

Study of COVID-19 Associated Fungal Infections at a Tertiary Care Hospital in Central India

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Abstract:

Background: The coronavirus infection pandemic of 2019 (COVID-19) has grown into a new worldwide health catastrophe, impacting thousands of humans. COVID-19 produces a variety of symptoms, involving severe various organ failures, acute respiratory distress syndrome (ARDS), and loss of life. Several occurrences of fungal co-infection as well as super infection have been described in various researches.

Materials and Methods: We included samples that were presented to the Department of Microbiology, Gandhi Medical College, Bhopal, for diagnosis of suspected fungal infection in the time period of April to June 2021 of individuals who had therapy for COVID-19 in the previous duration of three months or were presently receiving therapy for COVID-19. Samples were processed by Potassium hydroxide (KOH) wet mount preparation and fungal culture, microscopic identification was done, and data was collected in tabulated form.

Results: Overall, out of 332 samples, 235(70.8%) showed fungal elements by potassium hydroxide wet mount preparation, and 204(61.4%) were culture-positive. Out of culture-positive specimens, 61(29.9%) were *Rhizopus* spp., 31(15.2%) were *Mucor* spp., 41(20.1%) were *Aspergillus* spp., 11(5.4%) were *Candida* spp., 32(15.7%) were *Mucorales* with *Aspergillus*, 19(9.3%) were *Syncephalastrum* spp., and 3% were other fungal species.

Conclusion: Early screening and culture identification will aid in diagnosis, management, and treatment with anti-fungal agents, resulting in an improved prognosis in COVID-19 patients.

Keywords: COVID-19, Mucormycosis, Aspergillosis, *Candida*, Fungal co-infections.

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Introduction

The 2019 coronavirus infection outbreak (COVID-19) has grown into a major worldwide health epidemic impacting millions of people. COVID-19 produces a variety of symptoms and has the potential to result in severe organ failure across multiple organs, acute respiratory distress syndrome (ARDS), and loss of life. [1]

Previous research has found reports of infections, bacterial or fungal in origin together, which could raise fatal outcomes and morbidity from COVID-19. There have been multiple reports of simultaneous infections with COVID-19 and Mucormycosis. [2] Systemic steroids and antibiotics could lower the risk of severe COVID-19 adverse effects, but they also potentially promote infections that are opportunistic in nature. Such as Mucormycosis and Aspergillosis. There is a possibility. [3] Lymphocytes serves a vital function in ensuring immunological homeostasis

and providing a defence towards microbes that invade within the human body, hence it is possible that a low lymphocyte count is a contributor when it comes to secondary infections caused by fungi. [4] Patients admitted to the hospital for COVID-19 have a number of risk factors in common, including chronic respiratory disorders, corticosteroid medication, intubation or ventilatory support, and diabetes, immunocompromised states, neutropenia, organ transplantation (particularly lung transplantation), cytokinin storm, etc., that put them at risk of invasive fungal infections. [5,6] Infection can spread via spore inhalation (Aspergillosis), percutaneous inoculation (Dermatophytosis), commensal organisms like *Candida albicans* penetrating the mucosa, and ingesting a toxin from infected foods or beverages (gastrointestinal disease). [7] Virulence and pathogenicity are also contributed by enzymes such as extracellular

phospholipases, lipases, and proteinases; melanin formation, mannitol release, superoxide dismutase, rapid development and affinity for circulatory system, sensitivity to heat, and toxin generation.⁸ The most common medically important infectious agents of fungal origin in healthcare environments are *Aspergillus* spp. and *Candida* spp., Mucorales (e.g., *Rhizopus* spp.), Hyalohyphomycetes (e.g., *Fusarium* and *Scedosporium* spp.), or Phaeohyphomycetes (e.g., *Alternaria* spp. and *Cladophialophora bantiana*). [2,9,10]

Materials and Methods:

Our study is a hospital-based observational prospective study conducted from April 2021 to June 2021 in the Department of Microbiology, Gandhi Medical College, Bhopal. Patients who reported with clinical symptoms or who had received therapy for COVID-19 during the previous three-month period of manifestation (recent COVID-19) or who were receiving treatment for COVID-19 were considered as cases. Clinical characteristics, potential risk variables, physical findings, diagnostic workup, and medical treatment data were all gathered. The study comprised 338 patients. Samples of patients received in formalin or mismatched requisition forms were excluded from the study. Samples were then processed through standard microbiological procedures.

Specimens, namely, aspirate, pus, exudate, tissue, tissue biopsy, and bronchoalveolar lavage (BAL), were collected in a sterile universal container. Specimens were collected, and a small amount of sterile saline was added and transported to the Microbiology laboratory at the earliest. On receipt, all the samples were subjected to direct microscopic examination by Potassium hydroxide (KOH) wet mount preparation. The hyphae were observed according to their diameter, septate or aseptate, branching angle (right or acute), and their colour. For Primary isolation on fungal culture media, the sample was directly inoculated on one set of Sabouraud dextrose agar (SDA) with chloramphenicol and gentamicin and incubated at ambient temperature and 37 °C.

Cultures were studied for growth every day during the first week and then every third day thereafter. The texture and surface colour of the colony, colour of the reverse (underside) of the colony, rate of growth, and pigment production were observed.

Microscopic examination of the growth was done by Tease Mount Lactophenol Cotton Blue preparation (LPCB) and was observed under the microscope with low-power (10X) and high (40X) magnifications. Samples that could not be identified by LPCB were subjected to Slide culture. (Figure 1)

Results:

After the application of exclusion criteria, 332 samples were included in the study and processed. Our study shows that out of 332 samples received, 235(70.8%) showed fungal elements by potassium hydroxide wet mount preparation, of which 204(61.4%) were culture-positive.

There were 128(38.6%) samples that showed no growth on culture. Out of culture-positive specimens, 61(29.9%) were reported as *Rhizopus* spp., 31(15.2%) *Mucor* spp., 41 (20.1%) *Aspergillus* spp., 11(5.4%) *Candida* spp., 32(15.7%) Mucorales with *Aspergillus*, 19 (9.4%) *Syncephalastrum* spp., and 3% as other fungal species. (Figure 2 and Figure 3).

Discussion

COVID-19 was classified a public health emergency of global importance by the World Health Organisation (WHO) in February 2020. Since that time, it has grown into a global pandemic with significant morbidity and mortality. COVID-19 manifestations can vary from moderate to potentially fatal incidents caused by bacterial and fungal mixed infections (Mehta S, Pandey A.2020). [11]

Although invasive fungal infections are uncommon in healthy people, they are predisposed to the same by a number of immunodeficient states. Uncontrolled diabetes, haematological and other cancers, organ transplantation, persistent neutropenia, immunosuppression medication, and treatment with steroids are all examples, which are robustly discussed in multiple literatures (Seyedjavadi SS, Bagheri P et al., 2022). [12] Pathophysiological factors such as a decrease in lymphocyte count, enzymes like extracellular phospholipases, lipases, and proteinases, the rapid development and affinity for the bloodstream, tolerance for heat, and toxin synthesis all play significant roles in the pathological process of these diseases. Our observational study was carried out from April to June 2021. According to a study in 2021, India reported nearly 47,000 mucormycosis patients that were associated with COVID-19 in just three months (Muthu et al., 2021). [13] Our study shows that out of 332 samples received, 235(70.8%) showed fungal elements by potassium hydroxide wet mount preparation, of which 204(61.4%) were culture-positive. There were 128(38.6%) samples that showed no growth on culture. Out of culture-positive specimens, 61(29.9%) were reported as *Rhizopus* spp., 31(15.2%), *Mucor* spp., and 41(20.1%) *Aspergillus* spp. and 11(5.4%) *Candida* spp. were more or less in agreement with the findings of (Ramaswami A, Sahu AK, et al. 2021), [14] but far from near the findings of (Seyedjavadi SS, Bagheri P et al.,2022) [12]: *Aspergillus* spp. (82 isolates), *Mucor* spp. (69

isolates), *Rhizopus* spp. (24 isolates), and *Candida* spp. (21 isolates). Similar outcomes of the highest prevalent fungal infections that occur together in patients with COVID-19, such as *Aspergillus*, invasive candidiasis, and mucormycosis, were also

documented in research undertaken in China and Spain. (Song et al., 2020; Pemán J et al., 2020). [15] We have also found 32 (15.7%) Mucorales with *Aspergillus*, 19 (9.4%) *Syncephalastrum* spp., and 3% of other fungal species.

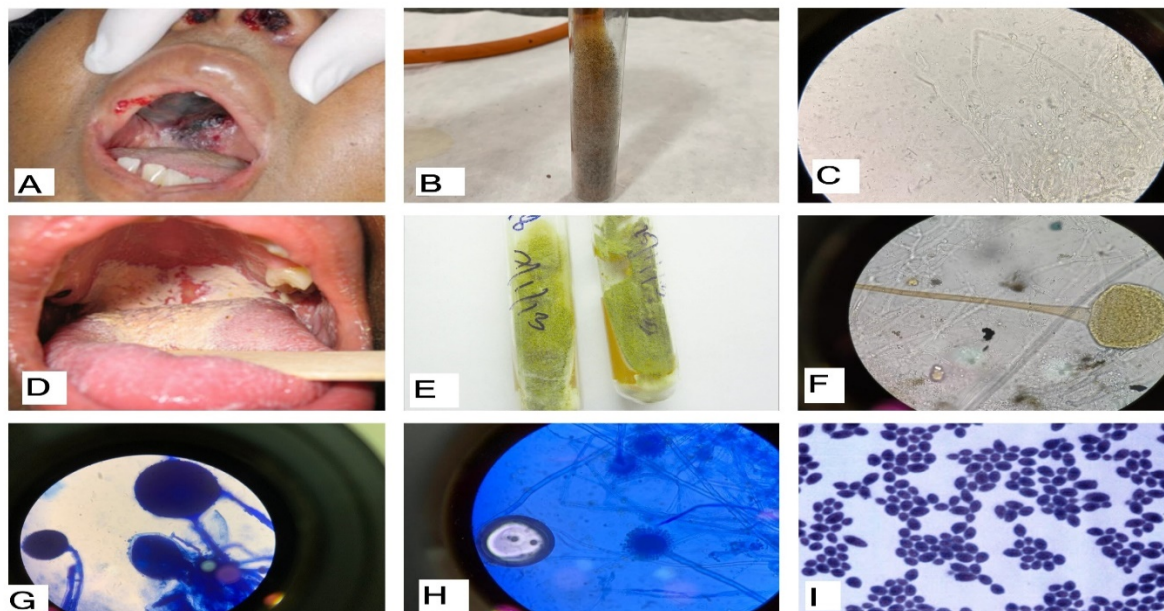


Figure 1: (A) Gross appearance of Mucor lesion in oral cavity; (B) SDA tube with salt and pepper appearance of *Rhizopus* spp.; (C) KOH wet mount preparation showing broad aseptate hyaline hyphae of Mucorales at 40X magnification; (D) Gross appearance of candida lesion in oral cavity (curdy white in appearance); (E) SDA tube with lemon green powdery colony of *Aspergillus flavus*; (F) KOH wet mount preparation showing hyaline septate hyphae with acute angle branching of *Aspergillus* spp. at 40X magnification; (G) Mucor on LPCB staining showing broad aseptate hyphae with columella extending into sporangium at 40X magnification; (H) *Aspergillus flavus* on LPCB staining showing thin long conidiophore and vesicle at the apex bearing phialides radiating from the entirety of the vesicle at 40X magnification; (I) Gram staining showing gram positive budding yeast cells, suggestive of *Candida* spp.

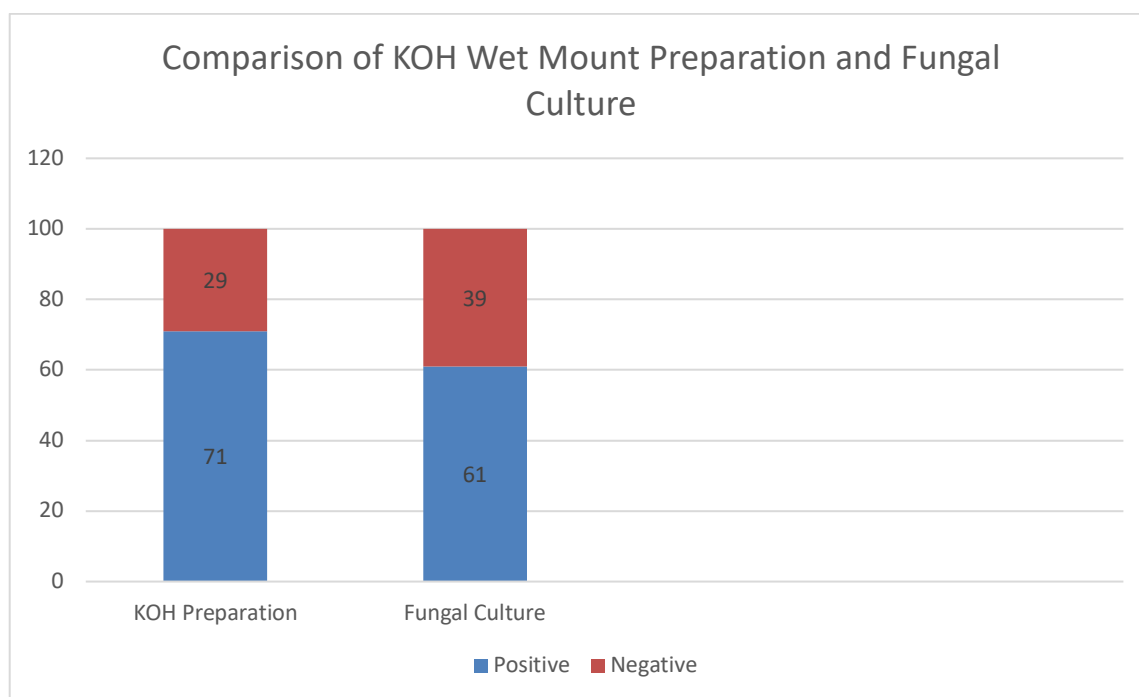


Figure 2: Bar chart showing comparison of KOH Wet mount preparation and fungal culture

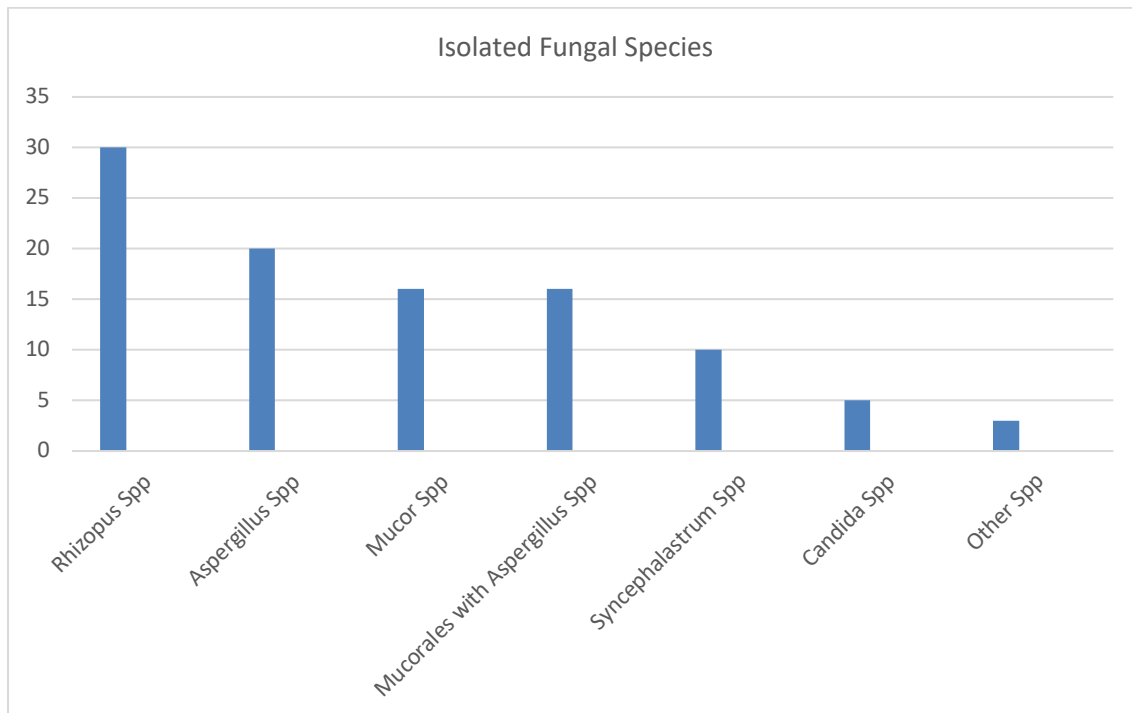


Figure 3: Histogram showing distribution of isolated fungal species

Conclusion

In the course of the global COVID-19 outbreak, increasing invasive fungal infections with comorbid conditions are linked to increased mortality as well as morbidity. In conclusion, doctors need to be aware of the potential risk of mucormycosis in patients with COVID-19 who are also recovering from it, particularly those who are receiving improper steroid therapy and have uncontrolled diabetes mellitus. Additionally, the responsible use of steroids must be emphasised. So early screening and culture identification will aid in diagnosis, management, and treatment with anti-fungal agents, resulting in an improved prognosis in COVID-19 patients. Our study concludes that fungal co-infections are associated with COVID-19 patients, Mucorales species being the most prevalent, followed by *Aspergillus* spp. and *Candida* spp. An extensive, multi-centric planned study would provide valuable information.

Authors' Contribution: All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

Data Availability: All datasets generated or analyzed during this study are included in the manuscript.

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