The Pathogenesis of Fungal Coinfections in COVID-19 Cases

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a respiratory disease carriedon by the SARS-CoV2 virus, and is responsible for lower respiratorytract infections called Acute Respiratory Distress Syndromes (ARDS). The first case of COVID-19 reportedly happened in the traditional market located in Wuhan, South China. The cause of several pneumonia cases with an unknown cause in December 2019 has been identified as a novel coronavirus, according to reports. WHO designated these respiratorytract infections Coronavirus disease-2019 (COVID-19) in February 2020. The significant rise in cases made WHO state the novel coronavirus as an emergency case for international concerns until March 11, 2020, and COVID-19 was officially stated as a pandemic. The earliest time of the COVID-19 outbreak, Wenzhou, one of the busiest cities in China, has the highest number of reported cases, with 499 of 1162 cases reported in China on February 15, 2020. Wenzhou’s worst cases and death rate compared to Hubei are 2.6% vs 0% (1457/54,406 vs 0/499) and 15.2% vs 5.2% (8276/64,406 vs 26/499), respectively. This stark contrast highlights the significant difference. Many problems can occur in COVID-19 patients, including complications due to fungal coinfection that can aggravate and even cause death inpatients infected with COVID-19. Several fungal coinfections in COVID-19 patients, such as Aspergillosis, Candidiasis, and Mucormycosis, have been found in patients with COVID-19 infections. Forty-one cases of COVID-19 associated with mucormycosis (CAM) were reported in March 2021, and 70% of them come from India. Telangana disclosed 50 cases per day, and 1196 cases were reported until June 9th, 2011, in Tamil Nadu. Another finding states that until June 2021, in a total of 275 cases of CAM, 223 cases
were found in India and the other 42 cases from other countries. Studies in the United Kingdom and Spain reported 12.6% and 0.4% of patients experienced an incidence of COVID-19-associated candidiasis (CAC), respectively. Furthermore, 23.3% of Aspergillus coinfection with COVID-19 (CAPA) was reported in China. However, research on the invasive mechanism and fungal coinfections in COVID-19 patients, especially CAPA and Mucormycosis, is still limited. Nevertheless, researchers have revealed several possible mechanisms of fungal coinfections in COVID-19 patients.

This review aimed to summarize these several possibilities of the related mechanisms. The recognition of finding the pathway is crucial for the specific management of affected patients.

METHOD

This article was compiled using a literature search approach from several kinds of literature, such as books and articles that published in English. Pubmed and Google scholar searches were performed from August 2021 until November 2022. The keywords were ‘COVID-19’, ‘fungal coinfection’, ‘COVID-19’ AND ‘coinfection fungal’, ‘mucormycosis’ AND ‘COVID-19’, ‘aspergillosis’ AND ‘COVID-19’, ‘candidiasis’ AND ‘COVID-19’, ‘invasive fungal infection’. All the studies related to the pathogenesis of fungal co-infection in COVID-19 patients were used. The information was explained as text and figures.

RESULT AND DISCUSSION

SARS-COV2 and Covid-19

One of the CoVs, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2), has a 79% genomic sequence identity with SARS-CoV and a 50% genomic sequence identity with MERS CoV. The virion of SARS-COV-2 has a 29.9 genome size, and the nucleocapsid contains RNA genomic and phosphorylated nucleocapsid (N). The coronavirus genome (ORF) presents numerous open reading frames. 16 non-structural proteins (NSP-16) were created after a protease digested the 5′ ORF (ORF1a/b) that was translated by the host endoplasmic reticulum into pp1a and pp1b. The 3′ ORF, which comprises one-third of the genome, is responsible for decoding the structural and auxiliary proteins. One of the four essential structural proteins is the surface spike protein (S), which recognizes the angiotensin-converting enzyme 2 (ACE2) receptor on the host cell, binds to it, and helps the virus enter the host cell. The binding of the RNA by the envelope protein (E), matrix protein (M), and nucleocapsid protein (N) is the basis for virion formation.

The six accessory proteins that are encoded by ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10, and whose functions are still unknown, are another characteristic of SARS-COV-2. Most of the proteins that ORF1a and ORF1ab encode are necessary for virus replication and, at the very least, host adaptation. The SARS-COV-2 genome’s 5′ and 3′ untranslated regions (UTR) have also been discovered. NSP 12 is a dependent RNA polymerase that replicates the viral RNA as part of a complex replica/transcriptase formed by some NSPs. But for it to work properly, NSP 7 and NSP 8 are needed. More than 85% of the amino acid sequences in the 12 structural proteins of SARS-COV-2 are identical to those of SARS-CoV. The board receptor of SARS-COV-2, angiotensin-converting enzyme 2 (ACE 2) could be recognized in various types of animals apart from humans, which emphasizes the wide range of infections.

The pathogenesis of COVID-19 could be started from the transmission of the virus, which occurs through respiratory droplets, direct contacts, and the fecal-oral route. The first suspected primer virus multiplication occurred on the upper tract mucosal epithelium and continued to the lower tract and gastrointestinal tract, which can eventually lead to mild viremia. Some patients would show asymptomatic symptoms, while others show non-respiratory signs such as diarrhea, kidney failures, and other symptoms involving several organs in the body.
Once SARS-COV-2 invaded the lower lungs, various cells, including vascular endothelial cells, alveolar airway epithelial cells, and alveolar cell macrophages, would get infected. When this virus enters the body, it will be detected by the innate immune sensor cytosolic and Toll-like receptor (TLR) on endosomes signaling the production of interferon type I/III (IFN) and proinflammatory mediators. The increasing seven inflammatory cytokines amplify tissue damage through endothelial dysfunction and vasodilatation, causing the presence of macrophages and neutrophils. The vascular leakage and the barrier function disruption increase endothelin and lung edema, restricting the gas exchange and causing hypoxia that eventually led to respiratory failure. The lung’s hyperinflammation further induces transcriptional alterations in neutrophils and macrophages, which makes the tissue damage permanent and leads to irreversible lung damage. The latest report shows that systemic inflammation inducts a long-term effect on the heart.

The cytokine storm is reputed as the leading cause of death in COVID-19 patients. Patients with older age (>60) and pre-existing disease (comorbid) have a higher risk of developing ARDS and death. The deadly symptoms of ARDS prevent oxygen from entering the lung and circulating in the body, causing grave harm to respiratory tract disorder and lung disease. Many things are associated with ARDS, namely genetic susceptibility and inflammatory cytokines, as well as several related genes such as ACE2, Interleukin 10 (IL-10), tumour necrosis factor (TNF), and vascular endothelial growth factor (VEGF).

**The Mechanism of Fungal Infection**

Less than 500 species of fungi are responsible for human and animal infections. The more days passed, the more opportunistic fungi mixed up, causing severe disease in the host with a compromised immune system. The genus Candida is currently included in the order Saccharomycetales of Ascomycetes. This genus is commensals to humans and often isolated from urine, skin, and gastrointestinal tract, which opportunistically causes candidiasis. Candida is pathogenic due to many virulence factors that help in adhesion to the mucosa, the ability to evade host defences, and the production of tissue-damaging hydrolytic enzymes. One of Candida’s important virulence factors is its ability to produce mucosal adherence and biofilms. By preventing the drug from penetrating the matrix and shielding it from the human immune response, it offers excellent resistance to antifungal treatment. Additionally, the ability of Candida to switch between yeast and hyphal growth is strongly linked to its virulence. Aspergillus, the genera of the Trichomaceae family, secretes enzymes with various functions, such as protease, hydrolase, and catalase. For instance, these enzymes directly influence the expression of virulence-related attributes in Aspergillus fumigatus by activating various pathways. Aspergillus’s virulence is influenced by a combination of biological traits unique to the fungus and the immune health of the host. Another genus Cryptococcus, are one of the Basidiomycetes with capsules oval yeast-like fungus. One of the Cryptococcus genera, C. neoformans has degradation enzymes such as asprotease and lipase as its virulence factor. C. neoformans are infamous for expressing two virulence factors that disrupt the host immune system: capsule and melanin. The prominent of these components is the double function not only as virulence factors, but also responsible for protecting the fungus from several immune response attacks.

Mucorales is responsible for rhinocerebral infections, pulmonary, skin, or disseminated infections characterized by angioinvasion, necrosis, and severe prognosis despite antifungal therapy and surgery today. Mucorales are commonly found in soil, spoiled food, manure and dust. In the fungus, high-affinity iron permease (FTR1) plays a role in iron absorption and transport, particularly in an environment with low ferritin levels. Another virulence factor of Mucorales is the spore mantle (CotH) detected in all of Mucorales fungus but absent in other species, playing the role of invasion agent and disrupting the host immune. Mucormycosis is distinguished by the development of hyphae on and around blood vessels that lead to disrupting the blood vessel, tissue damage, thrombosis and necrosis.
Innate immune cells play a role in the host’s response after fungi invade the mucosa. The innate immune defense is quite specific. The pattern recognition receptor (PRR) activates the response because it can recognize the microorganism’s conserved structure. The most important PPRs in the immune response are C-type lectin receptors (CLRs) that is Dectin-1, pentraxin-3, toll-like receptor, and Nod-like receptor (inflammasome).29 In the patient with the immunocompromised, the abundance of Fe creates a favorable environment for fungal growth. Mucorales use its high iron affinity permease and use it for their growth.31 The spore coat protein (CotH) in the spore surface penetrates, interferes, and damages the immune cells.34

The epithelial cells are the front barrier that contacts the fungus, especially Mucorales, which disrupts the cells with the increased signaling of platelet-derived growth factor receptor B (PDGFRB) and serve as a proper environment to grow.31 The neutrophils are the first barrier against fungi that activate the innate immune defense and regulate the adaptive immune systems.35 In diabetes mellitus patients or with steroid usage caused by ketoacidosis or hyperglycaemic, the chemotactic released by the neutrophil is decreased, causing hyphae growth increase. After entering the host cells, Mucorales produces the Mucorales-specific T-cells, which produce the proinflammatory cytokines such as interleukin (IL4, IL-10, and IL-17) and IFN-γ that stimulate T-cell CD4+ to disrupt the host’s cells. Additionally, the hyphae prevent the release of some immunomodulatory molecules secreted by NK cells in the early stages of infections, including RANTES (regulated upon activation, normal T-cells expressed and secreted) and IFN-γ. These mechanisms explain why immunocompromised patients are susceptible to mucormycosis and the potential in the host’s cell mechanism of action.31
Figure 1. *Mucormycosis Mode of Action*: Spores that enter immunocompromised patients through the respiratory route will attach to epithelial receptors and be detected by CotH receptors, then the things needed for the growth and development of fungal hyphae will be provided through the PDGFRB signaling pathway. When the fungus develops, the body will produce Mucorales-specific T cells with various proinflammatory cells, triggering a cytokine storm that causes cell damage.31

**Fungal coinfection in COVID-19 cases**

There are numerous potential causes of mucormycosis in COVID-19 patients, one of which is a variety of changes in the patient’s lung condition, which is suspected to be a focal point for fungal initiation.1,8,31,36 Furthermore, COVID-19 is linked to weakened immunity.2,22,31,37 Patients with heavy symptoms who have been hospitalized for a long time tend to use a ventilator, another way for spores to enter. On the other hand, high ferritin levels cause the release of ROS (Reactive Oxygen Species) that damages the surrounding tissues.8 The cytokines released while infected also increase the likelihood of intracellular iron entering circulation, increasing the risk of
Viewed from the patient’s immune response, the failure of the initial inflammation response could cause tissue damage and the spread of infections. The function of macrophages and neutrophils that directly act as a barrier against the entry of pathogenic fungi, killed and phagocytize spores and hyphae by producing and releasing perforins, antimicrobial enzymes, reactive oxygen metabolites, and cationic peptides are disrupted. The decrease of pro-inflammatory cytokines allows the spreading of fungal infections. Like the CAPA mechanism and its relation with COVID-19, mucormycosis also requires further research and understanding. A severe COVID-19 high-risk factor related to COVID-19 death risk is diabetes mellitus. Diabetes significantly increases glycemic control, disrupts phagocytic function, and delays the activation of the adaptive immune response, all of which severely damage the innate immune system. COVID-19 receptor (ACE2) was discovered in the lungs and pancreas. These proteins allow the entry of SARS-COV-2 and harm the pancreatic islet cells. Severe symptoms may increase insulin resistance by secreting stress hormones (cortisol and others) and cytokines.
Figure 3. Mucormycosis and COVID-19: COVID-19 patients are considered more susceptible to mucormycosis because their weakened immune condition and the use of suppressants as therapy will reduce the ability of phagocytic cells to fight fungi as foreign objects in the body.31

An important predisposition for CAM is corticosteroid. They are strong immunosuppressants with various effects on innate and adaptive immunity. The short-term glucocorticoid can trigger hyperglycemia and is proven as a predisposition to mucormycosis. Dexamethasone completely inhibits Aspergillus and Rhizopus phagocytosis in Drosophila melanogaster models. Dexamethasone and corticosteroids are commonly used to treat COVID-19, and their contributions to CAM pathogenesis appear undeniable.8

Prevention and therapy

Fungal coinfections in COVID-19 patients can trigger due to various things, such as the length of hospitalization and the severity of the patient’s illness, errors in treating patients, and the lack of supervision and early diagnosis of infection.40 Therefore, various preventive measures are recommended to reduce the chance of fungal infection in COVID-19 patients. One way to detect invasive aspergillosis in the body of COVID-19 is by detecting galactomannan, a polysaccharide antigen on the cell wall of Aspergillus spp., from bronchoalveolar lavage fluid (BALF). This method is known as precise and rapid detection to see IA in patients.29,41–46 In addition, PCR testing can be an alternative for early diagnosing fungal infections.47 Another way of screening for Candida spp. Routinely to reduce risk factors and improve routine treatment protocols.48

The treatment for fungi infection has been a broad topic mainly related to COVID-19. The Infectious Disease Society of America’s 2016 update guidelines discussed the treatment of Invasive Aspergillosis (IA) using alternative medicines or drugs. Despite theazole interaction effect, most patients treated with thiazole group drugs still need further observation. Amphotericin B is also commonly used as one of the IA treatments, as well as invasive candidiasis (IC). Another therapy uses echinocandins, liposomes, and monitoring for azole use for its effect optimization and
reducing its toxicity. 1 Treatment options for invasive mucormycosis (IM) include (i) an induction phase of amphotericin B deoxycholate + flucytosine, followed by fluconazole (or fluconazole + flucytosine or amphotericin B deoxycholate + fluconazole), (ii) a consolidation phase for fluconazole, and (iii) a maintenance phase (secondary prophylaxis) for fluconazole. A study that associated better survival with medical combination treatment using amphotericin b followed by isavuconazole or posaconazole reported a lower mortality rate in India, 36.5% lower than non-COVID-19 cases before, and to the latest study of CAM, 45.7%.

In addition to the antifungal combination, maintaining environmental conditions such as air circulation in hospital wards and oxygen therapy machines must be considered. The recovering patient was also advised to remain in the hospital for a few weeks to revive the immunity and prevent further complications. Waste disposal from patients is also one of the essential things to notice. The importance of fungal coinfection should be considered a priority to prevent further fungal transmission in COVID-19 patients.

CONCLUSION

The critically ill patients who are mainly admitted to ICU and require ventilators or are treated in the hospital for a long time are susceptible to fungal coinfection and have added up a worrying mortality rate in COVID-19 cases. The ability of fungi to evade host defenses, the adhesion sites in mucosa, and the production of tissue-damaging hydrolytic enzymes all contribute to their pathogenicity. The inflammatory mechanism is essential in the immune defense system and virulence agent from the fungus. Antifungal combinations, such as amphotericin B, fluconazole, flucytosine etc, should be considered for COVID-19 patients, especially those with immunity deficiency. In addition, the cleanliness of the medical equipment, especially the ventilators, the hospital ward, and the handling waste of COVID-19 patients are necessary to prevent fungal transmission in COVID-19 patients.

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CONFLICT OF INTEREST

There are no conflicts of interest, according to the authors.

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