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Risk factors for Coronavirus disease-associated mucormycosis

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ARTICLE INFO

Article history:

Accepted 27 December 2021

Available online 30 December 2021

Keywords:

Mucormycosis

COVID-19

Risk factors

Case control

SUMMARY

Background: The epidemiology of the Coronavirus-disease associated mucormycosis (CAM) syndemic is poorly elucidated. We aimed to identify risk factors that may explain the burden of cases and help develop preventive strategies.

Methods: We performed a case-control study comparing cases diagnosed with CAM and taking controls as recovered COVID 19 patients who did not develop mucormycosis. Information on comorbidities, glycemic control, and practices related to COVID-19 prevention and treatment was recorded. Multivariate regression analysis was used to identify independent predictors.

Results: A total of 352 patients (152 cases and 200 controls) diagnosed with COVID-19 during April-May 2021 were included. In the CAM group, symptoms of mucormycosis began a mean of 18.9 (SD 9.1) days after onset of COVID-19, and predominantly rhino-sinus and orbital involvement was present. All, but one, CAM cases had conventional risk factors of diabetes and steroid use. On multivariable regression, increased odds of CAM were associated with the presence of diabetes (adjusted OR 3.5, 95% CI 1.1–11), use of systemic steroids (aOR 7.7, 95% CI 2.4–24.7), prolonged use of cloth and surgical masks (vs. no mask, aOR 6.9, 95%CI 1.5–33.1), and repeated nasopharyngeal swab testing during the COVID-19 illness (aOR 1.6, 95% CI 1.2–2.2). Zinc therapy was found to be protective (aOR 0.05, 95%CI 0.01–0.19). Notably, the requirement of oxygen supplementation or hospitalization did not affect the risk of CAM.

Conclusion: Judicious use of steroids and stringent glycemic control are vital to preventing mucormycosis. Use of clean masks, preference for N95 masks if available, and minimizing swab testing after the diagnosis of COVID-19 may further reduce the incidence of CAM.

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Introduction

A recent upsurge in the cases of mucormycosis has been evident during the waxing and waning course of the COVID-19 pandemic, resulting in a syndemic named the Coronavirus diseases-associated mucormycosis (CAM).¹

CAM, Coronavirus-associated mucormycosis; RT-PCR, Real time polymerase chain reaction; CB-NAAT, Cartridge based nucleic acid amplification test.

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Mucormycosis is an opportunistic disease caused by angio-invasive fungi of the order *Mucorales*, with an estimated prevalence of 140 cases per million population in India.² It is characterized by the rapid development of tissue necrosis manifesting as rhino-orbital-cerebral, pulmonary, cutaneous, gastrointestinal, or disseminated disease. It is almost exclusively seen in patients having immunocompromised status due to diabetes, malignancy, chemotherapy, or immunosuppressive drugs.²

A multicentric cohort from India reported a two-fold rise in the prevalence of mucormycosis between September–December 2020 compared to the same time period in 2019 as a consequence of the increased burden of CAM.¹ Moreover, the number of cases rose even higher during the period of April–May 2021.³ High rates of morbidity and mortality, and exorbitant out-of-pocket expenditure for antifungal therapy with liposomal amphotericin B, posaconazole, and isavuconazole have made it imperative to prevent the occurrence of this disease.⁴

CAM occurs during or soon after convalescence from COVID-19. The epidemiological link with the COVID-19 infection suggests that novel non-conventional risk factors may have a role in CAM infection. Hypotheses abound in medical literature based on information available from mucormycosis before the onset of COVID-19, particularly existing knowledge of factors required for growth or inhibition of *Mucorales* in-vitro. We performed a case-control study comparing CAM patients with patients who have recovered from COVID-19 without developing mucormycosis, to investigate putative risk factors that may have contributed to the development of CAM.

Methods

An unmatched case-control study was performed at a tertiary care center in Delhi, India during the second wave of COVID-19 pandemic in the months of April–May 2021. Patients were selected either as cases of Coronavirus disease-associated mucormycosis or controls who had recovered from COVID-19 without developing mucormycosis. Informed consent was obtained from patients and the study was approved by the Institute Ethics Committee. [IECPG-353/28.05.21]

Patients above 18 years of age diagnosed with CAM were identified as cases. CAM was defined as mucormycosis based on clinico-radiological or microbiological evidence with fungal staining or culture. As per the consensus criteria, definite mucormycosis was defined as the demonstration of aseptate ribbon-like broad hyphae on histopathology of biopsied samples (with or without culture) and probable mucormycosis patients with suggestive clinical symptoms and radiologic imaging along with isolation of *Mucorales* from nonsterile samples such as swab sample from the nasal area.⁴ Controls were defined as patients who recovered from COVID-19 with symptom onset at least one month before recruitment and no clinical features suggestive of CAM. Controls were selected consecutively from records of the ward as well as outpatient clinic. The case definition by the World Health Organization (WHO) for definite or probable cases of SARS-CoV-2 infection was used to select both cases and controls, including requirement for a positive test among RT-PCR, rapid antigen test, or CB-NAAT.⁵ We limited both cases and controls to those who developed symptoms or tested positive for COVID-19 illness during the months of April–May 2021 to account for potential confounding factors including change in the viral strains. Patients who did not give consent were excluded.

We assessed the risk factors associated with the development of mucormycosis in patients with COVID-19 using a structured form that included relevant information regarding demographic details, comorbidities including diabetes mellitus⁶, glycemic control (poor glycemic control defined as blood glucose >200 mg/dL), COVID-19 vaccination status, COVID-19 related practices (mask-wearing, steam inhalation), COVID-19 infection severity as per WHO guidelines (oxygen or hospitalization requirement), and treatment given during COVID-19 illness particularly steroid therapy. Cases were interviewed during their admission while controls were contacted telephonically. An investigator-guided online form and digital consent was used for discharged patients. Missing information was supplemented using telephonic contact with patients' family members, discharge records, prescription slips, and hospi-

tal charts if needed. HbA1c and ferritin levels, if measured during the management of COVID-19, were recorded. Doses of various steroids (methylprednisolone, prednisone) were converted to equivalent doses of dexamethasone.⁷

Categorical variables were expressed as frequency and proportion of patients in the two groups and were compared using the chi-square test. Continuous variables were checked for approximate normality using the Shapiro Wilk test. Normally distributed variables were expressed as mean (SD) and compared using unpaired *t*-test, otherwise median (IQR) were presented and compared using Wilcoxon rank-sum test. A *p*-value of less than 0.05 was considered statistically significant. Univariate logistic regression for all potential risk factors was performed for the occurrence of mucormycosis in patients recovered from COVID-19. Variables with a *p*-value of less than 0.2 as well as known risk factors for mucormycosis were included in the multivariable model. Multivariable logistic regression analysis was performed with backward elimination to develop the final regression model. Statistical analysis was performed using Stata version 14 (TX, USA) and graphical representation using R version 4.0.2 and RStudio1.4.1717.

Results

A total of 163 cases of CAM were screened for inclusion. Eleven patients were excluded; seven did not give consent, three were diagnosed with *Aspergillus* on fungal culture, and one patient had suffered from COVID-19 more than six months before the diagnosis of mucormycosis. Finally, 152 patients of CAM (cases) and 200 patients of COVID-19 without mucormycosis (controls) were included in the study. Among the 152 CAM cases, 79% (*n*=120) were definite cases and 21% (*n*=32) were probable cases of mucormycosis. The spectrum of involvement among 152 CAM cases included rhino-sinus (*n* = 44, 29%), rhino-orbital (*n* = 72, 47.3%), rhino-orbito-cerebral (*n* = 22, 14.5%), isolated orbital (*n* = 2, 1.3%), rhino-orbital and palatal (*n* = 8, 5.3%), cutaneous (*n* = 1, 0.6%), isolated pulmonary (*n* = 2, 1.3%), and disseminated with rhino-sinus and pulmonary involvement (*n* = 1, 0.6%). Cases were interviewed at a median of 46 days (range: 30 – 70) and controls 48 days (range: 30 –72) after the onset of COVID-19 symptoms. The time period when COVID-19 symptoms began were distributed over April–May 2021 in both groups. (Fig. 1A) In patients with CAM, symptoms of mucormycosis began at a mean of 18.9 ± 9.1 (Range: 2–47) days after symptom onset of COVID-19. (Fig. 1B) The mean age of the study population was 48.2 ± 14 years with 226 males (64.2%). Age, sex, and body mass index (BMI) were similar in the two groups. A greater proportion of CAM cases resided in rural areas as compared to controls (*p* <0.001). (Table 1)

The most frequent comorbidities seen in our study population (*N* = 352) were diabetes (*n* = 196, 55.7%), hypertension (*n* = 125, 35.3%) and chronic kidney disease (*n* = 12, 3.4%). Diabetes was significantly more frequent among cases than controls (92.1% vs 28%, *p* <0.001). A third of the diabetic patients with CAM (*n* = 54, 35.5%) were newly diagnosed with diabetes during hospital admission for COVID-19 based on elevated blood glucose or abnormal HbA1c levels. None of our patients were diagnosed with malignancy or neutropenia, nor were receiving immunosuppression or chemotherapy prior to onset of COVID-19 symptoms.

Most of the patients in both groups had mild COVID-19. (Table 2). The requirement of hospitalization and oxygen therapy was similar in both groups. Patients with severe COVID-19 were more common amongst the controls (21% vs 9.9%, *p* <0.001). Ferritin levels during the acute phase of COVID-19 disease were available for 174 patients, which were higher in the CAM group (*p* <0.001). More than half of the study population received systemic steroids for the management of COVID-19, being more frequently used in CAM cases than controls (65.8% vs. 48%, *p* = 0.001).

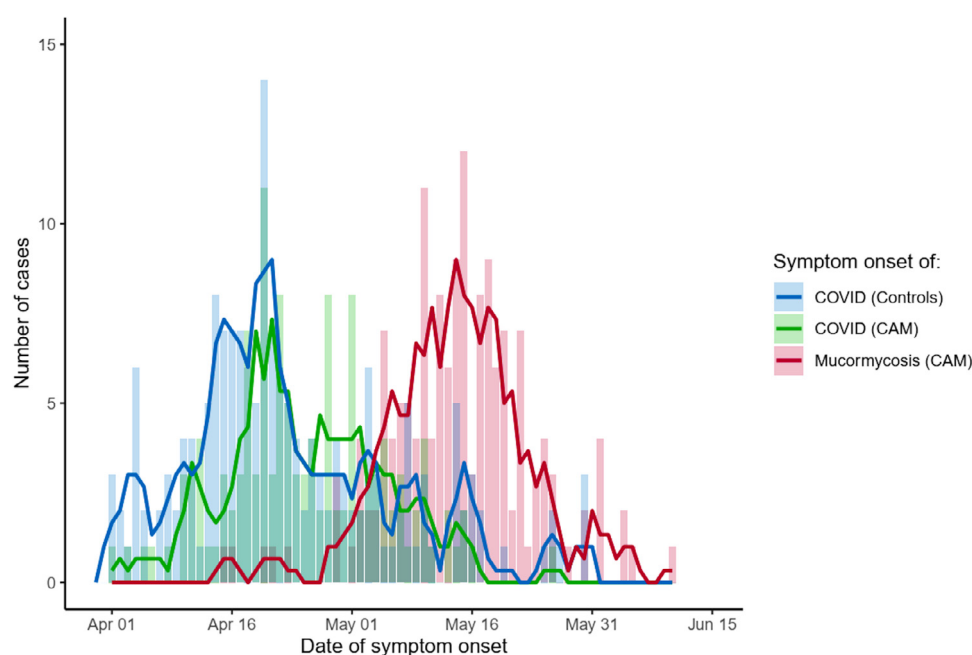


Fig. 1. Panel A: Date of symptom onset of COVID-19 illness for cases (green) and controls (blue), along with date of onset of mucormycosis symptoms for consecutive cases of CAM (red). The peak of symptom onset of COVID-19 for both groups was 20 April 2021 and of mucormycosis for cases was 15 May 2021. Panel B: Cumulative time to symptom onset of mucormycosis after the onset of COVID-19 symptoms in CAM. The first (Q1), second (Q2) and third quartile(Q3) represent 12, 18 and 26 days, respectively. CAM patients who were asymptomatic for COVID-19 are not included ($n = 29$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

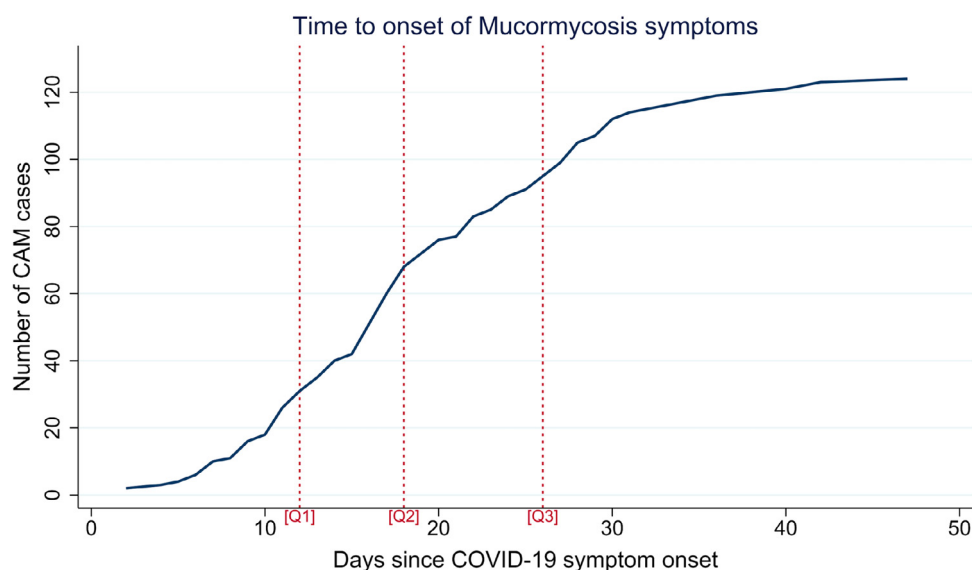


Fig. 1. Continued

Zinc use was more common in the control group (79.9% vs 53.8%, $p < 0.001$). Poor glycemic control was observed in a higher proportion of cases than controls (90.6% vs 51.5%, $p < 0.001$). Information on the occurrence of diabetic ketoacidosis was available for 176 patients. Six patients presented with DKA, and all belonged to the CAM group (5%). The overall distribution of patients between the two groups, according to the best established risk factors diabetes and systemic steroids is depicted in Fig. 2.

N95 mask use was more frequent among controls (42.5% vs 18%), while CAM patients tended to use cloth masks (52% vs 36%). (Table 3) The use of N95 masks was observed to be protective in comparison to not using a mask ($p < 0.05$). Use of cloth masks more than four hours (4–6 h, $p = 0.002$; >6 h, $p < 0.0001$) and use of sur-

gical masks for more than six hours ($p = 0.002$) was found to be associated with a higher risk of CAM when compared to the use of respective masks for less than two hours or N95 for any duration. (Fig. 3) The findings were similar even after excluding health care workers ($n = 40$) as confounders, who were limited to the control group and used N95 masks exclusively. Due to diversity in practices of mask disinfection, reuse, and discarding, this information could not be analyzed. The two groups did not differ in their practice of steam inhalation vis-a-vis the frequency of steam inhalation and the time of starting steam inhalation in relation to the onset of COVID-19 symptoms.

Mask use practices were divided into four risk categories based on the univariate analysis. The use of N95 masks for any

Table 1Demographic and comorbidity profiles of cases compared to controls. All values represented as n(%) except for *mean $\pm$ SD and **median (IQR).

	Total (N = 352) n(%)	CAM (N = 152) n(%)	COVID-19 Recovered (N = 200) n(%)	p-value
Age*	48•3 ($\pm$ 14•0)	49•4 ($\pm$ 11•0)	47•5 ($\pm$ 15•9)	0•21
Males	226 (64•2)	105 (69•1)	121 (60•5)	0•1
BMI *(kg/m ²)	26 $\pm$ 4•2	25•9 $\pm$ 4•2	26•1 $\pm$ 4•3	0•75
High risk occupation (farmer, gardener or labourer)	26 (7•4)	16 (10•6)	10 (5)	0•051
Residence in Rural Area	63 (17•9)	45 (29•6)	18 (9)	<0•001
Residence or workplace close to a construction site	105 (29•8)	32 (21•1)	73 (36•5)	0•002
Type 2 Diabetes Mellitus	196 (55•7)	140 (92•1)	56 (28)	<0•001
Diagnosed > 1 year	115 (32•7)	73 (48)	42 (21)	
Recently, but before onset of COVID-19	19 (5•4)	13 (8•6)	6 (3)	
At the time of COVID-19 diagnosis	62 (17•6)	54 (35•5)	8 (4)	
Duration of diabetes, years**	7 (3–10)	5 (2–10)	8 (4–15)	0•002
HbA1c value at the time of COVID-19**	9 (7•3 – 11•2)	10 (8•1 – 11•9)	7•1 (6•6 – 8•1)	<0•001
Chronic kidney disease	12 (3•4)	6 (4)	6 (3)	0•626
Not on hemodialysis	2 (0•6)	2 (1•3)	0 (0)	
Maintenance hemodialysis	7 (2)	5 (3•3)	2 (1)	
Post- renal transplant	3 (0•6)	2 (1•3)	1 (0•5)	

Table 2Diagnosis, disease severity, and management of COVID-19 illness in cases compared to controls. All values represented as n(%) except for *mean $\pm$ SD and **median (IQR).

	Total (N = 352) n(%)	CAM (N = 152) n(%)	COVID-19 Recovered (N = 200) n(%)	p-value
Number of times swab testing done for COVID-19*	2•2 ($\pm$ 1•5)	2•6 ($\pm$ 1•4)	1•9 ($\pm$ 1•4)	<0•001
History of two or more swabs testing during COVID-19	230 (65)	116 (76•3)	114 (57)	<0•001
Severity of COVID-19 disease				<0•001
Asymptomatic	35 (9•9)	30 (19•7)	5 (2•5)	
Mild	190 (54•1)	65 (43)	125 (62•5)	
Moderate	70 (19•9)	42 (27•8)	28 (14)	
Severe	57 (16•2)	15 (9•9)	42 (21)	
Oxygen supplemented	113 (32•1)	53 (34•9)	60 (30)	0•33
Low flow devices	76 (21•6)	43(28•3)	33 (16•5)	0•003
High flow devices	37 (10•5)	10 (6•6)	27 (13•5)	
Duration of oxygen therapy, in days**	6 (4 –12)	7 (4•5–12)	6 (4–12)	0•16
Highest value of ferritin (ng/mL) during COVID-19 (N = 174)**	761•3 (395•5–1316•5)	1010 (637–1500)	450 (161•8–737•9)	<0•001
Number of patients requiring hospitalization	157 (44•6)	71 (46•7)	86 (43)	0•49
Patients admitted to ward	122 (34•8)	63 (41•7)	59 (29•5)	
Patients admitted to ICU	35 (10)	8 (5•3)	27 (13•5)	
Duration of hospital stay, in days**	10 (6–15)	10 (5–15)	10 (6–15)	0•33
Zinc use (N = 334)	229 (68•6)	78 (53•8)	151 (79•9)	<0•001
Dose of zinc, in mg/day**	20 (20–50)	20 (20–50)	20 (20–50)	0•13
Duration of zinc, days**	10•0 (8–15)	10•0 (7–15)	10 (10–15)	0•06
Cumulative dose of zinc, mg**	500 (250 –750)	500 (200 –750)	500 (250 –750)	0•68
Systemic steroid use	196 (55•7)	100 (65•8)	96 (48•0)	0•001
Dexamethasone	82 (41•8)	34 (34)	48 (50)	0•056
Methylprednisolone	91 (46•4)	51 (51)	40 (41•7)	
Prednisone	23 (11•7)	15 (15)	8 (8•3)	
Daily steroid dose(dexamethasone equivalent), mg**	6 (4•5–9)	6•0 (5•1–10•69)	6 (4–8)	0•18
Duration of steroids, in days**	10 (6–15)	10 (6–14)	10 (6–15)	0•41
Cumulative dose of steroids(dexamethasone equivalent), in mg**	60 (31•4–112)	60•0 (30–120)	60 (36–100•63)	0•86
Poor glycaemic control (BG >200 mg/dL)	178 (74•2)	126 (90•6)	52 (51•5)	<0•001
Highest recorded blood sugars during hospital or home stay (in mg/dL) (N = 240)				
<200 mg/dl	62 (25•8)	13 (9•4)	49 (48•5)	<0•001
200–300 mg/dl	60 (25)	39 (28•1)	21 (20•8)	
300–400 mg/dl	62 (25•8)	39 (28•1)	23 (22•8)	
>400 mg/dl	56 (23•3)	48 (34•5)	8 (7•9)	
Number of patients with DKA (N = 176)	6 (3•4)	6 (5)	0 (0)	0•09
Drug therapy for COVID-19				
Remdesivir (N = 352)	58 (16•5)	18 (11•8)	40 (20)	0•04
Tocilizumab (N = 352)	6 (1•7)	0	6 (3)	0•03

duration was considered as low risk, while use of no mask was categorized separately. Use of surgical masks for more than 6 h or cloth masks for more than 4 h constituted the third group with the highest risk, while shorter duration of either mask use (surgical mask <6 h or cloth masks <4 h) formed the fourth group. The multivariable model was limited to patients with symptomatic COVID-19 since the hospitalization policy excluded admission of asymptomatic COVID-19 patients. We also excluded residence in rural area in the model as we were unable to capture information on the household or environment determinants contributing to this risk.

Risk factors that were found to be independently associated with the risk of development of mucormycosis in multivariable analysis were diabetes, mild and moderate (vs severe) COVID-19 disease, highest blood glucose of more than 200 mg/dL at any time during COVID-19 illness, the number of swab tests performed, and systemic steroid use. (Table 4) The use of zinc was found to be protective. Among mask use, prolonged use of surgical masks (>6 h) and cloth masks (>4 h) were associated with increased odds of CAM (6.9, 95% CI 1.5 – 33.1, $p = 0.02$) compared to no mask use. A shorter duration of cloth or surgical mask usage was associated with higher odds of CAM vs N95 masks (4.9, 95% CI

Table 3

Mask use and steam inhalation practices in cases vs controls. All values represented as n(%).

	Total (N = 352)n(%)	CAM (N = 152)n(%)	COVID-19 Recovered (N = 200), n(%)	p-value
Type of mask used before diagnosis of COVID-19 (for most days in the preceding month) (N = 350)				< 0.001
N95 mask	112 (32)	27 (18)	85 (42.5)	
Surgical mask	58 (16.6)	29 (19.3)	29 (14.5)	
Cloth mask	150 (42.9)	78 (52)	72 (36)	
None	30 (8.6)	16 (10.7)	14 (7)	
Duration of mask use per day(N = 319)				0.037
<2 h	101 (31.7)	33 (24.6)	68 (36.7)	
2–4 h	83 (26)	31 (23.1)	52 (28.1)	
4–6 h	39 (12.2)	19 (14.2)	20 (10.8)	
>6 h	96 (30.1)	51 (37.4)	45 (24.3)	
Frequency of steam inhalation				0.98
Two or more times per day	191 (71.5)	78 (72.2)	113 (71.1)	
Once a day	41 (15.4)	16 (14.8)	25 (15.7)	
Infrequently	35 (13.1)	14 (13)	21 (13.2)	
Timing of initiation of steam inhalation with respect to COVID-19 illness (N = 261)				0.98
Since before symptoms of COVID-19	71 (27.2)	28 (27.7)	43 (26.9)	
During COVID-19 illness alone	175 (67)	67 (66.3)	108 (67.5)	
Continued after recovery from COVID-19	15 (5.7)	6 (5.9)	9 (5.6)	

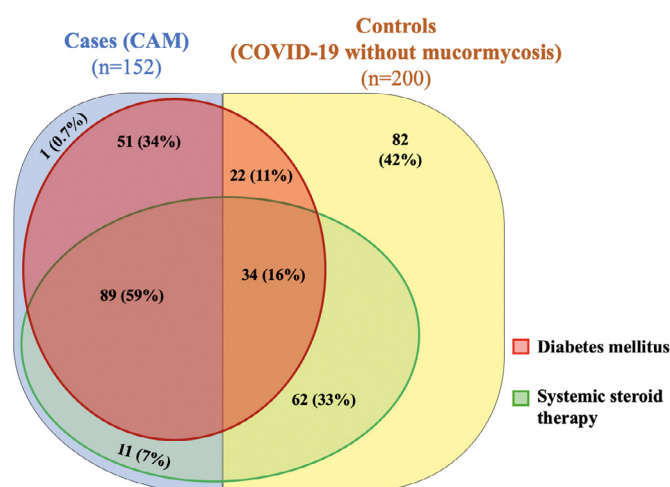


Fig. 2. Venn diagram depicting the distribution of cases with coronavirus disease-associated mucormycosis (CAM, blue) and controls (COVID-19 cases recovered without mucormycosis, yellow) in relation to the two main risk factors (diabetes mellitus, red; and systemic steroid therapy, green). Percentage out of the cases and controls are represented for each region in the parentheses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

1.9–16, $p < 0.001$), but lower than prolonged surgical or cloth mask use (0.28, 95% CI 0.08–0.97, $p = 0.046$). The findings were consistent after subgroup analysis of the same mask use categories among patients residing in urban areas.

Discussion

Mucormycosis is a sporadic disease occurring almost exclusively in immunocompromised patients.⁸ The sudden spike in the incidence of CAM in the wake of the COVID-19 pandemic raises the possibility that COVID-19 infection may itself predispose to mucormycosis. This may occur directly through its impact on immune system or indirectly due to the interventions related to COVID-19 prevention and management.

Even in the pre-COVID-19 era, India reported the highest prevalence of mucormycosis worldwide, nearly 70 times that of global estimates.² This disproportionate burden has been attributed to the high prevalence of both patient factors (diabetes) and environ-

mental factors (hot humid environment).⁹ Residence in a rural area was more common among CAM cases, possibly because of a referral bias for a difficult to treat disease. Our study found that CAM was more common in males in the fifth and sixth decades. The disease occurred three weeks after the onset of COVID-19 symptoms and involved mainly the rhino-orbital areas, similar to reported literature.¹ Poor glycemic control, systemic steroid therapy, and presence of diabetes are associated with increased risk of CAM.⁹ However, 2–32% of reported cases of CAM may lack any of these associations.^{1,10} This is a departure from our finding, where a single case of CAM (out of 152) was neither diabetic nor had received steroids.

Immune system modulation due to COVID-19, diabetes, and steroids

Neutrophils and macrophages kill sporangiospores and hyphal forms of *Mucorales* and constitute the major barrier to invasion.¹¹ An adaptive immune response is stimulated characterized by strong Th-17 activation that initiates a stronger neutrophil response. Simultaneously, the immune response to COVID-19 is complex. Neutrophils in the nasopharyngeal epithelium demonstrate markers of premature activation,¹² while the cells of adaptive immunity (T cells, NK cells, and B cells) are reduced in numbers.¹³ These abnormalities tend to peak in the second week, which may explain the clustering of cases of CAM in the third week after COVID-19 symptom onset. Also, endothelial dysfunction and vasculopathy due to COVID-19 may support angioinvasion and the spread of *Mucorales*.¹⁴ Influence of the mutated variants of COVID-19 on the development of CAM remains to be explored.

Diabetes or impaired glucose tolerance is reported in the majority of patients with mucormycosis with or without COVID-19. Hyperglycemia inhibits neutrophil chemotaxis, phagocytosis by macrophages, and degranulation of NK cells.¹⁵ It promotes surface glucose-regulated protein (GRP78) expression on the endothelium which is essential for invasion by *Mucorales*.¹⁶ The prevalence of diabetes in CAM in our study was higher than historical cohorts of mucormycosis not associated with COVID (92% vs 76%).¹⁷ Newly detected diabetes is reportedly more prevalent in CAM compared to mucormycosis not associated with COVID-19 (21% vs 10%, $p = 0.02$).¹ However, previous studies did not report measuring HbA1c for mucormycosis patients, which may have led to the underreporting of diabetes in previous cohorts. Additionally, supra-physiological stress during COVID-19 illness and viral-mediated islet cell damage may contribute to hyperglycemia.¹⁸ Di-

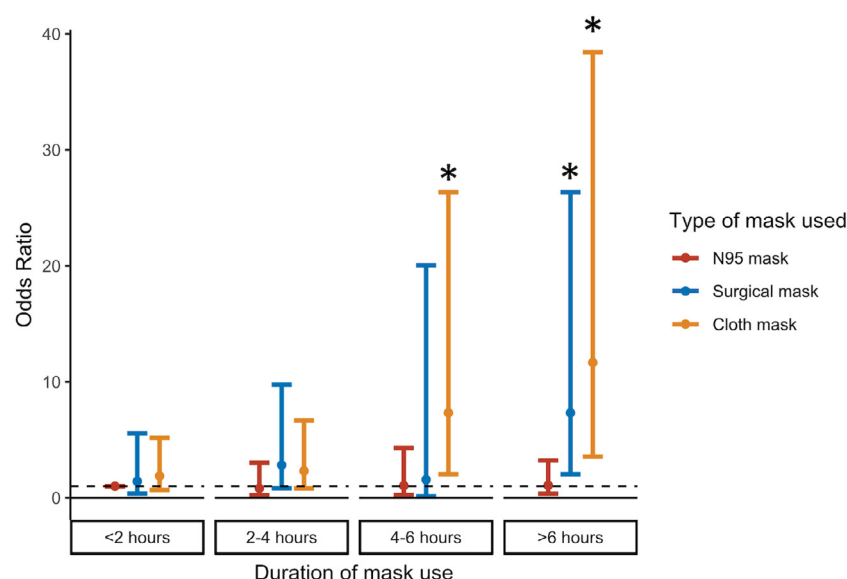


Fig. 3. Risk of Coronavirus disease-associated mucormycosis depends on the type of mask and duration of usage. * represents significant difference compared to the use of N95 mask for <2 h.

Table 4

Multivariable analysis of risk factors for development of Coronavirus disease-associated mucormycosis.

Risk factor	Adjusted odds ratio (95% CI)	P-value
Type 2 Diabetes mellitus	3•5 (1•1–11)	0•032
Steroid use	7•7 (2•4–24•7)	0•001
Severity of COVID-19 disease (vs mild)		
Moderate	1•4 (0•4–4•2)	0•58
Severe	0•17 (0•05–0•56)	0•004
Highest recorded blood glucose levels (vs ≤200 mg/dL)		
200–299 mg/dL	5•4 (1•2–24•8)	0•03
300–399 mg/dL	10•3 (2•7–46•6)	0•03
≥400 mg/dL	27•0 (5•3–136•1)	<0•001
Number of swab tests prior to onset of mucormycosis	1•6 (1•2–2•2)	0•001
Zinc use	0•05 (0•01–0•19)	<0•001
Mask use (vs no mask use)		
N95 mask use (any duration)	0.39 (0•94 – 1.66)	0•21
Short duration use of surgical (<6 h) or cloth mask (<4 h)	1.96 (0.48–8.06)	0•35
Prolonged use of surgical (>6 h) or cloth mask (>4 h)	6.9 (1•5–33•1)	0.02

abetic ketoacidosis is detected in 8–22% of mucormycosis patients at presentation, being a rare occurrence in the natural history of type 2 diabetes otherwise.¹⁹ However, DKA was previously identified to be uncommon among CAM compared to mucormycosis not associated with COVID-19 (8.6% vs 27%, $p < 0.001$).¹ Additionally, we found a progressively higher risk of mucormycosis with higher blood glucose levels suggesting a dose-response relationship, with those having blood glucose >400 mg/dL, at the highest risk.

Prolonged corticosteroid intake is a risk factor for the development of mucormycosis.¹⁶ This may be mediated by inhibition of macrophages and neutrophils and the tendency to cause hyperglycemia. Steroid-induced hyperglycemia (SIH) primarily raises post-prandial glucose levels and their measurement is imperative for glycemic control. High dose steroid use for short term does not raise HbA1c in those without diabetes or prediabetes, and higher pre-steroid therapy HbA1c predicts the development of SIH.²⁰ We found that patients in the CAM group had 7.7 [95% CI 2.4–24.7] times higher odds of being administered steroids when compared to the control group. However, if prescribed, the cumulative dose of steroids was similar in both groups. This was probably related to the well-defined regimens for steroid administration in COVID-19. The absolute risk of CAM among patients recovering from COVID-19 is low and should not prevent appropriate prescription

of steroids in moderate or severe COVID-19 due to their proven benefit.²¹ Our findings call for avoiding the use of steroids in mild COVID-19 in view of the risk of CAM. Overall, it appears that neutrophil dysfunction (due to COVID-19, hyperglycemia, and steroids) and endothelial dysfunction (due to diabetes and COVID-19) may be the main pathogenetic mediators of CAM.

Although studies on zinc in COVID-19 have shown equivocal results, it is widely used owing to its good safety profile and low cost.²² Zinc is essential for normal functioning of immune cells in our body. Further, zinc supplementation has been hypothesized to reduce pro-inflammatory cytokines, blunt lymphopenia, and reduce airway inflammation in COVID-19 patients.²³ We found that zinc supplementation was associated with a significant reduction in the risk of mucormycosis, but a dose-response relationship was not seen. This may be related to the beneficial immunomodulatory role of zinc, especially in COVID-19 patients who are likely to be deficient.²⁴ Prospective clinical trials are needed to confirm these findings.

Role of COVID-19 disease severity including ferritin

More than one-third of patients in the CAM arm (37.7%) had recovered from moderate to severe COVID-19. This is in contrast to the COSMIC study in which 72% of patients recovered from mod-

erate or severe COVID-19 disease.¹⁰ Our results suggest that severe disease was associated with a lower risk of developing mucormycosis. This may be related to the selection bias of recruiting mainly hospitalized patients in both arms, which may have enriched the control arm with patients having severe disease. Secondly, survivorship bias may have played a role since patients of severe COVID-19 may have died prior to the development of symptoms of CAM. A prospective study design is required to evaluate the same.

The overall proportion of patients requiring oxygen therapy as well as the duration of oxygen therapy was similar in cases and controls. However, exposure to high flow oxygen devices was lower in the CAM group in line with the proportion of patients with severe COVID-19 illness.

COVID-19 disease severity correlates with raised ferritin levels reflecting the inflammatory state. Ferritin levels were found to be higher in cases of CAM compared to controls. Iron uptake correlates linearly with the growth of *Rhizopus* in serum and is essential for its pathogenicity in the presence of siderophores.²⁵ Higher ferritin levels have been associated, albeit weakly, with the risk of mucormycosis in hematopoietic allograft recipients.²⁶ The pathogenetic role of iron metabolism in mucormycosis merits further evaluation as elevated ferritin does not directly imply increased intracellular iron available to *Mucorales*.

Practices related to COVID-19 prevention and diagnosis

Rhizopus, the dominant species responsible for mucormycosis, is thermotolerant and grows well at or above body temperature and in a moist environment.²⁷ This prompted us to evaluate the role of mask use in modulating the risk of CAM. The use of surgical or cloth masks for prolonged periods was found to be associated with an increased risk of CAM in our study. It has been shown that temperature of the perioral skin rises after use of face masks for even one hour.²⁸ Cloth masks increase the risk of respiratory tract infections compared to other masks, as well as no mask use.²⁹ The reuse of cloth masks, variation in effective cleaning practices, and retention of moisture are proposed as factors for this increase. N95 masks are made of fluid-resistant synthetic materials that inhibit the growth of fungal elements.³⁰ Organic cloths used commonly in Indian masks, like cotton and silk, do not share similar properties. The organic matter contaminating usual cloth masks may allow implantation of *Mucorales* spores within the nasal cavity. Also, duration of mask use may be a surrogate for increased outdoor exposure and consequently soil and dust that harbor *Mucorales* spores. Our findings may require further evaluation in prospective studies since a large proportion of our controls used N95 masks (42.5% vs 18% in CAM), probably due to the disproportionate number of healthcare workers in that arm ($n = 40$). We also could not obtain reliable information about the frequency of discarding or laundering of masks, socio-cultural factors influencing the preferred mask type, or change in mask use practices during recovery from COVID-19 – at a time when CAM is in incubation. Nevertheless, the use of unclean cloth or surgical masks should be avoided.

Physical factors that may increase the risk of mucormycosis were also evaluated. Repeated nasopharyngeal swab testing for COVID-19, if done more than twice, was found to be independently associated with a higher risk of CAM. Development of cutaneous mucormycosis at sites of local trauma such as infected intravenous catheters, dressings, and needles is well reported.³¹ Repeated nasopharyngeal swab testing may cause micro-trauma to nasal and nasopharyngeal mucosa, predisposing to angioinvasion. Similarly, steam inhalation has been hypothesized to cause heat injury to the nasal mucosa, whereas we did not find any association between the risk of CAM and frequent steam use or continued steam use beyond recovery from COVID-19.³²

Our study is the first large case-control study evaluating the risk factors specific to the development of CAM. We hypothesized and investigated several risk factors for CAM unique to the prevention and management of COVID-19, beyond those already established for mucormycosis prior to the COVID-19 pandemic. Information was obtained and corroborated from several sources to mitigate recall bias and missing information. Restricting ourselves to the second wave of COVID-19 between April–May 2021 prevented discrepancies in healthcare access due to the overwhelming rise in cases. The peak incidence of COVID-19 symptoms for both groups occurred on 20th April 2021 (Fig. 1A), coinciding with the peak number of 28,395 COVID-19 cases reported in a single day in the region of Delhi, India as per crowd-sourced trackers.³³ However, this study has a few limitations. Firstly, recall bias is inherent to the case-control study design and cannot be completely eliminated. Specifically, duration of mask use along with discarding and laundering practices is subject to recall and interviewer bias. Further prospective studies would be required to affirm its association with increased risk of CAM, although this may be impractical. Secondly, differences in COVID-19 treatment practices may exist since CAM cases were treated at other hospitals while controls were mainly treated at our tertiary care center. Patients with CAM may have differed from control arm with respect to residence in rural areas due to a possible referral bias, since treatment of CAM was limited to select tertiary centers. Thirdly, since it was a retrospective study, the robustness of our findings decreased due to lower sample size available for multivariable modeling in view of missing information particularly for blood glucose, HbA1c, and ferritin. Fourth, cases of CAM may occur infrequently beyond 30 days of COVID-19 onset ($n = 10$, 6.6%) and thus controls may remain at-risk of CAM. However, none of the controls have notified the authors of the occurrence of CAM even after study completion.

In conclusion, CAM is strongly associated with diabetes, poor glycemic control, and systemic steroid use. The requirement of oxygen therapy and hospitalization for COVID-19 did not affect the risk of CAM. Novel risk factors identified in our study include prolonged use of cloth and surgical masks vis-a-vis N95 masks and repeated nasopharyngeal swab testing. These are potentially modifiable and merit further prospective research.

Availability of data and materials

The authors would make the data available on specific requests keeping patient confidentiality in view.

IRB approval

The study was approved by the Institute Ethics Committee, AIIMS, Delhi, India. [IECPG-353/28.05.21]

Funding source

None to declare.

Authors' contributions

All listed authors meet the ICMJE criteria. We attest that all authors contributed significantly to the creation of this manuscript, each having fulfilled the criteria as established by the ICMJE.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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