Mucormycosis (black fungus) and its impact on the COVID-19 patients: An updated review

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ABSTRACT
Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a global pandemic of the century. The disease is wreaking havoc on human health, the world economy, society, and the environment. It has already caused the loss of millions of lives. Because of the mutation, the virus is constantly evolving itself, changing its nature including the disease transmission rate, virulence, pathogenesis, and clinical manifestations. It was recently reported that certain COVID-19 patients are also suffering from a fungal infection as co-infection commonly known as mucormycosis (black fungus). In India, the outbreak of black fungus in COVID-19 patients has already been declared an epidemic. Only a few reports are noticed in other countries. The focus must now be put toward better management and control of the COVID-19-associated fungal infection. In this review, we have discussed various aspects of black fungus particularly the etiology, taxonomy, risk factors, transmission, pathogenesis, clinical manifestations, diagnosis, and line of treatment to keep up to date on how to manage this fungal infection better.

INTRODUCTION
Coronavirus disease 2019 (COVID-19) is a serious ongoing public health problem throughout the world. Within a short period of time, the COVID-19 was declared a pandemic by the World Health Organization (WHO) [1]. The disease has become more hazardous due to its zoonotic nature [2, 3]. The COVID-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which is an extremely contagious RNA virus that affects the respiratory systems and associated organs including the heart, kidneys, and the brain [4]. The patients have a variety of clinical symptoms, and the majority of the complications are associated with respiratory organs such as pneumonia, dyspnea, and hypoxia, followed by respiratory failure, septic shock, and multi-organ dysfunction [5]. Other consequence includes diffuse alveolar damage which causes acute respiratory distress syndrome, disseminated intravascular coagulation depletion of white pulp in the spleen, etc. [6]. SARS-CoV-2 can affect people of any age, although a severe form of the disease has been reported predominantly in the elderly and those with additional comorbidities such as cardiovascular diseases, asthma, autoimmune diseases, gastrointestinal diseases, hypertension, immunosuppression, metabolic and neurologic diseases, obesity, renal diseases, etc. [7-10]. COVID-19 patients with comorbid conditions may also develop
immunosuppression conditions [11]. This immunosuppression can occur for various reasons, but the most important causes are the high dose and long-term usage of corticosteroids in the COVID-19 patients as a therapeutic measure [12, 13]. The COVID-19 patients might be co-infected with other microbial infections depending on the immune status [14].

The majority of the fungal pathogens are opportunistic. Fungal infections have been increasing due to the increased number of immunocompromised hosts and various types of mycoses may develop depending on the immune status of the patients [15]. COVID-19 patients were also shown to have fungal infections such as Aspergillus, Candida, mucormycetes, etc. [16, 17]. Mucormycosis, popularly known as the black fungus, is a fungal infection caused mostly by Mucorales species such as Rhizopus and Mucor [18, 19]. The disease is rare but may be serious around the globe, with a high incidence in India [20, 21]. The majority of invasive fungal infections occur in immunocompromised patients due to the poor health management system [21, 22]. Mucormycosis has been linked to a number of pre-existing health conditions, including diabetes mellitus, iron overload, cancers, organ transplant, kidney failure, co-infection with Mycobacterium tuberculosis, acquired immunodeficiency syndrome (AIDS), immunosuppressive therapy, and others [23-25]. In addition, COVID-19 patients may develop immunosuppression as well as increased blood glucose levels in both non-diabetic and diabetic cases. Therefore, COVID-19 associated mucormycosis (CAM) have been reported in the post-treated or recovered patients [17]. CAM reports are currently on the rise all over the world, especially in Asiatic regions where the fatality rate among COVID-19 patients is high [17, 26]. However, the information regarding the causal agents of mucormycosis and CAM is limited. Therefore, this review is focused on the taxonomy and genomic characteristics of mucormycosis etiologies, as well as transmission, clinical symptoms, pathogenesis, etc. In addition, we emphasized on pathogenesis and virulence factors of COVID-19/mucormycosis co-infections, as well as their epidemiology, diagnosis, and line of treatment in CAM patients.

ETIOLOGICAL AGENTS AND TAXONOMY

The etiological agents of mucormycosis belong to the order Mucorales under the phylum Glomeromycota and subphylum Mucoromycotina. The disease was described as zygomycosis under the Zygomycota phylum that includes two diseases: mucormycosis and entomophthoromycosis [19]. A comprehensive molecular phylogenetic re-analysis of fungi eliminated the phylum Zygomycota and replaced it with the phylum Glomeromycota that includes four subphyla e.g. Mucoromycotina, Entomophthoromycotina, Zoopagomycotina, and Kickxellales [27, 28]. In addition, the analysis showed that the subphyla Mucoromycotina and Entomophthoromycotina are evidently unconnected with separate clades. Therefore, the term zygomycosis has become obsolete [28]. Furthermore, the etiological agents are able to generate the word "Mucormycosis" over "Zygomycosis" due to their differences in morphology, epidemiology, clinical symptoms, and ecology [28].

Out of the 261 species in the Mucorales order, 38 are linked to human diseases [29]. Molecular phylogenetic researches have aided in the changing of their taxonomy in earlier years, and numerous taxa have seen repeated name modifications, e.g. (1) Rhizopus oryzae has changed to Rhizopus arrhizus, (2) from Rhizopus microsporus var. azygosporus, var. chinensis, var. oligosporus, var. rhizopodiformis, var. tuberosus to Rhizopus microsporus, (3) from Mucor ellipsoides, Mucor circinelloides f. circinelloides to Mucor ardilhaengiktus, (4) from Mucor circinelloides f. griseocyanus to Mucor griseocyanus, (5) from
Rhizomucor regularior, Rhizomucor variabilis var. regularior to Mucor circinelloides, (6) from Rhizomucor variabilis to Mucor irregularis, (7) from Mucor circinelloides f. lusitanicus to Mucor lusitanicus, (8) from Mucor circinelloides f. janssenii to Mucor lusitanicus, (9) from Absidia corymbifera, Mycocladus corymbifer to Lichtheimia corymbifera, (10) from Absidia ornata to Lichtheimia ornata, (11) from Absidia ramosa, Mycocladus ramosus, etc. [29, 30].

The common etiological agents of mucormycosis are Mucor spp., Rhizopus spp., and Lichtheimia spp., accounting for about 75% of all cases. Other genera including Rhizomucor, Cunninghamella, Saksenaea, Apophysomyces, Cokeromyces, Syncephalastrum, and Actinomucor are also causative agents of mucormycosis. However, their impacts on the cause of mucormycosis are minor [30]. The causative agents of mucormycosis may be different due to differences in the geographical distribution, e.g. in Europe, Rhizopus spp. were found in 34% of the cases, both Mucor spp. and Lichtheimia spp. were in 19% of the cases; in France, 52% cases were due to Rhizopus spp., and 29% cases due to Rhizopus spp.; in Mexico, Apophysomyces mexicanus was found as a common causative agent for mucormycosis; in India and Asia, Rhizopus spp. is the most common etiological agent [30-32]. Several emerging and uncommon species were also the causative agents for mucormycosis including Apophysomyces elegans, A. variabilis, Rhizopus homothallicus, Thamnostylus lucknowense, Mucor irregularis, and Saksenaea spp. [33-37]. Because of their fast-growing and thermo-tolerant qualities, as well as their ability to grow on decaying organic matter or agricultural and forest soils, these organisms are found all over the world [38].

**RISK FACTORS**

Mucormycosis is an opportunistic disease that has adverse consequences on immunocompromised individuals who have certain risk factors. However, immunocompetent hosts are also susceptible to mucormycosis with a very small percentage [34, 39]. The risk factors associated with mucormycosis weaken the hosts’ immune system, strengthen the growth of the agents, and permit their dissemination to the environment where they can cause significant invasive infections. Figure 1 illustrates the most major risk factors associated with mucormycosis.

Like etiological agents, risk factors of mucormycosis vary with the geographical areas e.g. hematological malignancy is the most common risk factor in Europe, and diabetes mellitus in India, Mexico, Iran, and different countries of the Middle East and North Africa [30, 31, 34, 40-45].
TRANSMISSION AND CLINICAL PRESENTATION OF MUCORMYCOSIS

Mucorales, also known as thermotolerant molds, are found widely in the environment, particularly in soil and decaying organic matter such as compost piles, leaves, rotten wood, and animal excreta. Mucorales sporangiospores with a diameter of 3-11 μm can easily be aerosolized and dispersed throughout the environment. Because the spores are airborne, they can be found on any human surface that comes into touch with air, and this is the primary mechanism of transmission. In addition, soil to skin penetration through any burned, cut, or wounded skin and another transmission mode like ingestion by contaminated food are also possibilities. Insects such as stings or bites can also take part in the transmission of fungal agents. Finally, nosocomial transmission occurs often via various transmissions route [46]. The transmission of mucormycosis to humans is illustrated in Figure 2.

Mucormycosis is known to be a threat for immunocompromised and immunocompetent patients (Figure 1). The symptoms of infection are mainly depended on the fungus growing site in the body. Mucormycosis has five major clinical presentations based on organ involvement: rhinocerebral (sinus and brain), pulmonary (lungs), cutaneous (skin), gastrointestinal (GI), disseminated, and rare conditions including kidney infection, osteomyelitis, peritonitis, and endocarditis [47, 48].
Figure 2. Transmission patterns of mucormycosis to humans.

**Rhinocerebral Mucormycosis**

A disease condition caused by Mucormycetes usually infect sinuses and can extend to the brain is called rhinocerebral mucormycosis. This is the most common among all forms of mucormycosis in patients having diabetes mellitus with ketoacidosis (70%) [49]. Rhinocerebral mucormycosis is rare but the disease may occur in patients who receive hematopoietic stem cells or solid organ transplants like kidneys [50]. Germination begins after inhalation of fungal spores into the paranasal sinuses, and it quickly spreads to the surrounding tissues. The initial sign is usually bloody nasal discharge [51]. Due to the disease, inflammation of the sinus and periorbital skin tissues occurs and can cause facial pain and numbness, blur or double vision, eyelid edema, and even vision loss. Because the fungus extends from sinuses to mouth, black, necrotic ulcers with pain in the hard palate are particularly prevalent. Moreover, the hallmark of rhinocerebral mucormycosis is the black, necrotic eschar on the face, however, the absence of this mark does not exclude the possibility. Fever and headache are common, but fever may not be found in up to 50% of cases. An elevated white blood cell (WBC) count is observed as long as the patient has active bone marrow. When the fungus reaches into the brain, partial paralysis, slurred speech, brain abscess, altered consciousness, and coma may be found [49].
Pulmonary Mucormycosis

Mucormycosis associated with the lungs is known as pulmonary mucormycosis. This form of mucormycosis is more common in leukemic patients who are receiving chemotherapy treatment and in hematopoietic stem cell transplant patients who have neutropenia [52]. Inhalation, hematogenous, and/or lymphatic dissemination are the most common causes of this infection. Because it is confused with pulmonary aspergillosis, the symptoms of pulmonary mucormycosis symptoms are nonspecific. Patients, on the other hand, frequently come to health care with high fever (38°C) and coughs that are resistant to broad-spectrum antibiotics. Chest pain, dyspnea, and hemoptysis are also present, but they are less common. Rarely, the infection might manifest as tracheal or endobronchial lesions, which can cause airways blockage, lungs collapse, and massive hemoptysis in diabetes patients. Besides lungs, the fungus may also invade the mediastinum, pericardium, and chest wall [53-55].

Cutaneous Mucormycosis

Immunosuppressed patients with a lack of normal protective skin barrier are at high risk of cutaneous mucormycosis. Typically, mucormycosis agents are incapable of penetrating the intact skin; however, if a burn or wound is present, the organism can easily infiltrate the skin and infect deeper tissues [50]. Catheter insertion and insulin injection sites increase the risk of cutaneous mucormycosis in immunocompromised diabetes patients. Mucormycosis agents are abundantly distributed in the soil, hence soil-associated penetration is common. Following penetration, the infection spreads to all the layers of the skin, including the fat and muscles, resulting in gangrene and hematogenous dissemination [56-58]. Clinical presentations include inflammatory reactions with tissue swelling, abscess formation, pus, and necrosis leading to tissue slough-off and large ulcer. Black eschars are often formed with the progression of the ulcer. Moreover, it has the potential to mimic gangrenous pyoderma and other bacterial or fungal infections [59]. Aerial mycelium is occasionally visible to the naked eye on cutaneous lesions [51].

Gastrointestinal (GI) Mucormycosis

Adults are less likely to develop GI mucormycosis, which is more common in preterm neonates and malnourished children. It is also found in patients with diabetes mellitus and a history of corticosteroid treatment [50]. The disease is promoted by spore ingestion through contaminated foods such as fermented milk, dried bread, and corn-origin alcohol [60, 61]. Furthermore, a scientist group reported healthcare-associated ingestion of sporangiospores during oropharyngeal examinations by tongue depressors [62]. The infection may occur in any part of the GI system, but the stomach, colon, and ileum are the most commonly affected. Clinical symptoms are nonspecific and vary depending on which part of the primary gastrointestinal tract is affected. Common symptoms include distended abdomen with pain, nausea, vomiting, and gastrointestinal bleeding. Patients often feel to have an intra-abdominal abscess. Fever and hematochezia can also be found [49].

Disseminated Mucormycosis

Mucormycosis that disseminate hematogenously from the primary site of infection is called disseminated mucormycosis. Lungs are the most common source of
mucormycosis-related dissemination. Dissemination can also occur from the GI tract, sinus, and skin. The brain, on the other hand, is the most frequent site of dissemination, followed by the spleen, heart, skin, and other organs. Rhinocerebral mucormycosis particularly, brain to cerebral dissemination is very common. Although disseminated mucormycosis has a mortality record of up to 100% if there is any involvement of the brain. The symptoms of this infection vary widely, as it is associated with the host, organ, and degree of vascular invasion [49, 50].

**MORTALITY/CASE FATALITY RATE**

Mucormycosis is a rare, but frequently deadly infection. The case fatality rate (CFR) of mucormycosis depends on different underlying locations and conditions, and the type of the fungus. Patients with disseminated infection and haematologic malignancies have a greater CFR of mucormycosis; also, patients on antifungal medication alone have a higher risk [63]. Since the disease is very rare, the determination of the exact data of morbidity and mortality rate of mucormycosis is difficult. However, previous several studies showed different data on CFR of mucormycosis (Table 1).

| Study number | Case fatality rate or mortality rate | References |
|--------------|-------------------------------------|------------|
| 1            | Overall 64% (11/17)                 | [64]       |
| 2            | Overall 54% (501/929)               | [39]       |
|              | - 96% of patients died with disseminated mucormycosis. |
|              | - 85% with gastrointestinal mucormycosis, and |
|              | - 76% with pulmonary mucormycosis |
|              | - 46% with sinus infection          |
| 3            | 32% (19/60)                        | [40]       |
|              | - 36% of patients were immunocompromised. |
|              | - 22% were immunocompetent.         |
|              | - 18% of patients treated with Liposomal Amphotericin B (L-AMB). |
| 4            | 11.5% (61/531)                     | [65]       |
|              | - 47.8% of patients with haematologic malignancies, and |
|              | - 36.4% with bone marrow transplantation. |
| 5            | 65% (20/31)                        | [66]       |
| 6            | 53% (10/19)                        | [67]       |
|              | - 75% of patient receiving antifungal treatment alone, and |
|              | - 14% receiving a combined treatment (antifungal and surgery). |
| 7            | 47% (108/230)                      | [30]       |
|              | - 76% of patients with Hematopoietic stem cell transplantation (HSCT) |
|              | - 58% with disseminated mucormycosis |
|              | - 56% with pulmonary mucormycosis |
|              | - 52% with haematologic malignancies |
|              | - 44% with diabetes.               |
| 8            | 44% (44/101)                       | [31]       |
|              | - 25% of patient with rhinocerebral mucormycosis, |
|              | - 79% with disseminated mucormycosis |
|              | - 48% with pulmonary mucormycosis, and |
|              | - 22% with cutaneous mucormycosis.  |
PATHOGENESIS AND GENETIC VIRULENCE FACTORS

Because the Mucorales fungus is so common in the environment, such as dirt and decomposing waste, humans are constantly exposed to it [68]. However, the disease-producing efficiency of these fungi is rare, as most of them are less virulent [68]. The mucormycosis may cause death in immunocompromised patients having diabetic mellitus, receiving glucocorticoids and organ transplantation, showing neutropenia, hyperglycemia, skin burning, and/or elevated serum iron level [38, 69]. The fungal spores firstly evade the host’s innate immune system after successful entry either through inhalation, ingestion, or traumatic skin, followed by occurring germination, angioinvasion, and tissue necrosis [68, 69]. Depending on the route of infection, mucormycosis lesions may develop at sites such a lung alveolus, eyes, gastrointestinal tract, cutaneous, etc. Spore coat protein homologs (CotH) of Mucorales can express on the fungal surface and interact with the host cellular receptor named glucose-regulated protein 78 (GRP78) of the endothelium. This phenomenon assists to intervene in the host cell invasion and thereby succeeding angioinvasion [68, 70]. Mucorales then down-regulated several host defense, pathogen identification, and tissue repair genes, resulting in the lethal mucormycosis [28, 68].

High-affinity iron permease (FTR1) of Rhizopus oryzae is an important virulent factor because it expresses during the infection in diabetic ketoacidosis (DKA) mice [71, 72]. Fungal alkaline Rhizopus protease enzyme (Arp) plays an important role to enhance the coagulation process of mucormycosis patients [73]. ADP-ribosylation factor (Arf) and calcineurin (CaN) are required for fungal growth and structural alterations respectively, and hence operate as virulent factors in mucormycosis-causing species [74, 75].

PATHOLOGIES AND OUTCOMES OF COVID-19 AND MUCORMYCOSIS CO-INFECTON

COVID-19, caused by the SARS-CoV-2, is a severe viral pandemic disease of international concern throughout the world, affecting people of all ages [76]. Patients with comorbid conditions, immunosuppression, and immunodeficiency are at higher risk of developing a more severe form of COVID-19 [77]. Patients with the severe form of COVID-19 may require hospitalization, intensive care, and breathing support, followed by recovery or death [78]. There are various kinds of comorbidities associated with COVID-19 severity such as diabetes, chronic kidney disease, chronic lung diseases, chronic obstructive pulmonary disease, asthma, interstitial lung disease, cystic fibrosis, pulmonary hypertension, heart failure, coronary artery disease, cardiomyopathies, hypertension, HIV infection, thalassemia, cancer, cerebrovascular diseases, thalassemia, etc. [79-81]. Patients’ immune systems may be harmed as a result of these medical problems. In addition, most fungal infections are associated with the health and immunosuppressive conditions of the patients [15, 78, 82]. The pathogenesis of CAM is audited in Figure 3.
Various studies reported that around 5% of COVID-19 associated co-infections are fungal, such as *Aspergillus* and *Candida* [16, 83]. Although COVID-19 associated mucormycosis (CAM) is rare, when it does occur, it can cause serious consequences in patients [17]. According to recent case reports and clinical data, most of the CAM patients had diabetes mellitus, hypertension, asthma, obesity, hypothyroidism, ischemic cardiomyopathy, or end-stage renal disease [17]. In addition, Garg et al. [17] analyzed a total of nine cases with CAM and found that only two patients were alive and the other seven were died due to various complications. Several vital organs of the patients such as lungs, heart pericardium, brain, kidney, rhino-orbito-cerebral, etc. were affected during the CAM [17, 84, 85]. A 41-year-old COVID-19 patient with a history of type 1 diabetic mellitus (T1DM) was diagnosed with rhinocerebral mucormycosis [86], however, the patient was able to recover after receiving proper treatment. A recent systematic review on 101 cases of CAM showed that around 80% of patients were suffering from diabetes mellitus followed by diabetic ketoacidosis (DKA) (~15%) [87]. Surprisingly, most of the CAM patients (76%) have been treated with corticosteroids [87]. In addition, high dosages and long-time use of corticosteroid treatment cause significant immunosuppression in the patients [88]. The COVID-19 patients developed mucormycosis about two weeks after being admitted to the hospital, regardless of their age [17]. A clinical study in India found that 16 of the 18 COVID-19 patients were using steroids, 16 had diabetes, and 16 had mucormycosis [89]. Similar types of findings have been published by different research groups [90, 91]. Therefore, COVID-19 patients treated with high dosages of corticosteroids for a long time might be a predisposing factor for the development of CAM. The overall scenarios of several CAM cases are documented in Table 2.
Table 2. Comorbidities, major clinical pathologies and final outcomes of various COVID-19 associated mucormycosis (CAM) patients

| Cases No | Type of CAM          | Comorbidities/ Pathologies/Complications                                                                 | Final outcomes | References |
|----------|----------------------|----------------------------------------------------------------------------------------------------------|----------------|------------|
| 1.       | Pulmonary mucormycosis | Long-standing diabetes mellitus, hypertension, and ischemic cardiomyopathy, end-stage renal disease     | Recovered      | [86]       |
| 2.       | Disseminated mucormycosis | Obesity and hypothyroidism, multiple sub-segmental pulmonary emboli, pericarditis, pericardial effusion, steatohepatitis | Died          | [92]       |
| 3.       | Mucormycosis          | Hypertension, asthma, mild tachycardia, tachypnea, moderate bilateral maxillary sinus mucosal and ethmoid sinus mucosal thickening, mucosal opacification | Died          | [93]       |
| 4.       | Rhino-Orbital mucormycosis | Diabetes mellitus, multiple patchy ground-glass opacities in lungs, bilateral lid edema, soft tissue swelling in the right preseptal, malar, premaxillary, and retrobulbar regions | Died          | [94]       |
| 5.       | Gastrointestinal mucormycosis | Arterial hypertension, giant gastric ulcers and deep hemorrhages,                                          | Died          | [95]       |
| 6.       | Pulmonary mucormycosis | Tension pneumothorax with bronchopleural fistula, necrotic empyema in lungs                                 | Died          | [96]       |
| 7.       | Rhino-Orbital mucormycosis | Diabetes, asthma, hypertension, hyperlipidemia, asymmetric retrobulbar fat stranding and extensive opacification of right maxillary, ethmoid, and frontal sinuses, conjunctival chemosis, erythema and edema of the eyelids, kidney injury | Died          | [84]       |
| 8.       | Pulmonary mucormycosis | Arterial hypertension, opacification of the left maxillary sinus and thickening with sclerosis of sinus walls | Died          | [85]       |
| 9.       | Sino-orbital mucormycosis | Malaise, proptosis, chemosis, peri orbits cellulitis                                                      | Recovered     | [97]       |
| 10.      | Paranasal mucormycosis | Diabetes, opacification of the left ethmoid, maxillary and frontal sinus, thickening of the left optic nerve | Recovered     | [98]       |
| 11.      | Mucormycosis          | Diabetes mellitus, hyperglycaemia, thickening and perineural enhancement of the left optic nerve         | Recovered     | [99]       |

COUNTRIES AFFECTED BY COVID-19 ASSOCIATED MUCORMYCOSIS

COVID-19 associated mucormycosis (CAM) and other fungal co-infections are on the rise all over the world [17]. Currently, CAM is significantly higher in India compared to developed countries [87]. However, recently published data showed that CAM has been also reported in the USA, UK, Brazil, Italy, France, Iran, Turkey, Mexico, Austria (Figure 4) [17, 87]. Though there is no scientific publication, the various international and national reputed newspapers reported the CAM in Bangladesh (Figure 4) [100].
DIAGNOSIS

Rapid and accurate diagnosis of mucormycosis is always challenging. An experienced physician can diagnose the cases of mucormycosis based on clinical findings and the patient’s history of immunosuppression resulting from medical conditions such as hematological malignancies, uncontrolled diabetes, chemotherapy, etc. Radiologically showing multiple (≥10) nodules accompanying pleural effusion are frequently found to be liked with pulmonary mucormycosis. Computerized tomography (CT) scan is also used for observing such lesions of the respiratory organs. Among the CT scan, the positron emission tomography-computed tomography (PET/CT) aided with [18F]-fluorodeoxyglucose (FDG) is one of the most common tools [72, 101].

Isolation and identification of Mucorales by culture are well-established protocols for the diagnosis of mucormycosis. However, a fungal culture is far more difficult to cultivate than bacterial culture and may not always be effective. Because fungal hyphae are particularly friable in nature and readily injured during tissue manipulation, inoculum production from tissue samples needs to be gentle [102]. When clinical samples are utilized, it may take 3 to 7 days or more to observe fungal colonies on a fungal medium such as Sabouraud dextrose agar or potato dextrose agar, which are commonly treated with antibiotics to reduce contaminated bacterial development. Culture plates are usually incubated at 25°C to 30°C under aerobic conditions and in the presence of moisture. The slide culture technique is a reliable method to culture fungi that allow studying colonies without changing their morphology too much [51].

Direct microscopy and histopathological study of the affected tissues is also useful technique for the diagnosis of the disease. Tissue stains such as H&E (Hematoxylin and eosin) stain, PAS (Periodic acid-Schiff), or Grocott-Gomori’s methenamine silver staining are commonly used to observe fungal hyphae and morphology under the microscope [103].

Serological tests have high diagnostic values in disease diagnosis. In the serological test, either antigen or antibody has to be known to identify the unknown. Despite the possibility of cross-reaction in some of the serological tests, these tests are reliable
diagnostic tools. Serological tests such as enzyme-linked immunosorbent assay (ELISA), immunoblotting, and immunodiffusion tests are available for the detection of fungal antigen or antibodies responsible for mucormycosis [104-106].

Confirmatory diagnosis is based on agent identification using fungal culture, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), and DNA/genome sequencing [102, 107]. The internal transcribed spacer or the 18S rRNA genes specific for the etiology of mucormycosis are usually targeted by PCR (standard PXR, real-time PCR, and quantitative PCR/qPCR) [108-110]. In addition, spore coat proteins encoding gene (cotH) has also been used as the target gene for the detection of Mucorales [111]. Many of these PCR has been successfully applied for the confirm diagnosis of mucormycosis. Experimental findings suggest that PCR is capable to detect Mucorales from fresh and formalin-fixed clinical samples, blood, serum, etc. [112, 113]. However, further optimization of these protocols is underway to validate their application for routine diagnostic purposes [114].

LINE OF TREATMENT

After more than a year since the first appearance of the novel coronavirus infection, a novel, definite and effective therapy is yet to be developed. Despite the recent development of vaccines which are still undergoing various phases of clinical trials to determine their efficacy and safety, many therapeutic agents are being tested including antiviral drugs used in other viral infections. The current treatments are mostly symptomatic and for associated complications. Therefore, treating COVID-19 patients with weak immunity and comorbidity remains challenging [115]. Due to the associated comorbidity and immune-compromised conditions, patients are susceptible to grow severe deceitful infections such as different fungal infections [116, 117]. Recent cases of mucormycosis infection in COVID-19 patients are like to slay the slain. The treatment strategies for CAM have been recorded in a number of case reports published recently. Currently, two main treatment strategies are followed to treat this deadly fungal co-infection: (1) medication and (2) surgery, in addition to continued antiviral and symptomatic treatments of SARS-CoV-2 disease.

When compared to drugs/medicines available to treat bacterial or other fungal diseases, mucormycosis has a limited number of treatment options. Polyenes and azoles are the only two groups of compounds being employed in therapeutic practice. Furthermore, because mucormycosis is a rare disease with a wide range of hosts and infection sites, as well as a wide range of offending Mucorales, it is difficult to obtain reliable therapy data [118].

The basis of mucormycosis treatment of COVID-19 patients

The mucormycosis treatment strategy incorporates either simultaneous application of multiple interventions or with different timing and intensity. The main steps of mucormycosis therapies include the assessment of disease severity and aggressive clinical and laboratory diagnosis, timely initiation of effective antifungal therapy (monotherapy and combination therapy) alongside intense surgical debridement of skin lesions, and immunosuppression reversal by withdrawing chemotherapy and immunosuppressive drugs [118]. Rapid diagnosis and early therapeutic intervention are critical to avoid progressive tissue invasion which ultimately might decrease the need for extensive surgical intervention to improve survival [119].
Antifungal mono or combination therapy

Due to high morbidity and mortality, quick and aggressive administration of antifungal drugs is usually given. A number of drugs choice are available including amphotericin B, voriconazole, caspofungin, isavuconazole, posaconazole, echinocandin, anidulafungin, nystatin, etc. After reviewing 80 individual mucormycosis infections of COVID-19 patients extracted from different case reports published until May 2021, amphotericin B and its lipid complex formulation were predominantly used [17, 84, 120-124]. Besides, isavuconazole and voriconazole have also recently been considered as primary drugs of choice depending on the types of molds. However, according to the case reports (12/17), this drug was used mostly in either dual or triple combination with other mold-active antifungals such as amphotericin B, caspofungin, isavuconazole, and anidulafungin [121]. Posaconazole is used as rescue therapy when the first-line drugs do not respond.

In the treatment of mucormycosis, amphotericin B is the key drug of choice [118]. Lipid formulations of amphotericin B have a greater therapeutic effect over the conventional amphotericin B deoxycholate [125, 126]. The fungicidal and fungistatic effect of amphotericin B is concentration-dependent. It binds to fungal cellular ergosterol and alters membrane permeability and the leakage of cellular components [127]. Posaconazole is a second-line treatment for patients who are intolerant to amphotericin B or who require long-term maintenance medication [128]. Because experimental findings show that posaconazole can cause a breakthrough infection when administered as a prophylactic, it is not recommended as a first-line mucormycosis treatment [129, 130]. Isavuconazole also showed potent activity against mucormycosis agents and received approval for therapy. Posaconazole and isavuconazole both suppress fungal ergosterol production. Caspofungin and anidulafungin are cyclic lipopeptide Echinocandins group of antifungals that have specific activity against mucormycosis when used in combination therapy. They inhibit the β-D-glucan synthase presented in R. oryzae and damage the fungal cell wall [131].

The use of a combination of antifungals in severely immunocompromised individuals is becoming more widespread due to synergistic effects and larger coverage. However, antagonism, drug-drug interaction, toxicity, and expense are the drawbacks [132]. An in vitro experiment showed that anidulafungin and posaconazole had a synergistic effect on Aspergillus fumigatus infection, with enhanced survival and lower fungal load when compared with their individual effects [133]. Moreover, the isavuconazole-micafungin combination exhibited synergistic activity against Aspergillus species ex vivo [134]. Furthermore, statins such as lovastatin exhibited anti Rhizopus spp. activity both in vitro and in vivo when combined with unreliable limited clinical evidence [120, 135].

Adjunctive therapy and surgery

It is well established that immunosuppressive patients are more prone to mucormycosis infection. Patients with COVID-19 immunity who also have diabetes mellitus are widely challenged. In addition to this, the use of steroids in moderate to severe COVID-19 patients further imposes immunosuppression. Immunosuppression setback strategy could be noteworthy for mucormycosis. Corticosteroid-mediated immunosuppression in COVID-19 patients should be lowered or switched to an alternative non-steroidal medication [118]. For individuals with uncontrolled diabetes and/or ketoacidosis, rapid glycemic management is critical. Sodium bicarbonate treated reversal of acidemia reasonably disabled the invasion ability of R. oryzae into the
endothelial cells [136]. Increased iron assimilation and serum levels cause enhanced growth of Mucorales and their pathogenesis as confirmed by in vitro and pre-clinical, and retrospective human studies [137, 138]. Therefore, iron chelators have been recommended as a possible supplementary treatment by researchers. Preclinical investigations demonstrated that when mice were administered deferasirox, they lived longer [139].

On the other hand, a large number of populations worldwide take zinc tablets to enhance their immune system and fight COVID-19. A recent report states that the sales of zinc supplements jumped by 93% in India during 2020. Zinc's importance in the therapy of COVID-19 has been related to its immunomodulatory properties, antiviral properties, and capacity to control the inflammatory response [140]. A number of studies have been reported that show the effectiveness of zinc therapy in the treatment of COVID-19 patients [141]. However, zinc acquirement is vital during the fungal life cycle for proper development when they are saprophytes or even during the infection process [142]. Therefore, zinc chelation is another approach to treat mucormycosis. Study reveals that different zinc chelators such as clioquinol, phenanthroline, and N,N,N',N'-tetakis (2-pyridylmethyl) ethane-1,2-diamine in combination with either amphotericin B or posaconazole showed inhibitory effect against 25 Mucorales strains, with the clioquinol-posaconazole combination being the most active [143]. Reduced Zn hinders fungal growth by inhibiting the activity of Zn-binding transcription factors which are involved in fungal biological processes [142].

Treatment with granulocyte (macrophage) colony-stimulating factor or interferon- has been proposed as adjunct therapy [144, 145]. In a recent case report, combined therapy of interferon-ϒ and nivolumab successfully treated an intractable mucormycosis in an immunodeficient, trauma patient [146].

During mucormycosis, blood vessel thrombosis and the resultant tissue necrosis can prevent antifungal medicines from reaching the infection site. As a result, debridement of necrotic tissues may be necessary for full mucormycosis eradication. Patients who did not have their mucormycosis surgically debrided had a far greater death rate than those who did [130].

A special observation to consider in pregnant COVID-19 patients with mucormycosis infection

The acute inflammatory response in COVID-19 causes erythrocytic damage, leading to increased iron loss from the body. This exacerbates iron deficiency anemia and may worsen coronavirus infection [147]. Iron deficiency anemia in pregnancy is common and iron salt supplement is generally given. However, a higher level of iron provides a favorable environment for mucormycosis development. The relationship between the growth of mucormycosis and iron level has been discussed above. Therefore, iron supplementation in pregnant COVID-19 patients may need careful monitoring. Early iron deficiency anemia diagnostics, which involves measuring serum ferritin and iron levels in COVID-19 positive pregnant women, as well as a proper data interpretation on serum ferritin levels in pregnant women with COVID-19 associated mucormycosis, is important.
New antifungal candidates in the pipeline

The search for new antifungal medication is still ongoing due to the restricted number of available drugs. Recently, a novel glucan synthase inhibitor SCY-078 demonstrated strong fungicidal activity against azole-susceptible strains of *A. fumigatus* when given alone. The activity was better than amphotericin B and voriconazole. It also exhibited synergy with voriconazole, isavuconazole, and amphotericin B [148]. Another novel fungal CYP 51 inhibitor VT-1161 possess in vitro activity against *R. oryzae, Lichtheimia*, and *Cunninghamamella*. APX001A (formerly E1210) is an antifungal agent which is under phase I clinical trials protected immunosuppressed mice with *R. delenar* infection [149]. Lastly, haemofungin hinders in vitro growth of a variety of fungi including *Rhizopus* [150]. The re-emergence of the CAM epidemic in India will undoubtedly push scientists hard to discover novel antifungal agents.

CONCLUSION AND RECOMMENDATIONS

Mucormycosis might be caused by various species of genus *Rhizopus, Mucor* and *Lichtheimia* and its incidence have been increasing recently due to the high number of patients with immunosuppression. There is a strong correlation among pre-existing risk factors for the development of mucormycosis and CAM. The CAM has been becoming an emerging threat to the patients suffering from various comorbid medical conditions such as diabetes and treatment with immunosuppressive drugs such as steroids. Therefore, the COVID-19 patients should be carefully treated with immunosuppressive drugs and if possible, it should be avoided in the cases of pre-existing factors for CAM. Immediate diagnosis and treatment should be started if the COVID-19 patients are suspected of CAM to prevent the severe course of the disease.

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AUTHOR CONTRIBUTIONS

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CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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