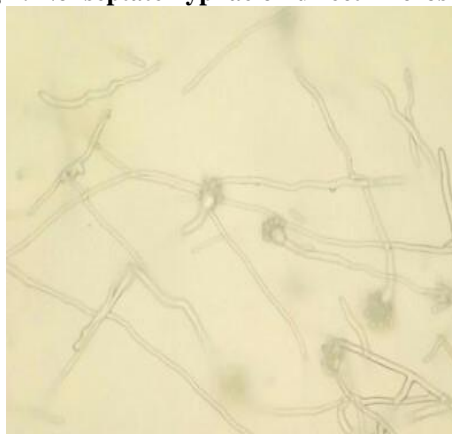


Fig 2: *Aspergillus fumigatus*



Fig 3: *Rhizopus* spp.



Fig4: *Schizophyllum* spp.

Discussion

The pandemic coronavirus disease 2019 (Covid19) continues to be a significant problem worldwide. India with over 20000 cases of covid associated mucormycosis remains the hardest hit country in the world [9]. Covid19 associated mucormycosis has been observed mainly in people with underlying diseases such as diabetes mellitus and on steroids [10,11]

In our study, out of 634 samples received from patients of mucormycosis, males outnumbered females with the male:female ratio of 2.25:1. The commonest age group affected was 41-50 years. In a study by A.Ramaswami *et al* from India, majority of the patients were middle aged 33-55 yrs, of which 2/3rd were males [12]. It has been hypothesized that the effect of oestrogen might be protective in systemic fungal infection which could have led to lower incidence in females [13]. In a study by Singh *et al*, mucormycosis was predominantly seen in males [1].

Many experts believe that the combination of high dose steroids and uncontrolled diabetes has led to this epidemic of mucormycosis in covid19 patients [14,15]. A systematic review of 101 cases of mucormycosis in covid19 by Singh *et al* noted that more than 80% cases had either preexisting or new onset hyperglycemia as a risk factor. Similarly, in our study, 72% cases of mucormycosis were diabetic. In a retrospective multicentric study conducted in India between September-December 2020, it was found that among 187 cases had covid associated mucormycosis of which, 78.7% were treated with glucocorticoids for covid19 and 60.4% had diabetes mellitus [16]. In our study, we also found that 62% patients with hypertension and 45% patients with other respiratory illnesses.

Other studies have demonstrated a notable rise in culture positivity from 72% to 89% [17]. Out of 154 culture positive samples, 46 (29.87%) were *Mucor* spp., 40 (25.97%) were *Candida* spp., 36 (23.37%) were *Aspergillus* spp. followed by 16 (10.38%) *Rhizopus* spp., 10 (6.49%) *Schizophyllum* spp. and 6 (3.89%) *Fusarium* spp. Most of the published cases identified *Rhizopus* as the most frequent species isolated, however, in our study, the most common isolate was *Mucor* spp. (29.87%) and we could isolate and identify 16 (10.38%) cases of *Rhizopus* [18,19]

Despite a lack of systematic screening for invasive aspergillosis, *Aspergillus flavus* or *Aspergillus fumigatus* is reported in 4% of patients with severe SARS Cov2 infections in Chinese patients [20, 21]. Another report from France similarly found *Aspergillus flavus* infection in patients with SARS Cov2 [22]. In our study, we found *Aspergillus* species in 23.37% cases, of which *A.flavus* in 38.88% and *A.fumigatus* in 30.55% cases with covid19 infection. Other species of *Aspergillus* like *A.terreus* (8.3%), *A.nidulans* (5.55%) and *A.glaucus* (2.77%) were also isolated in our study. More recently probable aspergillosis was diagnosed with 9 out of

27(33%) patients requiring mechanical ventilations using screening protocols aimed at early detection [23] These findings suggest that aspergillosis may be an important yet under recognized complication of SARSCov2 infection.

In our study, *Candida spp.* were isolated in 25.97% cases of which *C.albicans* (60%), *C.glabrata*(15%),*C.tropicalis*(15%),*C.parapsilosis*(5%) and *C.krusei*(5%) were isolated.Salehi *et al* also found coinfecting covid19 patients with *C.albicans*(70.7%),*C.glabrata* (10.7%),*C.dubliniensis* (9.2%),*C.parapsilosis*(4.6%),*C.tropicalis*(3%)and *C.krusei*(1.5%)in 53 hospitalised covid19 patients[24] .A retrospective study published in the Lancet also confirmed coinfection of *A.flavus*,*C.glabrata*and*C.albicans* in covid 19 patients[25] .

Conclusion

This study helped us to know about various fungi and risk factors associated with mucormycosis in covid and post-covid patients. In our study, not only *Mucor* and *Rhizopus*, but various other fungi like *Aspergillus*,*Candida*,*Schizophyllum* and *Fusarium spp.* were isolated and identified. Our purpose in this study was to assist clinician in the management and treatment of fungal coinfections in covid19 patients.Very few studies are available on mucormycosis in covid19 and no standard protocols have been developed and differentiating features have not been classified till date that can demonstrate the severity of SARSCov2 and mucormycosis.

Conflict of Interest

None

Acknowledgement

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