

A case series of Covid-19-associated fungal infections: The gruesome twosome

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ABSTRACT

Severe Acute Respiratory Syndrome Coronavirus-2(SARS-CoV-2) is the novel coronavirus responsible for causing Coronavirus disease (COVID-19). The pandemic has seen many ebbs and flows since its detection, particularly regarding various manifestations coupled with it. Secondary fungal infections, especially COVID-19-associated mucormycosis, have seen a summit, especially during the second wave of the pandemic. However, we reported a series of three cases of mucormycosis in association with another fungal mould from the sample received from patients infected with this virus that presented to our hospital during this pandemic. Our report is about the novelty of dual fungal infection caused by a *Zygomycete* group along with *Aspergillus* isolated from pulmonary as well as extra-pulmonary sites, all of which were confirmed on culture. Timely and multidisciplinary management of these patients helped patient survival.

Key words: *Aspergillus*, COVID-19, Mucormycosis, *Rhizopus*, *Zygomycete*

Coronavirus disease 2019 (COVID-19) has proven to be a medical malady globally. The diverse array of its presentation ranges from mild flu-like symptoms to serious conditions like pneumonia with multiorgan involvement [1]. Fungal infections have taken hold during the second wave of COVID-19 infection. Commonly associated fungal infections with it have been that of bloodstream and oropharynx by *Candida* and pneumonia by *Pneumocystis jirovecii* and *Aspergillus*. Unusual cases of rhino-orbital mucormycosis have also been reported [2]. Mucormycosis or Zygomycosis is a lethal opportunistic infection caused by a filamentous fungus of the order Mucorales of which *Rhizopus* and *Mucor* are the most common pathogens [2]. In India, the prevalence of COVID-19-associated mucormycosis was 80 times higher as compared to other developed nations worldwide. Around 71% of global cases of mucormycosis have been reported from India alone, Maharashtra being the second most common state to do so [3]. This could be attributed partly to the immunocompromised status caused by the disease, partly to associated patient comorbidities, and the rest to the hot and humid tropical climate. The omnipresent fungal spores in the humid climate get a grip in the warm and humid environment of the nose and paranasal sinus [2]. A case of mucormycosis is defined as one in which clinical and radiological features were consistent with


mucormycosis and fungus was demonstrated in the sample by KOH mount or culture or histopathological examination. There are various forms of presentation of mucormycosis such as rhinonasal, rhino-orbital, rhino-orbital-cerebral, pulmonary, cutaneous, gastrointestinal, and disseminated [3].

We report a series of three cases of COVID-19-associated mucormycosis during the period of May 2021–February 2022, one each of pulmonary, rhinonasal, and rhino-orbital type, in association with *Aspergillus flavus*. This unusual fact that all three cases were caused by a duo of fungi rather than Mucorales alone is yet another example of the unusual presentation associated with this pandemic.

CASE SERIES

Case 1

A 42-year-old male patient presented with expectorating cough since 5 days. He is a known case of juvenile diabetes mellitus on insulin. There were no other complaints. He was detected COVID-19 positive 3 weeks back but was not hospitalized for the same. On current visit, the sputum sample showed scanty acid-fast bacilli by Ziehl–Neelsen stain. GeneXpert/MTB Rif was also positive for the *Mycobacterium tuberculosis complex*. A high-resolution computed tomography scan of the chest revealed consolidation along with cavitation in the left lung. Other investigations were normal. His sputum was received for

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potassium hydroxide (KOH) mount and fungal culture. Gram stain of the sample showed a significant number of pus cells. KOH mount did not show fungal elements. After 24 h, growth on Sabouraud dextrose agar (SDA) and SDA with chloramphenicol and gentamicin without cycloheximide (SDAA) was green velvety with slight gray-colored growth over it. After 48 h, the growth increased in size and became black, dense, and woolly (Fig. 1). Lactophenol cotton blue (LPCB) mount from the growth showed septate branching hyphae with long conidiophores and phialides covering the entire vesicle pointing out in all directions and few broad aseptate hyphae without rhizoids and without distinct columella (Fig. 1). Slide culture from the growth also showed the same findings. Hence, growth was reported as *Mucor* species and *A. flavus*. He was given anti-tuberculosis treatment along with antifungal treatment to which he responded. There was no recurrence of symptoms till 6 months.

Case 2

A 76-year-old male presented with nasal blockage and bilateral nasal crusting for 10 days. There were no other complaints. He was detected COVID-19 positive 5 weeks back for which he was hospitalized for a month and was on oxygen support. He was also given methylprednisolone and remdesivir during that period. He is a known case of diabetes mellitus and hypertension for 15 years and ischemic heart disease for 5 years and is on medication. On examination, left infraorbital minimal puffiness was present. There was no other significant finding. The right nasal cavity had a dry hard crust and the left side had a greenish crust. Radiological findings suggested right maxillary, ethmoidal, and frontal sinus involvement. Crust from both nasal cavities was sent for KOH mount and fungal culture. KOH mount showed few broad aseptate hyphae. Gram stain showed a moderate number of pus cells. After 24 h, growth on both plates was green velvety with slight gray-colored growth over it. After 48 h, growth increased in size and became black dark grayish-brown, covering the entire plate (Fig. 2). LPCB mount from the growth showed septate branching hyphae with long conidiophores and phialides covering the entire vesicle pointing out in all directions, a few broad aseptate hyphae without rhizoids and without distinct columella, and a few brown-colored aseptate hyphae with rhizoids directly beneath the sporangiophores and ovoid columella (Fig. 2). Slide culture from the growth also showed the same findings. Hence, it was reported as *Mucor* species plus *Rhizopus* species and *A. flavus*. Surgical debridement was done along with antifungal treatment to which he responded. There was no recurrence of symptoms till 6 months

Case 3

A 57-year-old male presented with pain, redness, and watering of the left eye for 2 days. There were no other complaints. He is a known case of diabetes mellitus, hypertension, and hypothyroidism on medication. He was detected as COVID-19 positive 2 weeks back for which he was hospitalized for

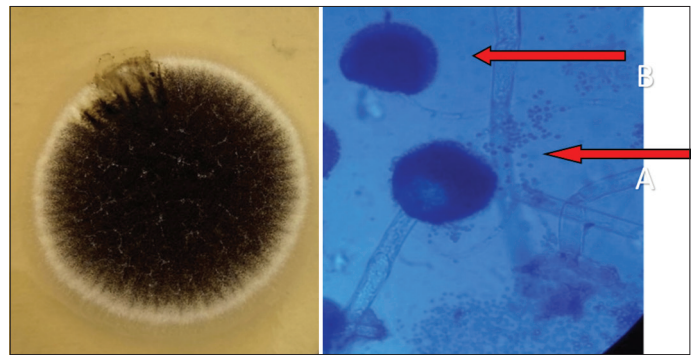


Figure 1: Growth on SDA and LPCB mount from growth showing *Mucor* (A) and *Aspergillus flavus* (B) ($\times 40$)

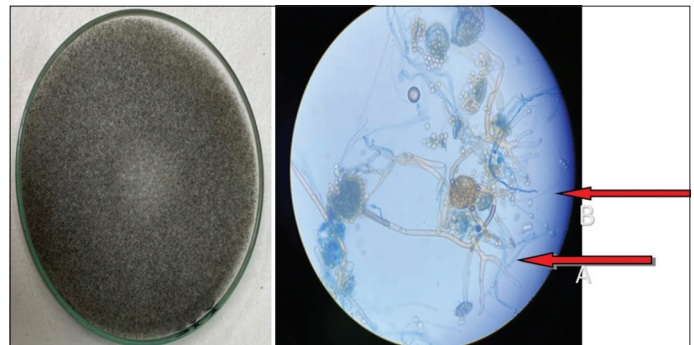


Figure 2: Growth on SDA and LPCB mount showing *Rhizopus* with rhizoids (A) and septate hyphae of *Aspergillus* (B) ($\times 40$)

10 days and given remdesivir, methylprednisolone, high-flow oxygen, and monoclonal antibody. The left eye was congested with upper eyelid edema. There was tenderness over the left frontal, ethmoidal, and maxillary sinus area. There was no other significant finding. Radiological findings suggested left maxillary and ethmoidal sinus and orbit involvement. Tissue from the left nasal cavity showed pus cells on Gram stain. KOH mount showed a few brown broad aseptate hyphae with ovoid columella and root-like structures (rhizoids) just below the sporangiophore (Fig. 3). After 24 h, growth on both plates was green velvety with slight gray-colored growth over it. After 48 h, growth increased in size and became black dark grayish-brown, covering the entire plate (Fig. 2). LPCB mount from the growth showed septate branching hyphae with long conidiophores and phialides covering entire the vesicle pointing out in all directions and a few brown-colored aseptate hyphae with rhizoids directly beneath the sporangiophores and ovoid columella (Fig. 2). Slide culture from the growth also showed the same findings. Hence, it was reported as *Rhizopus* species and *A. flavus*. Histopathological examination showed aseptate hyphae. Surgical debridement was done along with antifungal treatment to which he responded. There was no recurrence of symptoms till 3 months.

DISCUSSION

The increase in COVID-19-associated mucormycosis during the second wave has acted as fuel to the fire. This could be attributed to the complex interplay of environmental factors, etiological agents, and host factors. The tropical weather leading to a hot and

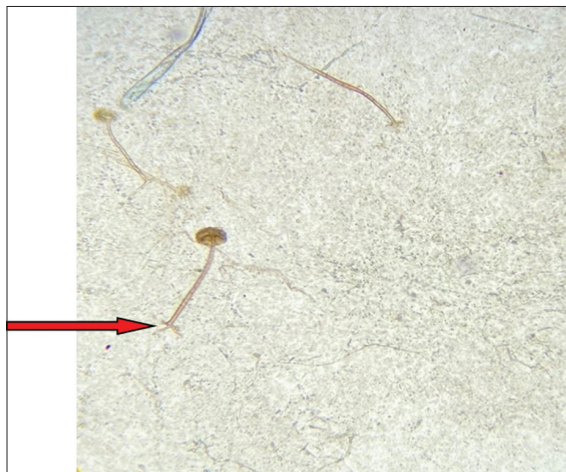


Figure 3: KOH mount from sample showing *Rhizopus* (×10)

humid climate provides a suitable environment for the growth of *Mucor* spores. *Mucor* is otherwise a saprophytic fungus with its spores omnipresent in the environment including nasal mucosa. These germinate inside the human body in the immunosuppressed and may spread to adnexa from the nose [4,5].

The chances of developing secondary fungal infections are increased in post-COVID-19 patients due to various reasons that cause an immunosuppressed state in the body [4]. This situation worsens in patients with comorbidities, especially diabetics due to metabolic events that occur in the body [5]. Diabetes mellitus is the most common predisposing factor for *Zygomycete* infection [4,6,7]. All three patients were known cases of long-standing diabetes mellitus and were infected with COVID-19 days before they presented with fungal infection. The presence of pus cells in all samples was suggestive of infection. The patients who require oxygen therapy and steroids for COVID-19 treatment are also potential candidates for fungal infection [4]. There is thrombo-inflammation and microvascular coagulation which provide a good media for intravascular growth, especially angioinvasive fungus like *Mucor* [4]. Two patients had a history of taking steroids and oxygen support for the treatment of COVID-19. The average period between COVID-19 infection and fungal infection is 3–42 days [8]. Our patients presented 21, 35, and 14 days after infection with this virus, respectively, during the second wave of the pandemic.

Among the *Zygomycete* group, *Rhizopus* followed by *Mucor* is the most common cause of mucormycosis. *Rhizopus arrhizus* is the most common species of this genus [9]. Among our cases, one sample grew *Mucor* and another *Rhizopus*. The third grew *Rhizopus* along with *Mucor*. The most striking finding of our cases was the growth of *A. flavus* along with the *Zygomycete* group. Among the COVID-19 patients who presented with a fungal infection, 5% were found to be infected with *Aspergillus* and 7% with *Mucor* [4]. However, reported cases of the association of these two fungi in the same sample are sparse. Invasive aspergillosis involving the lungs in such patients is known but the involvement of paranasal sinuses and orbit by it is scarce. *Aspergillus* species are ubiquitously present

in the environment and are opportunists [10]. Although KOH was negative in the first two cases and positive only in the third case, growth was seen in 48 h in all. Negative KOH does not rule out fungal infection and confirmation is by culture [9]. Successful management of mucormycosis includes clinician awareness, prompt microbiological diagnosis, and early initiation of medical and surgical treatment. Medical and surgical management include antifungals and debridement, respectively [4]. Our patients were also managed likewise. They responded to it and there was no recurrence till 3–6 months.

CONCLUSION

This article is a sneak into three cases of COVID-19-associated fungal infections reported in our institute during the second wave of the pandemic. The fact that makes our cases stand apart from other such reported cases is the combo of infection consisting of mucormycosis caused by *Zygomycete fungus* along with *Aspergillus flavus*. Their number was definitely higher in the above-mentioned period than before. All three were confirmed on culture and were known diabetics. Their successful management can be attributed to the clinician's high index of suspicion, on-the-dot laboratory diagnosis, and collaborative treatment including blood sugar level control.

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