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Mucormycosis in COVID-19 Patients: A Hospital-based Study

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Abstract

Background: Fungal infections are increasingly being seen and mucormycosis is increasingly being isolated in COVID-19 patients, especially in those needing prolonged hospitalization. This cross-sectional study was carried out to isolate different fungi and identify their species in COVID-19 patients admitted for more than one month.

Methods: Sputum samples were taken from all the consecutive COVID-19 patients admitted in the hospital for more than one month and their health condition not improving despite the administration of antibiotics during May to July 2020. The samples were subjected to 10% KOH mount and Gram's stain, and cultured onto Sabouraud Dextrose Agar. This was visualized under direct microscopy. Fungus was identified based on its morphology.

Results: Out of the 100 patients enrolled during the study period, 74 were male and 26, female. The different species of fungi were isolated from eight samples (8%) out of which two cases were confirmed to be mucormycosis (2%).

Conclusion: We conclude that fungal infections, including mucormycosis, are not uncommon in post-COVID patients with long-term hospital stay. We recommend that the investigation and identification of fungus may be routinely followed.

Keywords: COVID-19, Fungal infection, Mucormycosis

Declarations

Ethics approval and consent to participate: This study was conducted with prior ethical approval from Ethical Review Board of Nepalgunj Medical College (Ref. No. 765/ 077/ 078) and informed consent has been obtained from participants prior to the enrollment.
Consent for publication: Informed consent was obtained from the patient for the publication of identifying features along with the manuscript.
Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. All relevant data are within the manuscript.
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Coronaviruses are a diverse group of viruses infecting many different animals. They can cause mild to severe respiratory infections in humans. In 2002 and 2012, respectively, two highly pathogenic coronaviruses with zoonotic origin, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), emerged in humans and caused fatal respiratory illness, making emerging coronaviruses a new public health concern in the twenty-first century [1]. At the end of 2019, a novel coronavirus designated as SARS-CoV-2 emerged in the city of Wuhan, China, and caused an outbreak of unusual viral pneumonia. Being highly transmissible, this novel coronavirus disease, also known as coronavirus disease 2019 (COVID-19), has spread fast all over the world [2]. It has overwhelmingly surpassed SARS and MERS in terms of both the number of infected people and the spatial range of epidemic areas. The ongoing outbreak of COVID-19 has posed an extraordinary threat to global public health [3].

Respiratory fungal infection is a severe clinical condition especially seen in immune-compromised patients [4]. Emerging evidence suggests that patients infected with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) may develop secondary bacterial and fungal infections [5]. Invasive pulmonary aspergillosis is increasingly being recognized in association with coronavirus disease 2019 (COVID-19), especially in critically ill patients hospitalized in the intensive care unit [6].

Zygomycosis includes mucormycosis and entomophthoromycosis [7]. Zygomycosis is better called by its specific names: mucormycosis [8]. They are non-septate hyphae, found in food items, soil, air, manure and decaying vegetables. Pulmonary mucormycosis is a progressive severe pneumonia. Mucor is characterized by rapidly growing light dull to grey colonies [7]. There are only a few cases of COVID-19 associated mucormycosis reported on rhinosinusitis mucormycosis due to Rhizopus oryzae [9]. This study was conducted to isolate different fungi and identify their species in COVID-19 patients admitted for more than one month.

METHODS

This hospital-based cross-sectional study was conducted in Nepalgunj Medical College, Kohalpur from May 2020 to July 2020. The medical college was converted into COVID dedicated hospital during the second wave of COVID-19 in Nepal. The study was approved by the institutional ethical committee and informed consent was obtained from all the patients and their relatives.

Figure 1: Black, powdery, wrinkled colonies of fungal growth on Sabouraud Dextrose Agar (SDA)

We enrolled all the patients admitted to the hospital for more than a month with their health conditions not improving despite the administration of antibiotics during the study period.

The sputum samples were collected in leak-proof containers, tightly sealed and transported in leak-proof zip plastic bags. Patient name, hospital identity number, date and time of collection were written on the sample container and other relevant information about the patients were recorded.

The sputum samples were transferred to the microbiology laboratory and processed immediately following the standard protocol of biosafety. The sputum smears were prepared, fixed by heat, and stained by Gram's stain. The smears were examined under a light microscope using an oil immersion lens.

The sputum samples were cultured on Sabouraud Dextrose Agar (SDA). The culture plates were incubated at 37°C for 48 hours. If the growth was not apparent within 48 hours, then the plates were re-incubated for

another 2 weeks. The colony characteristic was recorded if growth occurred; otherwise, the culture media was discarded. At the same time, Gram's stain, 10% potassium hydroxide (KOH) mount and Lactophenol cotton blue (LPCB) stain were carried out [10].

The glass rod was placed on a sterile petri plate side, and a sterile glass slide was put on the glass rod. A 1-by-1-cm block of SDA cut with a sterile scalpel was then transferred to the glass slide. Using sterile wire needle the fungus was then inoculated from the culture plate to the four sides of the agar block. The sterile coverslip was put over the block with slight pressure to ensure adherence. Approximately two mL of sterile water was added to the bottom of the petri plate, and then the plate was covered and incubated at 37 °C. After 48 hours of incubation, two drops of 10% KOH and LPCB were poured on grease free slide and a coverslip from growth SDA block was transferred and placed on it. Fungal morphology was studied under a light microscope using a low-power lens followed by a high-power lens. The fungal morphology characteristic was recorded [11].

Data was collected in a preformed data collection sheet, coded as per variables, entered in Microsoft Office Excel Worksheet, and analyzed. Categorical variables were described with numbers and percentages.

RESULTS

During the study period, sputum samples from 100 patients were studied and analyzed. There was no missing data. Their age ranged from 17-83 years. Fourteen patients were in the 0-30 age group, 64 patients in the 31-60 age group, and 22 patients in the 61-90 age groups (**Table 1**).

Different species of fungi were isolated from 8 samples (6 males and 2 females) (**Fig. 1**). Mucormycosis was isolated from the two female patients (2%) (**Fig. 2**). Rhizopus species was encountered in both cases of Mucormycosis species. Penicillium species was isolated from one male patient (**Fig. 3**) and Aspergillus species was isolated from five male patients (**Fig. 4**). All the isolated fungi were identified based on their morphology. All the fungi were isolated from patients of the age group 31-60 years.

DISCUSSION

In this cross-sectional study conducted among the 100 COVID-19 patients in a COVID dedicated hospital during the second wave of COVID-19 in Nepal, fungus was isolated from eight patients. All the fungi were isolated from 31-60 years age group.

The incidence of mucormycosis varies from 0.005 to 1.7 per million population [12]. The global mucormycosis case fatality rate is 46% [13]. The reported cases of secondary mucormycosis are limited. A case study

Table 1: Demographic profile of patients and fungi isolated (n = 100). Values are expressed as number.

Particulars		**No. of patients (N)**
Sex	M/ F	74/ 26
Age distribution(years)	< 30	14
	30 – 60	64
	> 60	22
Fungi isolated	Mucormycosis	2
	Penicillium	1
	Aspergillus	5
	None	92

Figure 2: Non-septate, sporangiospores, branching mycelia with hyphae, stolons, rhizoids of Rhizopus species (Mucormycosis species)

Figure 3: Hyphae with septa of Penicllium species

reported Rhinocerebral mucormycosis in a COVID-19 patient from Nepal [14].

An article about mucormycosis surge with the second wave of COVID-19 in India till April of 2021 revealed 68 cases of mucormycosis in patients of COVID-19. Rhino-ocular-cerebral infections comprised the majority (60/68). Less common sites included pulmonary mucormycosis (5/68) [15].

An author suggested that imminent threat has emerged as a challenge in the form of coronavirus disease-associated mucormycosis has risen more rapidly during the second wave compared to the first wave of COVID-19 in India, with at least 14,872 cases as of May 28, 2021 [16]. The state of Gujarat alone contributed to the highest number of cases, with at least 3726 cases of mucormycosis in patients with active and recovered COVID-19, followed by the state of Maharashtra, 90 deaths attributable to mucormycosis. Other states such as Rajasthan, Andhra Pradesh, Karnataka, Haryana, Madhya Pradesh, Uttarakhand, and Delhi have also shown a steady rise in the number of mucormycosis cases and deaths related to it. Even though no official figures about mucormycosis in COVID-19 cases were released by the Union Health Ministry during the first wave of COVID-19, India contributed to approximately 71% of the global cases of mucormycosis in patients with COVID-19 based on published literature from December 2019, to the start of April 2021.

A study summarized 28 articles reporting the original cases [17]. A total of 101 cases of mucormycosis (including confirmed [95/101] and suspected [6/101]) in people with confirmed (RT-PCR diagnosis) COVID-19 were retrieved. Largely, 82 cases (81.2%) of mucormycosis in patients with COVID-19 were reported from India, followed by 9 cases (8.9%) from the USA and 3 cases (3.1%) from Iran.

A report of 11 cases of mucormycosis in people with COVID-19 showed that mucormycosis was predominantly seen in males (78.9%), both in people who were active (59.4%) or recovered (40.6%) from

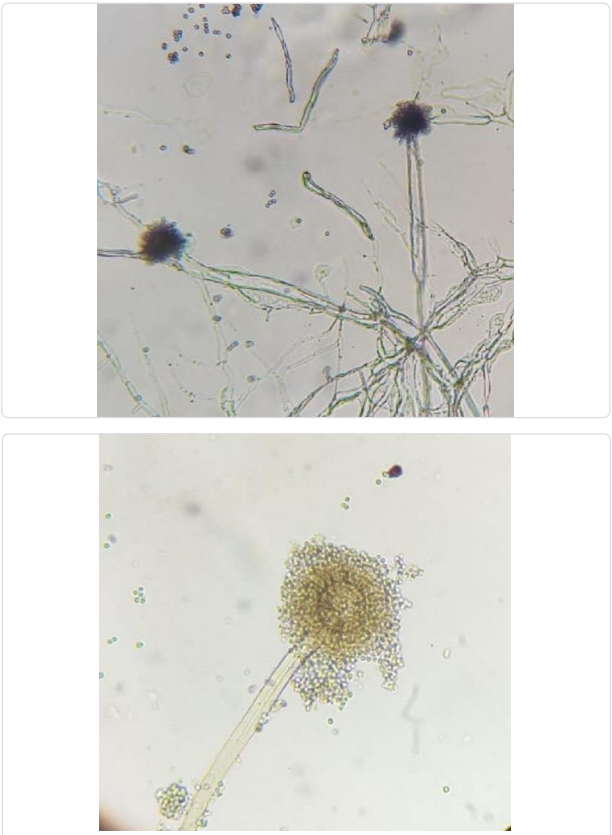

Figure 4: Conidiophores and spores of Aspergillus species

COVID-19 [18]. But our study showed mucormycosis was only found in female patients, which could be because of the small number of cases with fungal isolates.

Another limitation of the study was that identification of fungi was based only on the characteristic features of colonies and microscopic structure. Anti-fungal sensitivity tests were not performed.

CONCLUSION

Our study showed that different fungi were isolated from COVID-19 patients admitted for prolonged period in the hospital. Mucromycosis was predominant in females but other fungi like Penicllium species, Aspergillus species were predominant in males. Further studies are required to determine if respiratory fungal infection could be a cause of prolonged hospitalization after COVID-19 infection. We recommend work-up in line of fungal infection in such patients.

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