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The upsurge of mucormycosis at a tertiary care centre in Himalayan region, North India during the second wave of covid-19

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ABSTRACT

Background: Fungal infection especially mucormycosis seems remarkably upsurged in theCOVID era, especially during the 2nd wave peak of the pandemic raising the concern of the clinician for the admitted patient. Steroid therapy, diabetes and other immunocompromised states whenever associated more commonly involved with Covid Associated Mucormycosis(CAM).

Aim and Objective: To determine the prevalence of mucormycosis during the second wave of the COVID-19 pandemic and identify the risk factors associated with the same.

Methodology: Samples were received in the microbiology lab from all clinically suspected mucormycosis patients during 2nd peak process for KOH wet mount, fungal culture in SDA and COVID RTPCR testing. All relevant clinical and associated risk factors were tabulated and analysed.

Results: Out of 107, the total number of suspected cases of mucormycosisfor COVID-positivewas39(36.4%)and for COVID-negative cases was 68(63.6%) respectively. Males were predominantly involved & the rhino cerebral system was the most commonly involved site observed. High mortality(33.4%) was observed in COVID-associated suspected mucormycosis than in COVID-negative (5.9%) with a significant p-value(0.0005). CAM patients needed more frequent ICU admission (77%) as compared to non-covid mucormycosis patients (21.4%); found to be statistically significant (p-value of 0.007). A statistically significant association was found between CAM and immunocompromised states of the patients (p-value- 0.0472), the diabetes state (p-value - 0.0213) and the administration of oxygen therapy (p-value = 0.0183). The mucormycosis group (48.27%) were the most frequently fungal isolates seen among COVID-19 patients.

Conclusion: Mucormycosis infection is more commonly seen in COVID-19-infected patients as compared to non-COVID patients especially with comorbidities like diabetes mellitus, steroid usage and other immunocompromised states.

INTRODUCTION

The occurrence of the COVID-19 pandemic led to a rise in worldwide infection rates as more individuals fell ill with a virus called SARS-CoV-2, which is responsible for a severe respiratory ailment. This resulted in prompting the need for whole genomic

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sequencing and epidemiological studies to understand the severity of infections caused by emerging variants of concern [1]. Scientists and diagnosticians have been urgently investigating the causes of increased transmissibility of these strains to help control the surge in cases and alleviate human suffering [2]. While battling the challenges of the pandemic's second wave, a concerning rise in an invasive and potentially life-threatening infection known as "Black Fungus," caused by the Mucorales family, has raised global alarm [3]. In India, during the peak dominance of the Delta variant B.1.617.2, around 187 cases of Covid Associated Mucormycosis(CAM) were reported from April to June 2021 [4]. India witnessed a record of approximately 28,252 cases of mucormycosis, with 24,370 cases having a history of COVID-19 and around 17,601 cases reporting a history of diabetes [5]. The highest number of mucormycosis cases recorded was 6,329 [6].

The term "Mucormycosis" was coined by Baker in 1957 [7]. The filamentous forms of these fungi can easily evade a weakened immune system, leading to infections. The infection can be transmitted through air-borne or contact like inhalation of fungal spores or inoculation of the fungi into cuts or abrasions [8]. Mucormycosis can be classified into six types based on the location of occurrence, with the most common being rhino-orbital cerebral mucormycosis (ROCM), followed by cutaneous, pulmonary, gastrointestinal, disseminated, and uncommon sites [9].

While the commonly associated risk factors for CAM are underlying immunosuppressive conditions such as uncontrolled diabetes mellitus, prolonged corticosteroid usage, organ transplant, and hematological conditions, COVID-19 infection itself can act as a trigger for mucormycosis [10]. Other risk factors studied and reported include prolonged hospitalization, oxygen therapy, antibiotic use, pre-existing paranasal diseases, and cytokine storm [11,12].

Aims And Objectives

The aim of this study was to determine the prevalence of mucormycosis during the second wave of the COVID-19 pandemic and identify the factors that can increase the likelihood of acquiring the infection.

METHODLOGY

An observational study was conducted in the Department of Microbiology at the Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Jolly Grant, Dehradun, between April 15th and June 15th, 2021, for a duration of three months.

Inclusion Criteria

All suspected cases of mucormycosis admitted to the hospital were included in the study, regardless of age or gender. Patients already under treatment for mucormycosis and non-consenting patients were excluded.

Ethical Statement

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The study received approval from the ethics committee of the institute (EC reg. No.: ECR/483/Inst/UK/2013/RR-16).

Experimental Analysis

Samples such as nasal crust, nasal swabs, mucopus, and sputum were collected from suspected cases of mucormycosis for microbiological diagnosis. The samples were subjected to KOH Mount and fungal culture on Sabouraud's dextrose agar (SDA) and incubated at 25℃ overnight. Fungal identification was performed through Lactophenol cotton blue mount (LPCB) on the fungal growth obtained on SDA.

RESULTS

A total of 107 suspected clinical cases of mucormycosis were evaluated. Among the suspected cases, there were 39 (36.4%) COVID-19 positive and 68 (63.6%) COVID-19 negative cases. The median age distribution for suspected mucormycosis was approximately 46 years in COVID-positive patients and 57 years in COVID-negative patients. Males were predominantly affected in both COVID-positive (74.35%) and COVID-negative (64.70%) suspected mucormycosis cases (see Table 1). Rhino-cerebral involvement was the most common site observed in suspected cases, followed by pulmonary involvement in both COVID and non-COVID patients. The mortality rate was significantly higher in COVID-associated suspected mucormycosis cases (33.4%) compared to COVID-negative cases (5.9%) (p-value of 0.0005).

Table 1. Frequency of Suspected cases of Mucormycosis cases among COVID-19 positive and COVID-19 negative patients.

	Covid-19 Positive	**Covid-19 Negative**	**Total**	**P-Value**
Clinically Suspected cases of Mucormycosis (n=107)	39 (36.4%)	68 (63.6%)	107	
Median age	46 yrs	57 years		
Sex distribution	Male : 29(74.35%) Female :10 (25.64%)	Male : 44(64.70%) Female : 24(35.29%)	73(68.2%) 34(31.8%)	0.3020
Distribution of clinical type of suspected mucormycosis	Rhinocerberal-30(76.9) Pulmonary - 06(15.4) Disseminated-02(5.1) Cutaneous - 01(2.6)	Rhinocerberal40(58.8) Pulmonary - 23(33.8) Disseminated- 05(7.4) Cutaneous - 00	70 29 07 01	

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Mortality among suspected mucormycosis	Alive-26(66.6%) Died -13(33.4%)	Alive-64(94.1%) Died -04(5.9%)	90 17	0.0005
Confirmed cases of Mucormycosis	13 (33.3%)	14 (20.6%)	27	
Distribution of Confirmed cases of Mucormycosis in Ward and ICU	ICU - 10(77%) Ward- 03(23%)	ICU -03(21.45) Ward -11(78.6%)	13 14	0.007

The prevalence of lab culture-confirmed mucormycosis cases was 13 (33.3%) among COVID-positive patients and 14 (20.6%) among COVID-negative patients. COVID-associated mucormycosis patients required more frequent ICU admission (77%) compared to non-COVID mucormycosis patients (21.4%), which was statistically significant (p-value of 0.007).

Table 2. Mucormycosis association among COVID-19 positive and COVID-19 negative cases.

	Covid +Ve (n-39)	**Covid -Ve(n-68)**	**P Value**
Mucormycosis	13	14	0.1689
Other fungal infection	16	28	
Culture negative	10	26	

Microscopic evaluation of KOH mounts showed broad aseptate fungal hyphae (Figure 1), and lactophenol cotton blue mounts confirmed the presence of Rhizopus species (Figure 2). COVID-19 positive mucormycosis showed statistically significant associations with immunocompromised states of the patients (p-value = 0.0472, Fisher exact test), diabetes (p-value = 0.0213), and administration of oxygen therapy (p-value = 0.0183). In 41% of suspected mucormycosis cases, other pathogenic fungi were isolated, while 25.6% were culture-negative (Table 3).

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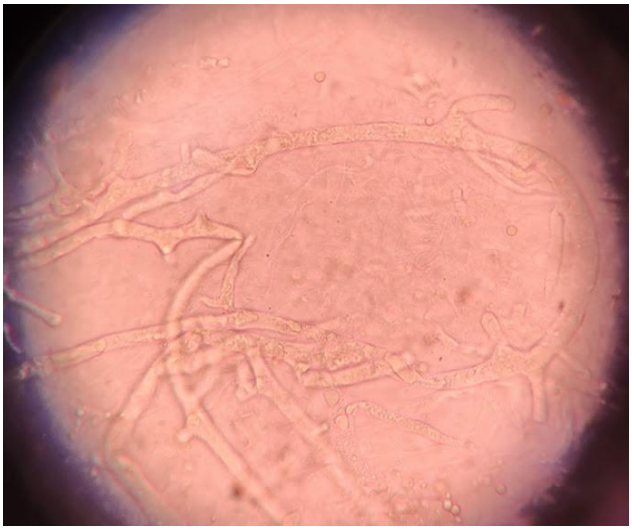

Figure 1: Broad aseptate fungal hyphae in KOH wet mount of nasal crust specimen

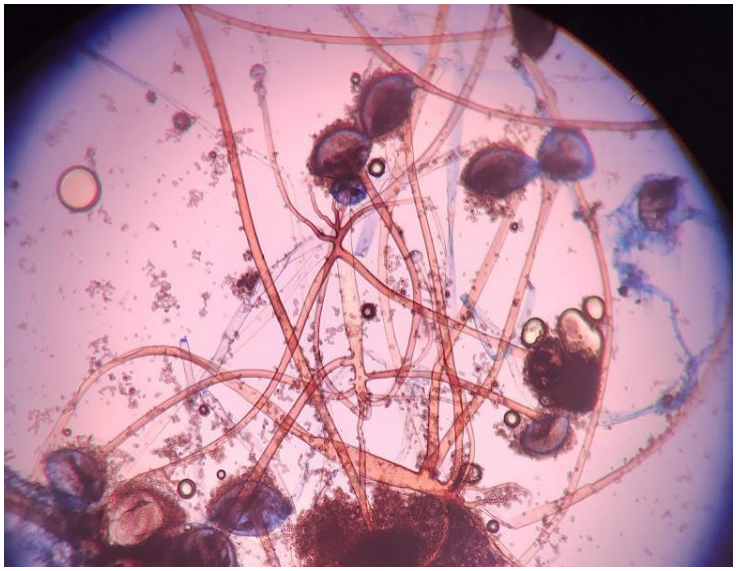

Figure2:Rhizoids, sporongiophore& columella of Rhizopus species in LPCB mount.

Table 3. Association between the covid-19 status of mucormycosis cases and various risk factors

	Immunocompromised	**Diabetes**	**Other comorbidities**	**Oxygen therapy**	**Steroid therapy**
Covid-19 positive mucormycosis	10 (77%)	09 (69.2%)	04 (31%)	08 (62%)	07 (54.5%)

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(n=13)					
Covid-19 negative mucormycosis (n=14)	04 (28.6%)	03 (21.4%)	04 (28.6%)	02 (14.3%)	00
p-value	0.0472	0.0213		0.0183	

Among the COVID-19 negative suspected cases, 41.2% had other pathogenic fungi isolated, and 38.2% were culture-negative. No statistically significant association was observed amongmucormycosis and COVID infection (p-value = 0.168). KOH wet mounts showed 89.74% sensitivity, while culture showed 74.35% sensitivity in clinically suspected mucormycosis patients. Among all the clinical samples received, mucormycosis accounted for 48.27% of the fungal isolates in COVID-19 patients, followed by Alternaria sp. (17.24%). Among mucormycosis patients, Mucor species (57%) were the most frequently isolated fungi in COVID-positive cases (Table 4).

Table 4: Sample wise distribution of fungal isolates recovered from suspected mucormycosis in Covid positive(n-39).

Sample	**KOH Findings**	**Frequency**	**Growth recovered on Fungal culture**	**No. of isolates**
Nasal Crust (n=17)	BAFH	14	Rhizopus species	6 (20.6%)
			Mucor species	4 (13.8%)
			Alternaria species	2 (6.9%)
			Fusarium species	2(6.9%)
			Trichophyton species	1(3.4%))
	SFH	01		
Nasal swab (n=20)	BAFH	05	Mucor species	3(10.3%)
			Alternaria species	3(10.3%)
			Candida species	2(6.9%)
			Fusarium species	2(6.9%)
			Cladosporium species	2(6.9%)
	NFES	06	Aspergillus species	1(3.4%)
Mucopus (n=01)	BAFH	01	Mucor species	1(3.4%)
Sputum (n=01)	SFH	01		0
Total(n-39)		35(89.74%)		29(74.35%)

Abbreviations: Broad aseptate fungal hyphae, SFH-Septate fungal hyphae, FH-Fungal hyphae, NFES-No fungal element seen.

The rate of secondary bacterial infection (Table 5) was higher in COVID-associated mucormycosis (48.2%) compared to non-COVID-associated mucormycosis (21.9%),

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and a statistically significant association was observed between bacterial coinfection and fungal-infected COVID patients (p-value = 0.0209).

Table 5: Bacterial co-infection association among COVID and non-COVID mycosis patient

	Bacterial coinfection (present)	**Bacterial coinfection (absent)**
Covid Associated Mycosis(n-29)	14	15
Non-Covid mycosis (n-41)	9	32
P value - 0.0209		

DISCUSSION

The sudden increase in mucormycosis cases during the COVID-19 pandemic has attracted the attention of researchers worldwide. In this study, the median age of suspected mucormycosis patients with COVID was relatively lower (46 years) compared to non-COVID patients (57 years), which is consistent with other studies [13,14,15]. Males predominated in suspected mucormycosis cases, similar to previous studies reporting male predominance [14,15,17,21]. The mortality rate among suspected mucormycosis cases was higher in COVID-positive patients (33.4%) than in COVID-negative patients (5.9%), and the prevalence of confirmed mucormycosis was higher in COVID-positive cases (33.3%) compared to COVID-negative cases (20.6%). COVID-associated mucormycosis patients had a higher need for ICU admission (77%) compared to non-COVID-associated cases (21.4%).

COVID-19 infection rates were notably higher among individuals with diabetes (69.2%), those undergoing oxygen therapy (62%), and those receiving steroid therapy (54.55%), when compared to those who do not have COVID-19. This research supports what other studies have found - that diabetes is a common health problem that occurs alongside other illnesses [13,18,19]. The lack of a statistically significant association between mucormycosis and COVID infection in our study might be due to the inclusion of acute COVID infections confirmed by RT-PCR, rather than previous infections with raised antibody titers as seen in other studies [20,27].

Mucormycosis, caused by various fungal infections within the order Mucorales and the family Mucoraceae, has been observed during the COVID-19 pandemic. In our study, Mucor species (27.5%) followed by Rhizopus species (20.6%) were the most frequently isolated fungi among COVID-positive patients, while other studies reported Rhizopus as the most common fungal isolate [19]. These fungi are typically non-pathogenic to immunocompetent individuals but can cause severe infections in immunosuppressed patients with risk factors like secondary infections, steroid intake and diabetes. Immune dysfunction, including decreased CD4 T cells, CD8 T cells, lymphocytes, delayed IFN-gamma response, and prolonged hyper-inflammatory states, exacerbates the cytokine storm and promotes the severity of COVID-19 infections [22,24]. Impaired

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macrophages and neutrophils' ability to kill fungal hyphae, along with increased fungal heme oxygenase enzyme promoting iron absorption required for fungal metabolism, contribute to severe infections, particularly in patients with uncontrolled diabetes [22,23].

Rhinocerebral involvement is the most commonly observed clinical form of mucormycosis, characterized by black necrotic eschar formation on nasal turbinates or palates [24].

The immune dysregulation mechanism also explains the increased rate of secondary bacterial infections in COVID-associated mucormycosis compared to non-COVID patients, as observed in our study (p-value < 0.05) [21,23]. The same mechanism justifies the increased rates of secondary bacterial (32.5%) and fungal coinfections (25.2%) in COVID patients reported in other studies [25], as well as the 21.8% secondary bacterial infection rate in CAM observed in another study [26].

CONCLUSION

The incidence of mucormycosis and other fungal coinfections has significantly increased in post-COVID-19 patients, particularly those with uncontrolled diabetes, immunocompromised states, steroid therapy, and living in unhygienic conditions. Strict monitoring of judicious use of antimicrobial drugs, early screening for fungal infections in suspected patients, and control of associated risk factors are crucial in preventing and reducing the overall morbidity of COVID-associated mucormycosis and COVID-associated aspergillosis.

Limitations of The Study

Our study lacks histopathological on the suspected mucormycosis patients.

STATEMENT AND DECLARATIONS

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Conflicts of interest: The authors have no conflicts of interest associated with the material presented in this paper.

Authors' contributions: Concept and Study design: GM and RS; Literature search: GM, RS and NK; Acquisition, analysis and interpretation of data: GM, RS, BK and SR.

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