Review Article

Coronavirus disease 2019-associated mucormycosis – A syndemic

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Abstract

Coronavirus disease 2019 (COVID-19) and COVID-associated mucormycosis (CAM) came as a syndemic that not only severely increased morbidity and mortality but also posed a serious challenge for the healthcare system of a developing country like India. Although mucormycosis is a rare disease with a worldwide incidence of 0.43 cases per million population/year, these two COVID-19 waves caused a total of more than 14,000 cases in India itself. Mucormycosis is an angio-invasive fungal infection with rapid progression. The three major modalities of treatment involve early surgical debridement, initiation of antifungal therapy and controlling the predisposing risk factor. A complex interplay of factors, including pre-existing disease such as diabetes mellitus, use of immunosuppressants and immunomodulators, risk of hospital-acquired infection and immune dysregulation post-COVID-19, may all predispose to the development of CAM. Future research regarding the efficiency of newer antifungal with lower side effect profiles and evidence-based establishment of risk factors for adopting preventing strategies is the need of the hour. The disease is known to have high mortality despite the best treatment. We review in this article the aetiopathogenesis, various diagnostic modalities and treatment considerations for this disease.

Keywords: COVID-19, COVID-19–associated mucormycosis, mucormycosis

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INTRODUCTION

The current pandemic of COVID-19 shook the world placing an unprecedented burden on the world economy, healthcare and globalisation. It has not only led to overstretching of health capacity but also jeopardised the hard-earned progress in global health goals through decades. With mortality crossing 4 million cases worldwide as of July 2021 with India contributing to approximately 1/10th of the cases, the rising incidence of mucormycosis in post-COVID patients mimics a silent storm lying at the footfall of this pandemic ready to ravage and mock the helpless situation in a country hard hit.[1]

The surge of COVID-associated mucormycosis (CAM) case has been more in the second wave compared to the first wave of COVID, summing to a total of 14,872 cases as of 28th May 2021, with multiple states already having declared it as an epidemic and a notifiable disease to the national health authorities.[2]

Mucormycosis is an angio-invasive fungal infection caused by the order *Mucorales*. These are ubiquitous organisms

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causing life-threatening infection almost exclusively in the immunocompromised. It presents as various syndromes with rhino-orbital cerebral and pulmonary being the most common forms. The incidence rate of mucormycosis globally varies from 0.005 to 1.7 per million population. In India, the prevalence of mucormycosis is estimated as 140 per million populations, 80 times higher than the prevalence in developed countries.[3] A review of published mucormycosis cases found an overall all-cause mortality rate of 54%. The mortality rate varied depending on underlying patient condition, type of fungus and body site affected (for example, the mortality rate was 46% among people with sinus infections, 76% for pulmonary infections and 96% for disseminated mucormycosis).[4]

In this review, we aim to discuss the microbiology, immunopathogenesis, diagnosis and management of the cases of CAM.

EPIDEMIOLOGY

These are organisms present in decaying vegetation, soil and frequently found as a contaminant in clinical microbiological samples. The genera in the order *Mucorales* most frequently causing human infections include *Rhizopus*, *Mucor*, *Rhizomucor*; *Cunninghamella*, *Absidia* (now reclassified as *Lichtheimia*), *Saksenaea*, and *Apophysomyces*, with *Rhizopus oryzae* being the most common isolated accounting for 70% of all cases of mucormycosis[5] and *Cunninghamella* being the most virulent of all strains. Although *Rhizopus arrhizus* continues to remain the most common causative agent isolated among the clinical forms in India and globally, evidence of invasive infections by *Rhizopus homothallicus* and *Rhizopus microsporus* has also been reported.

The major risk factors for mucormycosis include uncontrolled diabetes mellitus in ketoacidosis, other forms of metabolic acidosis, treatment with corticosteroids, organ or bone marrow transplantation, neutropenia, trauma, burns, malignant haematological disorders, intravenous drug use, HIV patients and deferoxamine therapy in patients receiving haemodialysis. Prolonged use of antifungal therapies such as caspofungin and voriconazole lacking activity against *Mucorales* is an emerging predisposing condition, leading to breakthrough infection.[6]

Rhinocerebral mucormycosis representing 39% of infections by *Mucorales* is common in diabetic population, whereas pulmonary involvement is commonly seen in neutropenic patients and those with graft versus host disease post-stem cell transplantation. Disseminated infection can occur from any primary site although lung is most commonly associated organ.[7]

The largest review in history of 929 cases of mucormycosis reported between 1940 and 2003 noted that diabetes mellitus was the most common risk factor (36%), followed by haematological malignancies (17%) and solid organ or haematopoietic cell transplantation (12%). In a later study in France between 2005 and 2007, haematological malignancy was found to be the most common risk factor (50%).[8]

Diabetes mellitus is the most common risk factor in India and the developing world, with an association varying from 17% to 88% globally. In India, among all mucormycosis cases, 23%–43% have diabetes defining illness. The most common risk factor in Europe and the United States is the presence of haematological malignancies and recipients of haematopoietic stem cell transplantation. The disease develops during the phase of neutropenia.

In India, overall prevalence of rhino-orbital mucormycosis was found to be followed by pulmonary mucormycosis (17%), gastrointestinal mucormycosis (13%), cutaneous mucormycosis (11%) and renal and disseminated mucormycosis (5% each).[7]

Isolated renal mucormycosis is a rare entity and has been described only in India and China. In a recently published case series[9] of 15 such cases of isolated renal mucormycosis in immunocompetent hosts, noted flank pain and persistent fever as the most common presentation. There were treated with initiation of amphotericin B and surgical debridement in the form of nephrectomy. The most common organism isolated was *Rhizopus arrhizus*.[9]

Mucormycosis is a defining illness of the immunocompromised. Factors that have been established to play a causal role in post-CAM include overuse of high-dose glucocorticoids, administration of highly immunosuppressive drugs such as inhibitors of the Janus kinase inhibitors or interleukin-6 (IL-6) receptor inhibitors, prolonged hospital stay of critically ill patients and the direct effect of COVID on immune dysregulation.

One review of case reports of mucormycosis in patients with COVID-19 included 101 cases, 80% of whom had pre-existing diabetes mellitus and 76% of whom had received glucocorticoids for the treatment of COVID-19. The majority of cases were from India; the reasons for this are unknown. Almost 90% of cases involved the nose and sinuses, and overall mortality was

31%.[10] The largest series of CAM patients from India comprises 2826 patients. Of these, 87% had received corticosteroids (21% for >10 days) and 78% of patients were diabetic. Most of the cases developed symptoms of rhino-orbital-cerebral mucormycosis between day 10 and day 15 from the diagnosis of COVID-19.[11] Similarly, in the series of 52 cases the authors concluded that steroid use and diabetes were important risk factors for CAM whereas anticoagulation and aspirin use was found to be protective (hypothesised as reduction in ischemic tissue necrosis).[12]

Despite aggressive therapy which includes disfiguring debridement and toxic antifungal therapy, the overall mortality rate remains >50%, approaching 100% among patients with disseminated disease or persistent neutropenia.[13] However, in another study[11] an overall mortality of only 14%, which means, with optimal management, mortality rates can be brought down in mucormycosis.

DIAGNOSIS

Prompt diagnosis and early treatment initiation is essential for preventing dissemination and improving patient outcome and survival. It is important to note that the agent of mucormycosis colonises the airways and may act as a contaminant, so appropriate interpretation in the background of clinical suspicion is important before starting the patient on treatment (Figure 1).

The diagnosis relies on identification of organism in tissue via histopathology followed by confirmation by culture. It is important to note that the tissue sample from the biopsy site should not be crushed since *Zygomycetes* are very fragile and may result in false culture-negative results. In patients with haematological malignancies, severe thrombocytopaenia may limit tissue biopsy. In such cases and when biopsy specimen cannot be obtained, the available clinical samples should be subjected to direct microscopy where demonstration of aseptate hyphae can suggest infection by *Mucorales*.

After treatment with potassium hydroxide which clears cellular material and helps in better visualisation of fungal elements, these samples can then be subjected to optical brighteners such as calcofluor white and Gomori methenamine stain. In the prototype, *Rhizopus*, the hyphae are broad (5–15 μ diameter), aseptate or pauci septate, ribbon like with irregular branching at 90° which is in contrast to ascomycetous moulds, such as *Aspergillus*,

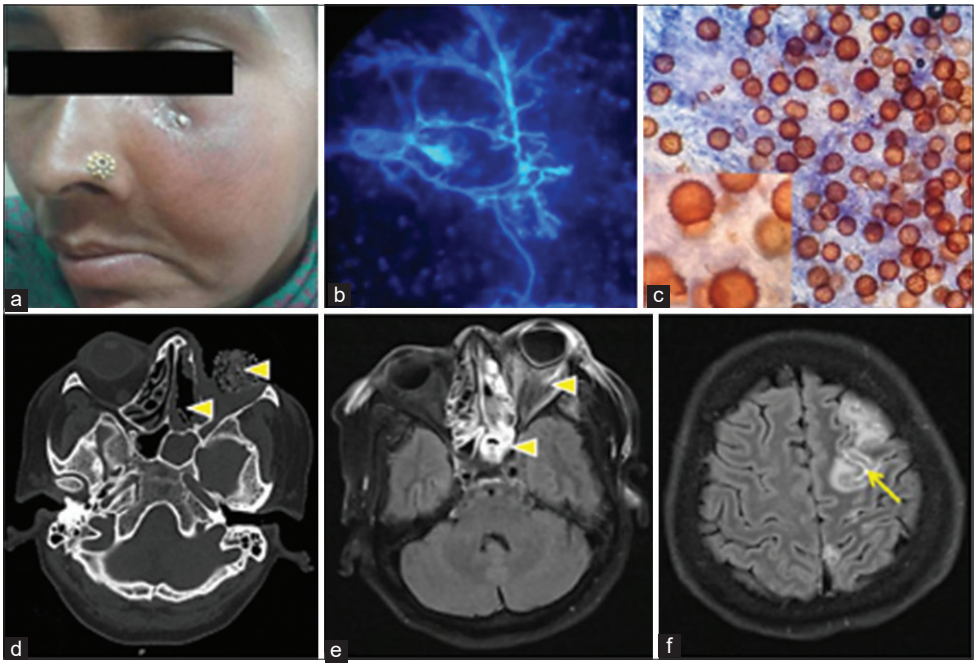

Figure 1: Clinical photograph showing erythematous lesion on left side of face with serous discharge (a). Photomicrograph showing broad aseptate hyphae (calcofluor stain with 10% potassium hydroxide, ×400) (b). Photomicrograph showing golden-to-brown–coloured zygospores having remnants of single suspensor cell typical of *Rhizopus homothallicus* (Lacto phenol cotton blue, X 400); inset shows the similar zygospores (Lacto phenol cotton blue, X 1000) (c). Non-contrast computed tomography of the head showing diffuse mucosal thickening in left maxillary sinus, bilateral ethmoid, frontal and sphenoid sinus (d); left eye shows pre-septal thickening with air foci (e and f). Magnetic resonance imaging of the brain, paranasal sinuses and orbit showing diffuse heterogeneously enhancing mucosal thickening of the left maxillary sinus, bilateral ethmoid, frontal and sphenoid sinus (e), T2-weighted flair magnetic resonance imaging showing hyperintensity with central hypointensity in the left frontoparietal occipital region (f)

where hyphae are narrower (2–5 μ diameter), exhibit regular branching and have many septations. Growth of these organisms on Sabouraud dextrose agar is rapid and usually occurs during incubation for 24 h at 25 °C – 37 °C. Biopsies stain with Gomori methamine silver or periodic–acid Schiff. Hyphae may be observed within necrotic tissue with signs of angio-invasion and infarction; neutrophilic infiltrates or granuloma formation may be present in patients who are not granulocytopenic or with more chronic infection, respectively.[14]

Culture from a sterile site confirms the infection which can then be subjected to species identification. Blood cultures are usually negative and their presence almost always indicates contamination due to the ubiquitous presence of these organisms. Even during cerebral involvement, these organisms are rarely found in the cerebrospinal fluid.[15]

When cultures are negative, molecular identification from tissue samples can confirm the histological diagnosis. Although no method has yet been standardised for molecular diagnostic testing, a recent study[16] noted high rate of concordance of polymerase chain reaction (PCR) of formalin-fixed paraffin-embedded tissue samples with tissue culture. Such molecular methods have the benefit of early diagnostic confirmation and species identification. The antifungal susceptibility is variable in different species; therefore, precise identification of *Mucorales* up to the level of species using molecular methods may help in knowing antifungal effectiveness.

Molecular identification of agents of mucormycosis can help confirm diagnosis and identify the fungus to the genus and species level. Different techniques have been reported: deoxy ribonucleic acid (DNA) probes targeting 18S subunit, ITS1 sequencing after PCR with pan-fungal primers, 18S-targeted semi-nested PCR and real-time PCR targeting cytochrome b gene.[17]

In a recent study, the use of PCR-RFLP on invasive clinical specimens, with assays targeting the 18s ribosomal gene, a suitable marker for taxonomic identification with a relatively low rate of molecular evolution, proved as good molecular method for species identification. Another molecular technique using Microseq D2 sequencing kit to amplify the D2 domain range of the large rRNA gene subunit was studied, but a recent report has shown difference in conventional phenotypic characterisation and identification of *Mucorales* using this kit.[18]

Imaging techniques such as computerised tomography (CT) can help in determining the extent and exact location of fungal infection. Reverse halo sign on the CT scan of lungs in patients with pulmonary mucormycosis has been defined with area of tissue necrosis appearing as ground glass on the film. Lass-Flörl in his study has also shown high efficiency of CT-guided percutaneous lung biopsy in differentiating pulmonary aspergillosis from mucormycosis.[14]

However, the diagnosis of mucormycosis remains challenging as there is no circulating antigen test similar to galactomannan detection for invasive aspergillosis. 1, 3 beta-D glucan detection test is usually found negative in *Mucorales* infection. These two tests can however help in differentiating from invasive aspergillosis a common differential in such patients.[19] Summary of cases of mucrmycosis in COVID-19 is shown in Table 1.[1,11-19]

MANAGEMENT

The principles of management remain rapid diagnosis, urgent surgical debridement, reversal of underlying predisposition and anti-fungal therapy. It is important to note that the morbidity and mortality in mucormycosis is directly related to the lag time before diagnosis and treatment.

The Global Guideline for the Diagnosis and Management of Mucormycosis by the European Federation of Medical Mycology strongly supports an early complete surgical treatment whenever possible, in addition to systemic antifungal treatment repeating resection and debridement if required. In a systematic review of 90 solid organ-transplant recipients, surgery was independently associated with decrease mortality (odds ratio = 0.12) in rhino-orbital mucormycosis.[20]

In a recent study[21] on the emergence of 80 CAM cases from 18 countries, they suggested a significant decrease in mortality in patients who underwent surgical debridement (62.5% *vs.* 13.8%); however, the impact was not statistically significant in those with central nervous system (CNS) involvement (71.4% *vs.* 57.1%).

The usage, dosage and duration for which antifungals are to be used remain a much debated issue. There exist no randomised control trials to assess the efficacy of different anti-fungal regimens. Liposomal amphotericin B has been proclaimed as the drug of choice in various published case series. The use of amphotericin B deoxycholate has been limited by the substantial nephrotoxicity caused restricting its use to resource limited settings. However, if LAmb is unavailable, amphotericin B deoxycholate can be used as an alternative.

Table 1: Mucormycosis in COVID-19 - Summary of cases

Study (country) (reference)	Study Design	DM association	Steroids	Other immuno supressants	Involvement	Mortality
Singh *et al*., India (Chandigarh) (1)	Systematic review (*n*=101)	83.3%	76.3%	Tocilizumab (4.1%)	Sinonasal (88.9%), rhino-orbital (56.7%) and rhino-orbital cerebral (22.2%)	30.7%
Sen *et al*., India (Mumbai) (11)	Case series (*n*=6)	6/6	5/6	ND	rhino-orbital (83.3%), cerebral (16.7%)	Improving
Sarkar *et al*., India (Puducherry) (12)	Case series (*n*=10)	10/10	10/10	ND	Rhino-orbital (100%)	40%
Mishra *et al*., India (Bangalore) (13)	Case series (*n*=10)	8/10	6/10	Tocilizumab 1/10	Sinonasal-80% , Rhinoorbital (20%),	40%
Satish *et al*., India (Bangalore) (14)	Case series (*n*=11)	Majority	ND	ND	Rhino-orbital (majority)	18%
Moorthy *et al*., India (Bangalore) (15)	Case series (*n*=17)	88.2% (15/17)	88.2% (15/17)	ND	Rhino-orbital (majority), cerebral (47%)	41%
Sharma *et al*., India (Jaipur) (16)	Case series (*n*=23)	91.3% (21/23)	100% (23/23)	ND	Sinonasal (majority), Rhino-orbital (43.4%), cerebral (8.6%),	Improving
Garg *et al*., India, Chandigarh (17)	Case series (*n*=8)	4/8	ND	ND	Rhino-orbital cerebral (37.5%), pulmonary (37.5%) GIT (12.5%), disseminated (12.5%),	12.5%
Dallelzadeh et al., USA (18)	Case series (*n*=2)	2/2	2/2	ND	Rhino-orbital majority, cerebral involvement in 1 case	50%
Veisi *et al*., Iran (19)	Case series (*n*=2)	2/2	2/2	ND	Rhino-orbital majority, cerebral involvement in 1 case	50%

ND = not described

The global guidelines recommend daily doses ranged from 1 mg/kg per day to 10 mg/kg per day. In CNS involvement and renal-transplant recipients, a dose as high as 10 mg/kg has been given, leading to increase response rate with a risk of increase in serum creatinine and drug-induced acute kidney injury. The basic principle of antifungal therapy in mucormycosis is to start at the highest possible tolerable dose and then de-escalate later rather than gradually increasing the doses. Moreover, there is no cut-off for cumulative doses of amphotericin and duration is guided by clinico-radiological response.

Although some animal models have shown benefit of combination antifungal therapy with polyene plus azoles or polyenes plus echinocandins in improving survival rates, we still lack substantial literature to justify the rational for combination therapy against potential risks of added toxicity, drug interactions and financial burden. Therefore, azoles of proven benefit such as posaconazole and isavuconazole continue to be used as a rescue or salvage therapy. Currently, we lack experience with isavuconazole in mucormycosis. However, owing to its better CNS distribution, it might prove to be valuable in cases with CNS involvement.[22]

The VITAL trial[22] conducted in 2008 was an open-label, single-arm study conducted to assess the efficacy and safety of isavuconazole for the treatment of patients with invasive aspergillosis and renal impairment, or with invasive fungal diseases caused by rare moulds, yeasts or dimorphic fungi. The trial not only concluded efficacy of isavuconazole but also suggested no loss of efficacy or drug-specific safety concerns in patients with renal function derangement.[22] Currently, we lack experience with isavuconazole in mucormycosis. However, owing to its better CNS distribution, it might prove to be valuable in cases with CNS involvement.

Treatment duration of antifungals continues to remain the decision of the treating physician. It is recommended

to continue intravenous treatment till a stable (clinical and radiological) disease is reached followed by switching to oral treatment with the use of isavuconazole and posaconazole delayed release tablets.

According to Deferasirox-AmBisome Therapy for Mucormycosis Mucor Study,[23] a randomized, double-blinded, placebo-controlled trial, the role of iron-chelating agents such as deferasirox as an adjunctive therapy to liposomal amphotericin B was discouraged. Analysis of 20 proven mucormycosis cases revealed higher mortality rate at 90 days in in the treatment arm receiving–LAmB plus deferasirox compared to the placebo arm treated with LAmB (82% vs. 22%, $P = 0.01$).[23]

The role of a multidisciplinary team consists of otorhinolaryngologists, ophthalmologists, anaesthetists and neurosurgeon for urgent assessment and individualisation of the treatment in a rapidly progressive disease where time is essential for saving vision, preventing cerebral involvement and reducing mortality.

As we continue to face spontaneous mutations and generation of different variants, the COVID-19 guidelines should be updated taking CAM as a diagnostic consideration. The surge in CAM cases has not only led to increased morbidity and mortality but also massive financial burden due to expensive anti-fungal therapy on the health sector already crippled due to the pandemic.

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Conflicts of interest
There are no conflicts of interest.

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