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Original Article

A Retrospective Study of Mucormycosis, Aspergillosis, and Candidiasis Coinfection with COVID-19 Positive Patients in Tertiary care Hospital

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Abstract

Introduction: Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), been sweeping across the globe. In the era of COVID-19 pandemic, the ever-increasing use of immunosuppressive drugs has dramatically increased the incidence of deep mycoses and substantially broadened the range of fungi causing potentially lethal disease.

Purpose: This study aimed to determine the patient demographics and risk factors, which include comorbidities, etiological agents, medications used to treat COVID-19, and the management outcome of COVID-19-associated invasive fungal infection.

Materials and Methods: The study was carried out over a period of 5 months from March 2021 to August 2021. We included 65 swab and tissue samples were collected from ENT department to microbiology laboratory of tertiary care hospital, Ahmedabad, Gujarat. Mucormycosis, *Aspergillus*, and *Candida* infections in COVID-19 patients will require early detection by a comprehensive diagnostic intervention (direct microscopic examination, culture, and histopathology) to ensure effective treatments.

Results: In our study, the most predominant isolate obtained from samples was *Aspergillus* (47.6%), followed by zygomycetes (42.8%) and *Candida* (9.5%). The predominant fungal organism identified from zygomycetes spp. was *Rhizopus*. Patients with elevated levels free iron (not bound to transferrin) are uniquely susceptible to infection in all of our zygomycetes cases, but not to other pathogenic fungi, such as *Candida* or aspergillosis.

Conclusion: Mucormycosis can occur among COVID-19 patients, especially with poor glycemic control, widespread and injudicious use of corticosteroids and broad-spectrum antibiotics, and invasive ventilation. Due to the high mortality, high index of suspicion is required to ensure timely diagnosis and appropriate treatment in high-risk populations.

Key words: SARS-CoV-2, Invasive fungus, Aspergillosis, Mucormycosis, Candidiasis

INTRODUCTION

As the human-to-human transmitted disease, coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been an emergency global public health events.[1] For the severe COVID-19 patients who have more opportunities to be treat with broad-spectrum antibacterial drugs, parenteral nutrition and invasive examinations, or the patients accompanied with prolonged neutropenia and other immune impairment factors, the risk of infection with Mucormycosis, *Candida* species, and *Aspergillus* species may significantly increase.[1] Critically ill patients, especially the patients who were admitted to the intensive care unit and required mechanical ventilation, or had a longer duration of hospital stays, were more likely to develop fungal coinfections.[1]

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Mucormycosis, a group of filamentous fungi in the subphylum Mucoromycotina that belongs to the order Mucorales can cause life-threatening infections in humans, especially in immunocompromised hosts. The first documented report of human mucormycosis is credited

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to Paltauf, who, in 1885, reported a disseminated infection in a patient with rhinocerebral involvement caused by angioinvasive, ribbon-like hyphae that he termed Mycosis Mucorina. Subsequent descriptions of the infection in the following decades relied on tissue morphology and, as often is the case today, were infrequently confirmed by culture. Hence, the findings of coenocytic (aseptate or pauciseptate) fungal hyphae in tissue invading blood vessels were assumed to be due to *Mucor* spp. and have become synonymous with the clinical term mucormycosis or, simply, *Mucor* infection.[2] Now, increasing incidence of Mucormycosis in COVID19 cases has become a matter of concern globally.[3] Around 11,000 cases of Mucormycosis have been reported all over India. The government of India declared it a notifiable disease on May 20, 2021.[3] Mucormycosis is an acute oppotunistic infection caused by several fungi belonging to phylum Glomeromycota[4] It is a potentially lethal, angioinvasive fungal infection in COVID-19 patients with diabetes mellitus, immunosuppressive drugs therapy, corticosteroids, and primary and secondary immunodeficiency disorder.[3] Amphotericin B lipid complex, liposomal Amphotericin B, and posaconazole oral suspension are treated as the first-line antifungal monotherapy.[1]

Candidiasis, the most interesting period in the history of *Candida* infections, began in the 1940s, when the widespread use of antibiotics was introduced. Since then, the incidence of practically all forms of *Candida* infections has risen abruptly. *Candida* spp. has been the fourth most common organisms recovered from blood of hospitalized patients in the United States during recent decades.[2] In general, patients who are suspected or confirmed with invasive candidiasis should be treated with azoles (fluconazole, voriconazole, and itraconazole), and Amphotericin B and its liposomes.[1]

Aspergillosis is systemic fungal infection found among immunocompromised as well as immunocompetent individuals. This is primarily pulmonary infection with involvement of other body sites such as paranasal sinuses and tissues.[4] In most serious type of infections, invasion of infections, invasion of lung tissue, and dissemination to vital body organs may take place leading to fatal consequences among the immunocompromised patients. It is the most common opportunistic fungal disease after candidiasis.[4] In general, drugs recommended for the treatment and prophylaxis of IA include triazoles (itraconazole, voriconazole, posaconazole, and esaconazole), Amphotericin B and its liposomes and echinococcins (micafungin or carpofenjing). Most patients can choose triazole drugs to treat invasive aspergillosis.[1]

Role of Iron Uptake in Mucormycosis Pathogenesis

Fungi can acquire iron from the host using low-molecular-weight iron chelators (siderophores) or high-affinity iron permeases, such as Ferrirhizoferrin. Of the two mechanisms, it is believed that high-affinity iron permeases play the more critical role for adaptive survival of the fungus in the human host.[2] Of interest, uptake of radiolabeled iron in the presence of deferoxamine is 8- to 40-fold lower in *Candida* and *Aspergillus* compared with R. arrhizus, suggesting this mechanism is a relatively unique pathogenic trait of this fungus. This observation has been confirmed in animal models, where administration of deferoxamine worsens survival of guinea pigs infected with R. arrhizus but not *Candida*.[2] Albicans and [Table 1 and Figure 1] Pathogenesis of invasive mucormycosis.

MATERIALS AND METHODS

We included that 65 samples collected from ENT department from 18 March, 2020, to 19 August, 2021, were six swab and 59 tissue samples.

Eligibility criteria were set as follows:

1. Patients must have had a proven diagnosis of COVID-19 either before or at the time of development of mucormycosis
2. Case with a diagnosis of mucormycosis was included
3. Patients having co-infections of candidiasis and aspergillosis were also included the methods used to confirm the infection are direct microscopy (KOH and Gram stain), culture, and serology. Samples for culture should be placed on Sabouraud dextrose agar in aerobic conditions and observed daily.

Invasive Mucormycosis

1. The microscopic examination of nasal discharge or biopsy material requires a microbiologic and/or histopathologic evidence Direct examination: (a) direct microscopic examination of clinical specimens with 10% KOH shows characteristic broad, non-septate, and ribbon like hyphae with wide angle or right-angle branching at irregular intervals[4]
2. Culture: The mucormycetes can be easily grown on

Table 1: The analyzed data have been summarized

Total	65 (%)
Male	48 (73.8)
Female	17 (26.15)
diabetes mellitus	44 (67.6)
Hypertensive	21 (32.3)
Diabetes mellitus and hypertensive	15 (23)
Angioinvasion	16 (24.6)
Tissue	52 (80)
Tissue, bone invasion with angioinvasion	11 (16.9)
Use of steroids	47 (72.3)
broad-spectrum antibiotics	49 (75.3)
Use of oxygen therapy	27 (41.5)

conventional media like SDA with antibiotics at both temperatures 25 and 37. The Mucorales grow on standard fungal culture media without cycloheximide.[4] [Table 2 and Figure 2] (a) *Rhizopus*, (b) *Mucor*, and (c) *Absidia*.

Invasive Aspergillosis

The diagnosis of invasive aspergillosis requires a microbiologic and/or histopathologic evidence.

(1) Direct examination: (a) Direct microscopic examination of clinical specimens with 10% KOH for demonstration of hyaline septate hyphae of *Aspergillus* species. The septate hyphae are 3–6 μm in diameter with dichotomous branching.[4] (b) Histopathological examination of biopsy material stained with hematoxylin and eosin and periodic acid-schiff stain show acute-angled (45°) branching and hyaline septate hyphae with neutrophilic to granulomatous response. The optical brightener methods, Calcofluor or Blankophor, which may increase the sensitivity and specificity for detecting *Aspergillus*-like features;[1] (2) Culture: The material is inoculated on Sabouraud dextrose agar with antibiotics and without cycloheximide at 25°C and 37°C, respectively. The cultures should be examined daily

Table 2: Age wise distribution

Age duration	Total cases (%)
21–30	2 (3.07)
31–40	11 (16.9)
41–50	15 (23.07)
51–60	18 (27.6)
61–70	11 (16.9)
71–80	8 (12.3)

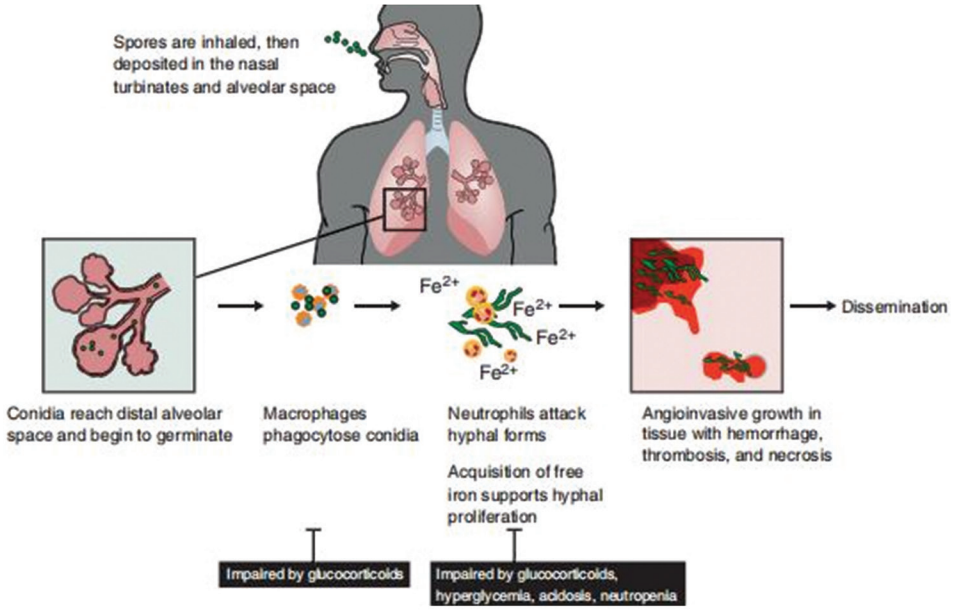

Figure 1: Pathogenesis of invasive mucormycosis[1]

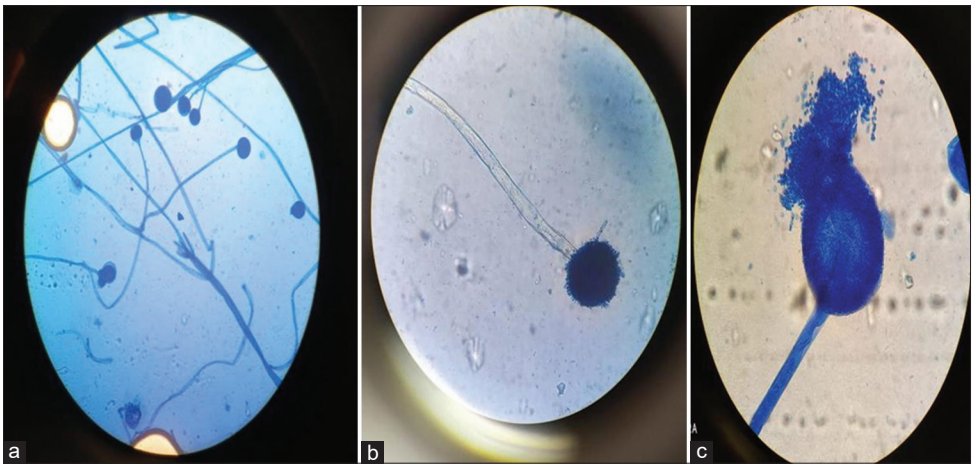

Figure 2: (a) *Rhizopus*, (b) *Mucor*, and (c) *Absidia* (Microbiology department of GMERS Medical college and hospital SOLA, Ahmedabad, Gujarat)

during 1st week and twice a week for further 4 weeks before being considered the plate/tube as sterile.[4] If positive, morphological features of *Aspergillus* can be identified under the microscope or the DNA sequencing may be used in reference laboratories to identify the species accurately, but usually culture yield is low and a negative result does not exclude the diagnosis of invasive aspergillosis.[1]

Table 3: Microbiological profile

Total	65 (%)
KOH positive	40 (61.5)
Culture positive	25 (38)

Table 4: Mixed fungal infection

Rhizopus and *Aspergillus niger*	3
Mucor and *Aspergillus niger*	1
Mucor, *Aspergillus*, *Syncephalastrum*, and *Candida*	1

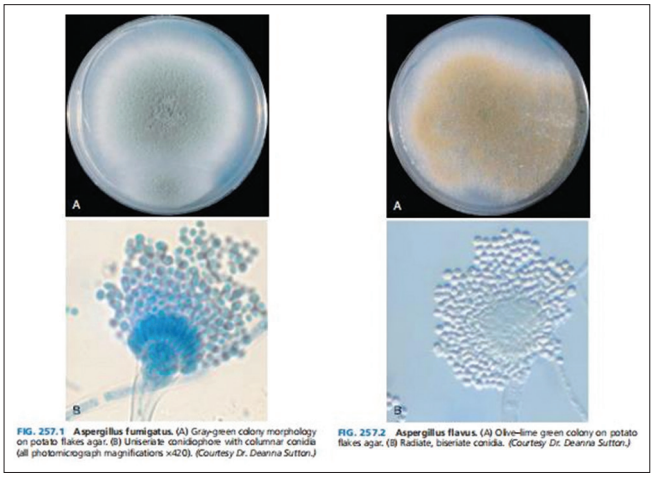

Figure 3: *Aspergillus fumigotus* and *Aspergillus flavus* (Courtesy Dr. Deanna Sutton.)

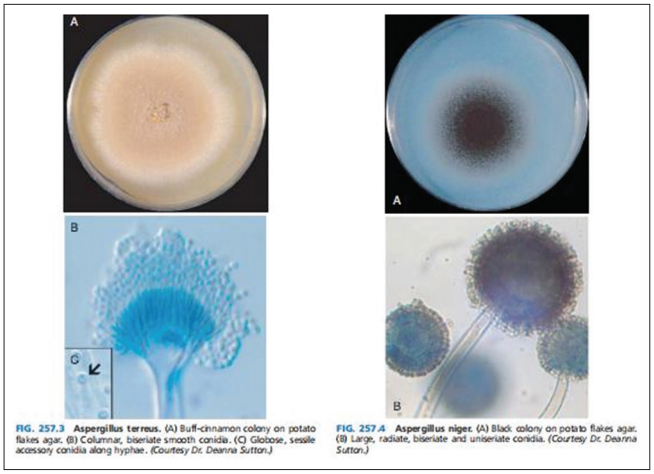

Figure 4: *Aspergillus terreus* and *Aspergillus niger* (Courtesy Dr. Deanna Sutton.)

[Table 3 and Figure 3] *Aspergillus fumigotus* and *Aspergillus flavus* (Courtesy Dr. Deanna Sutton.) [Table 4 and Figure 4] *Aspergillus terreus* and *Aspergillus niger* (Courtesy Dr. Deanna Sutton).

Invasive Candidiasis

The clinical specimens are collected depending on the site of involvement and examined in KOH wet mount or normal saline preparation.

(1) direct microscopic examination (a) Gram stain and KOH: Gram's staining is performed to see presence of yeast and pseudohyphae of *Candida* species. The yeast cells are approximately 4–8 μm with budding and pseudohyphae. The biopsy specimens are kept in tube containing KOH for an overnight period at 37°C and after mincing these are examined under the microscope for yeast cells and pseudohyphae.[4] (2) Culture: The clinical specimens can be cultured on SDA agar with antibacterial antibiotics and incubated at 25°C and 37°C. The colonized appear in

Table 5: Species wise distribution of fungus

Fungal	Total
Zygomycetes spp.(42.8%)	
Rhizopus	4
Mucor	3
Syncephalastrum	2
Aspergillus spp.(47.6%)	
Aspergillus flavus	4
Aspergillus fumigatus	4
Aspergillus terreus	1
Aspergillus niger	1
Candida spp. (9.5%)	
Non-albicans	1
Albicans	1

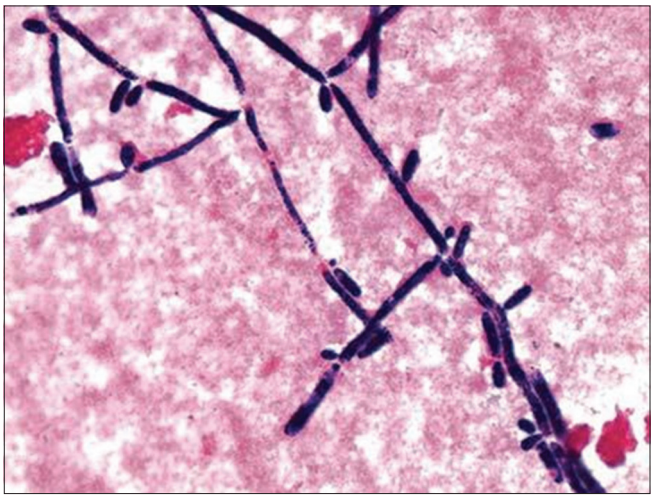

Figure 5: Morphology of *Candida* seen on Gram stain. (From Anaissie EJ, McGinnis MR, Pfaller MA. The laboratory and clinical mycology. In: Anaissie EJ, McGinnis MR, Pfaller MA, eds. Clinical Mycology. 2nd ed. New York: Churchill Livingstone; 2009:55–77.)

2–3 days as cream colored, smooth, and pasty.[4] [Table 5 and Figure 5] Morphology of *Candida* seen on Gram stain. (From Anaissie EJ, McGinnis MR, Pfaller MA. The laboratory and clinical mycology. In: Anaissie EJ, McGinnis MR, Pfaller MA, eds. Clinical Mycology. 2nd ed. New York: Churchill Livingstone; 2009:55–77).

Statistical Data Analysis

EXCEL sheet data analysis.

RESULTS

We included 65 samples collected from ENT department from March 18, 2020, to August 19, 2021, were six swab and 59 tissue samples. All of the patients had a previous history of COVID-19. The median time interval between COVID-19 diagnosis and the first evidence of mucormycosis infection diagnosis was 15 days; however, mucormycosis developed even as late as 42 days and 90 days following COVID-19 diagnosis.[5] In our study, there was a noticeable gender bias, with 73.8% of the cases being male. Mucormycosis is most commonly affects immunocompromised hosts, but are rarely reported in immunocompetent. In total, 65 patients with 44 (67.6%) were diagnosed with diabetes and 21 (32.3%) were diagnosed with hypertension. Although COVID-19 was severe/critical in most patients, mucormycosis developed even in 20% patients without any comorbidity. During management of COVID-19, 72.3% patients received corticosteroids. Imaging revealed tissue invasion 80% [Table 1]. Majority of the isolates were obtained from patients in the age group of 51–60 year [Table 2]. KOH positivity was seen in 61.5% of patients. Among the fungal culture was isolated in 38% of patients [Table 3]. In our study, 20% of patients had more than one invasive fungal involvement. Invasive fungal co-infection also seen in our study which is summerized in Table 4. The most predominant isolate obtained from samples was *Aspergillus* (47.6%) followed by zygomycetes (42.8%) and *Candida* (9.5%). The predominant fungal organism identified from zygomycetes spp. was *Rhizopus* [Table 5]. In our study, serum ferritin elevated in 69.2% of patients, CRP elevated in 78% of patients, and D-dimer elevated 58.4% of patients [Table 6].

Table 6: Inflammatory markers

Serum ferritin	45 (69.2%)
CRP	51 (78.4%)
D-Dimer	38 (58.4%)

Table 7: Comparison between two study

Supportive treatment during COVID-19	Other study Baghel *et al*. (%)	Present study (%)
Steroids	72.6	72.3
Antibiotics	73.4	75.3
Oxygen	55.6	41.5
Mortality rates	33.3	4.5

DISCUSSION

We report the clinical, microbiological, and radiological investigation of invasive fungal infections in a series of COVID-19 patients. Mucormycosis is a potentially fatal infection which arises from the invasion of blood vessels by fungal elements leading to thrombosis, ischemic infarction, and cause necrosis of affective host tissues and bone. Globally, the incidence of mucormycosis has been described to range from 0.005 to 1.7 per million population, where as in India, the reported prevalence was 0.14/1000, approximately 80 times higher than that in developed countries, making it the country with the highest burden of mucormycosis.[6] In one study, conducted by Baghel *et al*., a rate of steroids received 72.6% of patients, while 73.4% received antibiotics, and 55.6% received oxygen during COVID-19 management, which concords with our present study with 72.3%, 75.3%, and 41.5%, respectively.[7] In the similar study of Baghel *et al*., the prognosis of invasive fungal infection is poor, with reported mortality rates ranging from 33.3% to close to 100% in disseminated infections even if aggressive debridement and intravenous antifungal agents are utilized. However, in our study, only 4.5% mortality rates seen due to our early laboratory. Early diagnosis and early appropriate treatment is helpful for this deadly disease. Appropriate treatment with precautions and care to effectively manage infected patients which reflect with good prognosis [Table 7]. Over-the-counter sale of these antibiotics and steroids drugs should be prohibited.

CONCLUSION

Invasive fungal infections are most commonly affects the immunocompromised patients. Patients with elevated levels free iron (not bound to transferrin) are uniquely susceptible to infection by *Rhizopus* and other Zygomycetes, but not to other pathogenic fungi, such as *Candida* or aspergillosis. Therefore, fungal iron acquisition is a promising therapeutic strategy to impact clinical outcomes for this deadly and sever disease. Applicable treatment for management of Mucormycosis includes surgical debridement of infected tissue followed by first-line treatment with high-dose liposomal Amphotericin B; in addition, Voriconazole and posaconazol are also prescribed. Fungal infection can be prevented in healthy individuals by good hygiene and nutrition and can be treated with proper and timely

diagnosis. To prevent such a dangerous and costly disease, stricter policies and guidelines have to be implemented and followed to prevent injudicious antibiotic and steroid use. Over-the-counter sale of steroids drugs should be prohibited.

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