



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



ELSEVIER

Contents lists available at ScienceDirect

## International Journal of Infectious Diseases

journal homepage: [www.elsevier.com/locate/ijid](http://www.elsevier.com/locate/ijid)

## Review

## COVID-19-Associated Mucormycosis: An Opportunistic Fungal Infection. A Case Series and Review

Asma Al Balushi<sup>1,\*</sup>, Ahmed Al Ajmi<sup>2</sup>, Qais Al Sinani<sup>3</sup>, Varna Menon<sup>4</sup>, Zainab Al Berieki<sup>5</sup>, Asma Al Shezawi<sup>5</sup>, Saleh Al Azri<sup>6</sup>, Azza Al Rashdi<sup>6</sup>, Amina Al Jardani<sup>6</sup>, Thuwaiba Al Baluki<sup>7</sup>, Salem Al Ghaithi<sup>7</sup>, Ali Al Reesi<sup>7</sup>, Ahmed Talib Al-Za'abi<sup>8</sup>, Mohammed Abdullah Al' Balushi<sup>8</sup>, Talib Al Maqbali<sup>3</sup>

<sup>1</sup> Infectious Diseases Unit, Internal Medicine Department, Sultan Qaboos University Hospital, Muscat, Oman

<sup>2</sup> Oral and Maxillofacial Surgery Department, Sohar Hospital, Ministry of Health, Sohar, Oman

<sup>3</sup> Ear, Nose and Throat Surgical Department, Sohar Hospital, Ministry of Health, Sohar, Oman

<sup>4</sup> Histopathology Department, Sohar Hospital, Ministry of Health, Sohar, Oman

<sup>5</sup> Radiology Department, Sohar Hospital, Ministry of Health, Sohar, Oman

<sup>6</sup> Central Public Health Laboratories, Directorate General for Disease Surveillance and Control, Ministry of Health, Muscat, Oman

<sup>7</sup> Internal Medicine Department, Sohar Hospital, Ministry of Health, Sohar, Oman

<sup>8</sup> Clinical Pharmacy Department, Sohar Hospital, Ministry of Health, Sohar, Oman

## ARTICLE INFO

## Article history:

Received 27 February 2022

Revised 2 May 2022

Accepted 3 May 2022

## Keywords:

Mucorales

Rhizopus oryzae

COVID-19 associated mucormycosis

Rhino-orbital-cerebral mucormycosis

Invasive mold infections

COVID-19 variants

## ABSTRACT

**Background:** A surge in COVID-19-associated mucormycosis cases has been observed during the second wave of COVID-19 in summer of 2021. Most cases were reported from India. The Delta variant (B.1.617.2) was the most common variant circulating at that time. Mucormycosis is an opportunistic angioinvasive fungal infection with high morbidity and mortality.

**Methods:** We present 10 cases of COVID-19-associated rhino-orbital and rhino-orbital-cerebral mucormycosis managed in a secondary hospital in Oman.

**Results:** The median time for developing mucormycosis was two weeks after COVID-19 diagnosis. All patients were newly diagnosed or already known to have poorly controlled diabetes mellitus. Five patients received corticosteroid therapy for COVID-19. Three patients had severe COVID-19 and died of severe acute respiratory distress syndrome and septic shock. Another three patients died of advanced mucormycosis and cerebral involvement. Despite aggressive medical and surgical intervention, the mortality rate was 60% (6/10).

**Conclusion:** Mucormycosis is an aggressive opportunistic infection with high morbidity and mortality that requires prompt recognition and urgent intervention.

Uncontrolled blood sugar, the use of corticosteroids, and immune dysfunction due to COVID-19 are all important risk factors for development of mucormycosis. Worse outcomes are associated with poor glycemic control despite aggressive medical and surgical interventions.

© 2022 The Authors. Published by Elsevier Ltd on behalf of International Society for Infectious Diseases.

This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

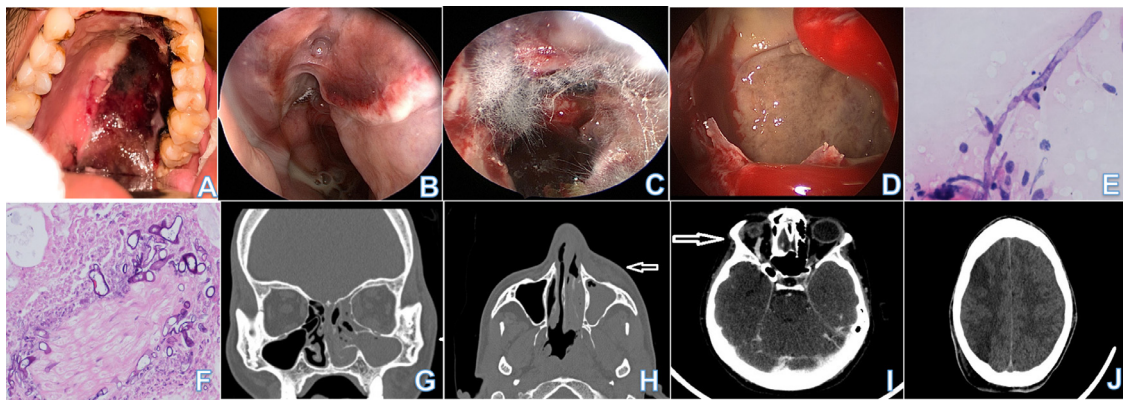
## Introduction

During the second wave of the COVID-19 pandemic, there was a sudden increase in the number of mucormycosis cases worldwide, presenting a new challenge to clinicians managing these patients

(Kumar et al., 2021; Mahalaxmi et al., 2021). Although the first case of COVID-19-associated mucormycosis (CAM) was reported in Chile, most cases were reported in India and were linked to infection with the Delta variant (B.1.617.2) (Rao et al., 2021). Diabetes mellitus (DM) is the most commonly identified risk factor for developing mucormycosis (Gupta et al., 2021; John et al., 2021; Kumar et al., 2021). Rhino-orbital-cerebral mucormycosis is the most commonly reported clinical form (Mahalaxmi et al., 2021; Singh et al., 2021). Mortality rates remain high, exceeding 50%, de-

\* Corresponding author: Asma Al Balushi: Address: Infectious Diseases Unit, Internal Medicine Department, Sultan Qaboos University Hospital, Muscat, Seeb, Alkhoudh, P. O. Box 35, Postal Code 123, Oman. Mobile number: (+968) 92319222.

E-mail address: [thebrightsoul2@gmail.com](mailto:thebrightsoul2@gmail.com) (A.A. Balushi).



**Figure 1.** A. Hard palate grossly involved with the disease. Black eschar is visible. B. Pre-operative endoscopic view of right-side posterior nasal cavity. C. Fungal hyphae growth in post-operative nasal cavity. D. Maxillary sinus mucosa appears pale and sloughy. E. Imprint smear showing broad, ribbon-like and twisted, non-septate fungal hyphae displaying right-angled branching (Hematoxylin and Eosin 200X). F. Histopathological section showing perineural infiltration by broad wide-angled branching fungal hyphae (Hematoxylin and Eosin 400X). G. Coronal computed tomography (CT) image shows unilateral mucosal thickening of left maxillary and left ethmoid sinuses. H. Axial CT image shows unilateral left nasal cavity and maxillary sinus mucosal thickening. There is soft tissue infiltration of left anterior periantral fat. I. Dilated right superior ophthalmic vein. J. Axial CT head shows right sided fronto-parietal ischemic infarct.

spite aggressive medical and surgical interventions (Kumar et al., 2021; Sharma et al., 2021).

We describe our experience in managing 10 consecutive COVID-19 patients admitted to our institution who presented within the same month with associated mucormycosis. The first case was reported on June 8, 2021.

Our hospital recorded the highest number of mucormycosis admissions within the country.

## Cases

We encountered a total of 10 cases of CAM. Eight patients were Omani and two were Indian. Three were female and seven were male, aged 16 to 67 years (median age, 44.5 years). Seven of 10 patients presented to the emergency department with three to seven days' history of headache and periorbital pain (10 days to three weeks from COVID-19 onset, except in one patient who presented within 3 days of COVID-19 onset). Clinical presentation included periorbital swelling, chemosis and ophthalmoplegia on examination. Three patients had palatal eschar (Fig 1). All of these seven patients had mild COVID-19 but two of them received one dose of dexamethasone before admission from another institution.

The other three patients were admitted for severe COVID-19 pneumonia/acute respiratory distress syndrome (ARDS) one to four weeks before diagnosis of mucormycosis. All three patients received high-dose dexamethasone (8 mg) once daily for the duration of hospital stay despite having poor glycemic control.

All patients had uncontrolled blood sugar on presentation or during hospital stay, and four of them had severe diabetic ketoacidosis (DKA). Six patients were known to have DM and four patients were diagnosed with DM at admission. Glycosylated hemoglobin (HbA1c) ranged from 9–15% for all patients and only one patient had controlled DM with HbA1c of 6.4% but high blood sugar on admission. Clinical characteristics of patients are shown in Table 1.

Seven patients had rhino-orbital mucormycosis (one of them did not have any imaging of the brain to assess for cerebral dissemination of the infection) and three had rhino-orbital-cerebral mucormycosis. The findings of radiological imaging are shown in Table 2 and Fig. 1.

Nasal endoscopy was performed for all patients showed edematous mucosa. Dusky necrotic mucosa was observed in seven of the 10 patients (Fig. 1).

Nasal squash imprint cytology of tissue samples was performed on six of 10 patients (patients 2, 3, 4, 5, 7, and 8), revealing mixed inflammatory cells and ribbon-like, non-septate, broad, and crinkled fungal hyphae with right-angle branching, consistent with *Mucorales*.

Histopathology of tissue samples from nasal turbinate and sinuses demonstrated tissue necrosis and angioinvasion with vessels thrombosed by non-septate fungal hyphae displaying right-angled branching, which was consistent with angioinvasive mucormycosis (Fig. 1).

Fungal cultures of tissue biopsy specimens from nasal turbinate and septum of all patients were sent to Central Public Health Laboratories, inoculated on Sabouraud agar (with and without antibiotics) and incubated at different temperatures. Tissue cultures of all samples grew *Rhizopus oryzae* except for those obtained from patient 1 and patient 7, which showed no growth. The isolates were identified by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF; MALDI Biotyper, Bruker Daltonics GmbH, Bremen, Germany) using MBT Filamentous Fungi Library (Bruker Daltonics). The score of identification ranged between 1.74 and 2.06, and isolates with low scores (three isolates) were sent for whole-genome sequencing to a reference laboratory abroad, and identification matched ours to the species level. Second and third best matches were the same (*R. oryzae*) in all isolates identified.

Tissue bacterial cultures were negative for all patients except those from patient 1, which grew *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis*.

Respiratory (nasopharyngeal and oropharyngeal swab) specimens were collected from cases as per the Ministry of Health national case definition. SARS-CoV-2 sequencing was performed on two patient's variants, one found to be the Alpha variant (B.1.1.7) (patient 1), and the other the Delta variant (B.1.617.2) (patient 8). The remaining variants were not sequenced either because of poor quality, low viral load, (cyclic threshold of >30), or lack of samples for sequencing because COVID-19 preceded the mucormycosis presentation.

## Polymerase chain reaction and sequencing method

The ribonucleic acid (RNA) extraction of samples was carried out by Viral RNA Isolation Kit with Liferiver EX3600 (Liferiver Biotech, Hangzhou Bay, China), following manufacturer's protocol. For the detection of the SARS-CoV-2 virus by a real-time polymerase chain reaction (RT-PCR) system, TaqPath™ COVID-19

**Table 1**  
Characteristics of COVID-19 associated mucormycosis cases, medical and surgical interventions and outcomes.

Case #	1	2	3	4	5	6	7	8	9	10
Age	65	42	47	67	36	35	47	16	51	29
Sex	M	F	M	F	M	M	F	M	M	M
Nationality	Omani	Omani	Omani	Omani	Indian	Omani	Omani	Omani	Omani	Indian
Days since COVID-19 symptoms started	3 weeks	3 weeks	3 days	4 weeks	10 days	9 days	2 weeks	10 days	2 weeks	3 weeks
COVID-19 strain	Alpha variant (B.1.1.7)	Not done	Not done	Not done	Not done	Not done	Not done	Delta variant (B.1.617.2)	Not done	Not done
Indication for admission	Mucor	Mucor	Mucor	COVID-19	Mucor	COVID-19	Mucor	Mucor	Mucor	COVID-19
Prior steroid therapy	X	X	1 dose	8 mg od × 21 days	X	8 mg od × 7 days	X	X	1 dose	8 mg od × 20 days
Known/ new DM (HbA1c)	Known (6.4%)	Known (13%)	Known (13.1%)	Known (9%)	New (15.38%)	Known (11.19%)	New (13.35%)	New (13.08%)	Known (12.95%)	New (9.38%)
RBS on presentation (mmol/L)	13	11	18	21	28	16.6	18.7	21	15	13.9
DKA	X	Yes	X	X	Yes	Yes	X	Yes	X	X
Comorbidities	-CKD on dialysis -Myelofibrosis	-HTN	Nil	-Morbid obesity -HTN -AF	Nil	Nil	Nil	Nil	Nil	Nil
Symptom/clinical findings	7 days -Headache -Left periorbital pain + swelling + chemosis - Ophthalmoplegia	5 days -Headache -Right facial numbness -Right periorbital swelling + chemosis - Ophthalmoplegia -Facial nerve palsy -Palatal eschar	3 days -Headache -Right periorbital pain + swelling+ chemosis -Proptosis	1 day -Right periorbital swelling -Proptosis -Chemosis	3 days -Headache -Left eye pain -Left periorbital swelling + chemosis -Palatal eschar -Confusion	2 days -Left eye proptosis + chemosis	5 days -Left facial numbness -Nasal fullness -Left periorbital swelling - Ophthalmoplegia	4 days -Tooth pain -Right periorbital swelling + chemosis -Palatal eschar -Confusion	5 days-Headache -Nasal fullness -Left eye chemosis + ophthalmoplegia -Left cheek induration	1 day -Left periorbital swelling + chemosis + proptosis
Extension of mucormycosis	Rhino-orbital	Rhino-orbital-cerebral	Rhino-orbital	Rhino-orbital	Rhino-orbital-cerebral	Rhino-orbital	Rhino-orbital	Rhino-orbital-cerebral	Rhino-orbital	Rhino-orbital
Nasal endoscopy	Congested edematous mucosa No necrosis	Dusky necrotic mucosa involving the septum	Dusky necrotic mucosa	Dusky necrotic mucosa	Necrosis of entire left-side mucosa extending into contralateral side	Dusky necrotic mucosa	Congested mucosa No necrosis	Necrosis of entire right-side mucosa extending into contralateral side	Dusky necrotic right-side mucosa involving contralateral side	Dusky necrotic mucosa

(continued on next page)

Table 1 (continued)

Case #	1	2	3	4	5	6	7	8	9	10
Tissue fungal c/s	No growth	<i>Rhizopus oryzae</i>	<i>Rhizopus oryzae</i>	<i>Rhizopus oryzae</i>	<i>Rhizopus oryzae</i>	<i>Rhizopus oryzae</i>	<i>Rhizopus oryzae</i>	<i>Rhizopus oryzae</i>	<i>Rhizopus oryzae</i>	<i>Rhizopus oryzae</i>
Tissue bacterial c/s	- <i>Klebsiella pneumoniae</i> - <i>Pseudomonas aeruginosa</i> - <i>Staphylococcus epidermidis</i>	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth
Tissue cytology	Not done	Done	Done	Done	Done	Not done	Done	Done	Not done	Not done
Histopathology consistent with angioinvasive mucormycosis	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Surgical intervention	Yes	Yes	Yes	Nil	Yes	Yes	Yes	Nil	Yes	Yes
Orbital exenteration	No	Yes	No	No	No	No	No	No	No	No
Antifungals	Monotherapy	Combination	Combination	Monotherapy	Combination	Monotherapy	Monotherapy	Monotherapy	Combination	Monotherapy
Initial therapy	IV AmBisome 5 mg/kg × 3 weeks	IV AmBisome 10 mg/kg + PO posaconazole × 3 weeks	IV AmBisome 7.5 mg/kg + PO posaconazole × 3 weeks	IV AmBisome 5 mg/kg × 1 day	IV AmBisome 10 mg/kg + PO posaconazole × 3 weeks	IV AmBisome 5 mg/kg × 5 days	IV AmBisome 5 mg/kg × 1 week	IV AmBisome 5 mg/kg × 2 days Then, PO posaconazole + IV caspofungin AmBisome was not available	IV AmBisome 7.5 mg/kg + PO posaconazole × 3 months	PO posaconazole + IV anidulafungin AmBisome was not available
Maintenance therapy: Oral posaconazole	6 months	4 weeks	3 months	NA	NA	NA	3 months	NA	3 months	NA
Other complications	Nil	-Septic shock -Bacteremia: MDR <i>Enterobacter cloacae</i> -VAP: MDR <i>Acinetobacter baumannii</i> + MDR <i>Pseudomonas aeruginosa</i> -Severe <i>Clostridioides difficile</i>	Nil	-Septic shock	-Septic shock -VAP: MDR A. <i>baumannii</i> -Severe <i>C. difficile</i> -AKI	-Septic shock	-CSF leak	-Septic shock -Bacteremia: MDR A. <i>baumannii</i>	Nil	-Septic shock -Bacteremia: CRE <i>K. pneumoniae</i> MDR <i>E. cloacae</i> MDR A. <i>baumannii</i> - Candidemia: <i>Candida albicans</i>
Outcome	Discharged after 3 weeks	<b>Died</b> (7 weeks after diagnosis)	Discharged after 3 weeks	<b>Died</b> (Same day of diagnosis)	<b>Died</b> (3 weeks after diagnosis)	<b>Died</b> (5 days after diagnosis)	Discharged after 3 weeks	<b>Died</b> (1 week after diagnosis)	Discharged after 3 weeks Recurrent admission	<b>Died</b> (2 days after diagnosis)
Cause of death	-	Advanced mucormycosis Septic shock	-	Advanced COVID-19 Septic shock	Advanced mucormycosis Septic shock	Advanced COVID-19 Septic shock	-	Advanced mucormycosis Septic shock	-	Advanced COVID-19 Septic shock

AF, Atrial fibrillation; AKI, acute kidney injury; c/s, culture; CKD, chronic kidney diseases; CRE, Carbapenem-resistant Enterobacteriaceae; CSF, cerebrospinal fluid; DKA, diabetes ketoacidosis; DM, diabetes mellitus; ESS, endoscopic sinus surgery; F, female; HbA1c, glycosylated hemoglobin; HTN, hypertension; IV, intravenous; M, male; MDR, multi-drug resistant; NA, not applicable; od, once daily; PO, per oral; RBS, random blood sugar; VAP, ventilator-associated pneumonia.

**Table 2**

Description of radiological findings of the described patients with COVID-19-associated mucormycosis and surgical interventions.

Patient #	Description of radiological findings	Surgical intervention
Patient 1	Contrast CT: total opacification of left maxillary sinus with central linear hyperdensities and opacification of left ethmoid air cells associated with early bone changes. There was associated subcutaneous thickening of left periorbital and pre-maxillary regions with mild left-eye proptosis.	ESS with left-side maxillary antrostomy and ethmoidectomy.
Patient 2	Contrast CT: mucosal thickening within right frontal, maxillary and sphenoid sinuses with opacification of right ethmoid air cells. Bulky right pterygoid muscle and muscles of right masticator space. Proptosis of right eye. Acute infarction in right posterior parietal region. Cerebral CT angiography with venogram: dilated right superior ophthalmic vein indicating impending right cavernous sinus thrombosis. MRI: perineural extension along right optic nerve and soft tissue infiltration with involvement of infra-temporal and pterygopalantine fossae and masticator space. Follow-up CT after 2 weeks: worsening infarct in the right parietal region and new infarct in the pons and complete right cavernous sinus thrombosis.	ESS debridement of all necrotic mucosa, including all right-sided sinuses and nasal septum. Right-eye exenteration.
Patient 3	Contrast CT and venogram: complete opacification in right maxillary sinus with mild mucosal thickening in right ethmoid, sphenoid and frontal sinuses. Periorbital edema and soft tissue infiltration anterior to right maxillary and cheek area. Mild right-eye proptosis.	ESS medial maxillectomy with ethmoidectomy. Second surgery: debridement of facial necrotic material anterior to maxilla.
Patient 4	Not done	Nil
Patient 5	Contrast CT: complete opacification and mucosal thickening of left maxillary, ethmoid and frontal sinuses with bone refraction. Soft tissue infiltration of left periorbital area and left-eye proptosis. Soft tissue infiltration in sphenopalatine and nasopharynx. Second CT after 1 week: multiple hypodensities seen in left fronto-parietal, left parieto-temporal, left brainstem, pons, left cerebellum and right frontal lobe. Distal branch of left MCA, ACA, PCA are occluded. Occlusion of left internal carotid artery. Left cavernous sinus thrombosis.	Bilateral ESS debridement with exenteration of all paranasal sinuses.
Patient 6	Contrast CT: dense opacification of left nasal cavity, frontal and ethmoid sinuses. Mucosal thickening in both maxillary sinuses with bone remodeling. Prominent left ophthalmic vein and intraorbital fat stranding. Left-eye proptosis.	ESS debridement of all necrotic tissue involving all left paranasal sinuses and posterior nasal septum.
Patient 7	Contrast CT: high-density mucosal thickening of left maxillary, sphenoid and frontal sinuses and left ethmoid air cells. Bulky left lateral pterygoid muscle.	ESS including maxillary antrostomy with ethmoidectomy and sphenoidectomy. Then, medial maxillectomy and sphenoid CSF leak repair.
Patient 8	Contrast CT: mucosal thickening and high-density fluid in both sides of maxillary, frontal and sphenoid sinuses and ethmoid air cells. Bone erosion in right lamina papyracea and medial orbital wall. Bulky right temporalis, masseter and pterygoid muscles and bulky right parotid gland. Proptosis of right eye with pre-orbital, retro-orbital and pre-septal enhancing soft tissue thickening with fat stranding. Bulky right medial and inferior rectus muscles. Left periorbital soft tissue thickening and enlarged optic nerve. Dilated right superior ophthalmic vein with right cavernous sinus thrombosis. Ischemic infarct in right temporal region. Follow-up CT after 24 hours: new infarcts in right frontal, parietal, temporal and pontine regions and both cerebellar hemispheres. New left cavernous sinus thrombosis.	Nil
Patient 9	Contrast CT: mucosal thickening in left maxillary and sphenoid sinuses. Complete opacification of left ethmoid sinus and refraction of left maxillary sinus wall. Periorbital edema and bulky pterygoid muscle in both sides. Follow-up MRI: progression of disease and involvement of left zygomatic bone, orbital floor and medial orbital wall. Later MRI: involvement of right pterygoid and temporal bone.	ESS debridement included bilateral maxillectomy, ethmoidectomy and sphenoidectomy with posterior septectomy. Second surgery: removal of zygomatic bone, maxillectomy, orbital floor removal and medial orbital wall, along with zygomatic bone and pterygoid on the left side. Third surgery: further debridement and removal of parts of skull base.
Patient 10	Contrast CT: complete opacification of left maxillary and frontal sinuses, ethmoid air cells and both sphenoid sinuses. Refraction of ethmoid air cells and left lamina papyracea. Left-eye proptosis and thickened inferior rectus muscle with fat stranding.	ESS debridement included medial maxillectomy with ethmoidectomy.

ACA, anterior cerebral artery; CSF, cerebrospinal fluid; CT, computed tomography; ESS, endoscopic sinus surgery; MCA, middle cerebral artery; MRI, magnetic resonance imaging; PCA, posterior cerebral artery.

CE-IVD RT-PCR Kit (Thermo Fisher Scientific, Waltham, MA) was used in accordance with the manufacturer's instructions. The assay targets three genomic regions of the SARS-CoV-2 (S, N2, and ORF1ab). Amplification was performed on the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument or the QuantStudio 5 Dx Real-Time PCR Instrument (Thermo Fisher Scientific). SuperScript VILO cDNA Synthesis Kit (Thermo Fisher Scientific) was used to reverse-transcribe the SARS-CoV-2 RNA with a quantitative PCR program (42°C for 30 minutes, 85°C for 5 minutes, and

hold on 10°C). To ensure enough complementary DNA content for next-generation sequencing workflow, we re-quantified it on the Qubit 3.0 Fluorometer (Thermo Fisher Scientific). The complementary DNA (60 ng/ul) was used to prepare libraries with Ion Xpress Barcode Adapters (Thermo Fisher Scientific) through the Ion AmpliSeq Library Kit Plus and pre-defined Ion AmpliSeq SARS-CoV-2 Research Panel (Thermo Fisher Scientific). Workflow was conducted according to manufacturer's guidelines; libraries were prepared and quantified, and templates were enriched and

sequenced on Ion 530 Chip Ion Torrent S5 (Thermo Fisher Scientific). The Torrent Suite Software (version 12; Thermo Fisher Scientific) with SARS-CoV-2 plugins (COVID19AnnotateSnpEff, IRMAreport, and AssemblerTrinity) was installed and pre-optimized with the reference sequence (ion\_ampliseq\_sarscov2) and target regions (Ion\_Ampliseq\_SARS-CoV-2.2020323. Designed.bed) to trim, filter, quality check, assemble, and annotate the samples.

## Management and outcomes

Three patients were intubated for low level of consciousness (patients 2, 5, and 8). Three patients were already intubated for severe COVID-19 pneumonia/ARDS (patients 4, 6, and 10), and the other four patients did not require intubation.

Blood sugar control and management of DKA was challenging. Four patients had DKA (patients 2, 5, 6, and 8) with very high blood sugar above 20–25 mmol/l that required high doses of insulin therapy. Daily insulin doses in these cases exceeded 200 IU.

Eight of the 10 patients underwent endoscopic sinus surgery and debridement of all necrotic tissue from nasal and sinus cavity, and one patient (patient 2) had right-eye exenteration. Two patients did not have surgical intervention. Patient 4 died on the day of mucormycosis diagnosis. Patient 8 had very extensive disease with cerebral involvement, and family declined surgery to prevent disfigurement or permanent disability although the high mortality rate was explained. Further elaboration of surgical interventions is shown in Table 2. Four patients with rhino-orbital mucormycosis survived (patients 1, 3, 7, and 9) and the rest died.

All patients received liposomal amphotericin B (AmBisome) except patient 10 who received oral posaconazole because of unavailability of amphotericin B. AmBisome was started at 5 mg/kg for milder cases and increased to 7.5 mg/kg in cases that progressed while on lower doses of AmBisome or 10 mg/kg in cases of cerebral involvement. AmBisome was combined with oral posaconazole suspension of 200 mg every 6 hours in these cases.

The duration of intravenous AmBisome treatment differed between patients according to severity of disease and availability of AmBisome. Average AmBisome treatment duration was 3 weeks except in cases of patients who died earlier.

The mortality rate among our patients was 60% (6/10). Three patients died because of severe COVID-19 pneumonia/ARDS, and all of them had septic shock. Three of the other non-severe COVID-19 patients died of advanced rhino-orbital-cerebral mucormycosis, whereas those without cerebral extension survived. Those who died had worse blood sugar control, and four of them had severe DKA.

## Discussion

During the second wave of the COVID-19 pandemic, a sudden and rapid rise in mucormycosis incidence was observed. CAM has been identified as a deadly complication of this viral infection. Nearly 70% of CAM cases have been reported from India (Kumar et al., 2021; Mahalaxmi et al., 2021). Before the outbreak of the COVID-19 pandemic, global prevalence of mucormycosis varied from 0.005 to 1.7 per million population (Sen et al., 2021a,b; Sharma et al., 2021; Singh et al., 2021). In India, the prevalence is 80 times higher than that recorded in developed countries, estimated as 0.14 per 1000 population (Kumar et al., 2021; Mahalaxmi et al., 2021; Singh et al., 2021). Scarce data are available about the epidemiology and prevalence of mucormycosis in Middle Eastern countries. Al Shahawey et al. reported that an overall rising incidence of mucormycosis has been observed, with variability among countries. Reported incidence in Iraq, Jordan, and Algeria was 0.2 cases/100,000 individuals, whereas it was higher

in Qatar and Lebanon, with 1.2 cases/100,000 individuals and 1.18 cases/10,000 admissions, respectively (Al Shahawey et al., 2022).

Mucormycosis is an aggressive, angioinvasive infection caused by ubiquitous filamentous fungi of the order *Mucorales* (Kumar et al., 2021; Singh et al., 2021). Paltauf reported the first case of human rhino-cerebral mucormycosis in 1885 (Kumar et al., 2021; Sharma et al., 2021; Singh et al., 2021). The most commonly reported species are *Rhizopus* species, followed by *Mucor*, *Rhizomucor*, *Lichtheimia*, *Cunninghamella*, and *Absidia* (Mahalaxmi et al., 2021; Rao et al., 2021; Sen et al., 2021a; Singh et al., 2021). As it has been observed in our cases, *R. oryzae* is the leading cause of rhino-orbital-cerebral mucormycosis, constituting 90% of cases in the world (Mahalaxmi et al., 2021; Singh et al., 2021).

*Mucorales* are found in the environment, especially in decayed organic material such as bread, fruits, vegetables and soil (Kumar et al., 2021; Mahalaxmi et al., 2021; Richardson et al., 2020; Sharma et al., 2021). *Mucorales* can tolerate high temperatures, thus infections occur more in hot, dry summer when the humidity is low, as it has been observed in India, a country with very high prevalence (Kumar et al., 2021). The similar weather conditions in Oman might partially explain the increasing incidence observed in this country during the second wave of the pandemic. Inhalation of fungal spores followed by repeated ingestion of contaminated products in immunocompromised patients, such as patients with diabetes, or occasionally traumatic skin injury are the most common routes of acquisition of *Mucorales*. (Mahalaxmi et al., 2021; Rao et al., 2021).

The disease ranges from local sinusitis to a severe and aggressive form with dissemination and cerebral involvement. Fungal hyphae invade blood vessels, causing inflammation and necrosis of the vascular wall, which results in thrombosis, infarction or hemorrhage (Mahalaxmi et al., 2021; Sen et al., 2021a,b; Sharma et al., 2021; Singh et al., 2021; Rao et al., 2021).

Patients can present with facial pain or swelling, paresthesia, headache, or visual loss. On clinical examination, periorbital swelling, chemosis, proptosis, ophthalmoplegia, and facial nerve palsy might be found. Later, signs like nasal septum and palatal necrosis eschar may develop (Sen et al., 2021b; Sharma et al., 2021). The most common presentation reported by our patients were headache, periorbital pain, proptosis, and chemosis, all of whom had rhino-orbital mucormycosis, and three cases were complicated by intracranial extension.

Mortality rates can be as high as 50–80%, which is similar to the mortality rate of our patients. Mucormycosis mortality depends on site of involvement. Higher mortality is associated with intracranial involvement, disseminated disease and presence of underlying comorbidities (Kumar et al., 2021; Mahalaxmi et al., 2021; Sharma et al., 2021; Singh et al., 2021).

## Risk factors

DM is the most common risk factor predisposing patients to mucormycosis. Up to 85% of mucormycosis cases are associated with uncontrolled DM (Gupta et al., 2021; John et al., 2021; Kumar et al., 2021). Previously reported HbA1c among these patients was approximately 10%. India has reported the highest number of mucormycosis cases in the world, which might be partially explained by the high incidence of DM in India, as it constitutes 15% of the diabetic population globally (Kumar et al., 2021; Singh et al., 2021).

Among our patients, six were known to have DM, mainly uncontrolled, and the other four were newly diagnosed. Most patients had HbA1c of approximately 13%.

DM can affect innate immune response by reducing natural killer cell and T-cell activity. High blood sugar decreases phagocytic activity and impairs neutrophil activity required to kill or-

organisms. In DKA, these organisms can use ketones to grow. In addition, the binding of iron to transferrin is impaired in a low-pH environment of less than 7.4, thus more iron is available for fungal metabolism, which plays a major role in fungal survival and virulence (Goddanti et al., 2021; John et al., 2021; Mahalaxmi et al., 2021; Perner et al., 2003; Revannavar et al., 2021; Singh et al., 2021). All of our patients had DM and presented with rhino-orbital mucormycosis with or without cerebral involvement. Those with poor glycemic control and DKA had worse outcomes and died.

Other identified risks for developing mucormycosis are underlying hematological malignancies, organ or bone marrow transplantation, neutropenia, broad-spectrum antibiotic use, and malnutrition (Kumar et al., 2021; Mahalaxmi et al., 2021; Sen et al., 2021a,b). Patients with these conditions have higher risk of pulmonary mucormycosis (Singh et al., 2021).

Up to 8% of COVID-19 cases are complicated by secondary bacterial and invasive fungal infections. The use of broad-spectrum antibiotics, steroids, and tocilizumab increased the risk for such complications (Sen et al., 2021a,b; Sharma et al., 2021).

CAM has been identified as a serious complication during the second wave of the COVID-19 pandemic (Sen et al., 2021b; Singh et al., 2021). Most reported cases were from India, with a median age of 45–50 years, and male patients being predominantly affected. As was the case with patients included in this study, DM and steroid use are the most common risk factors for CAM (Kumar et al., 2021; Sen et al., 2021a,b).

The SARS-CoV-2 virus can itself infiltrate beta cells in the pancreas, which causes necroptosis cell death and thus metabolic dysregulation. This contributes to new-onset hyperglycemia and poor control of pre-existing DM (Gupta et al., 2021; Rao et al., 2021). Cytokine storm can also lead to insulin resistance (John et al., 2021). The reduction in absolute lymphocytes and T-cells (especially CD4+ and CD8+) associated with SARS-CoV-2 infection affects the production of cytokines such as interleukins (IL-4, IL-10, and IL-17) and interferon-gamma cytokines. These cytokines can damage fungal hyphae, but this mechanism is impaired in COVID-19 patients (Sen et al., 2021a; Sharma et al., 2021; Singh et al., 2021; Revannavar et al., 2021).

Steroids are widely used in the management of COVID-19 pneumonia. They reduce phagocytic activity and thus invasion of pathogens (Mahalaxmi et al., 2021), and also worsen glycemic control (John et al., 2021; Kumar et al., 2021). This is challenging, especially when patients require steroids for the management of severe COVID-19 pneumonia/ARDS.

Three of our patients were on high-dose steroids during their admission, and two patients with mild cases received at least one dose of steroids. Thus, COVID-19, DM, and the use of high steroid doses were the main risk factors contributing to the development of mucormycosis in our patients.

Studies showed that the median duration of developing mucormycosis is approximately 10–15 days after COVID-19 diagnosis (Gupta et al., 2021; Sen et al., 2021a,b).

Delayed mucormycosis cases have also been reported 4–6 weeks after COVID-19 diagnosis (Sen et al., 2021a). Most of our patients presented with mucormycosis within 2–3 weeks after COVID-19 diagnosis.

Another link between the SARS-CoV-2 virus and mucormycosis is the same entry route, namely glucose-related protein 78, which is required for *Mucor* fungi to enter nasal and paranasal sinus mucosa. The highest number of CAM cases was reported during the second wave of the COVID-19 pandemic, when the Delta variant (B.1.617.2) predominated (Goddanti et al., 2021).

Survival and outcomes of patients with mucormycosis are affected by early diagnosis and appropriate management (Singh et al., 2021). Diagnosis of mucormycosis is challenging when there is low clinical suspicion and difficulty in fungal iso-

lation from tissue cultures (Garg et al., 2021; Sen et al., 2021a,b). Assessing paranasal sinuses for opacification, thickening of mucosal lining, bony erosions, and extra-sinus spread suggestive of mucormycosis with or without cerebral extension using computed tomography or contrast-enhanced magnetic resonance imaging can aid diagnosis (Cornely et al., 2019; Sen et al., 2021a,b).

Nasal endoscopy is used to look for mucosal inflammation or necrosis. Targeted biopsy of affected areas is important for microbiological and histopathological diagnosis (Sen et al., 2021b).

Fungal culture is used to identify fungal species and antifungal susceptibility (Cornely et al., 2019). In eight of our 10 cases, microscopic examination revealed broad non-septate/sparsely septate hyphae with random branching, sporangia, and rhizoids. The growth of dense, cottony, white to dark colonies covering the full plate was detected 24–48 hours after incubation, best visible at 37°C, and was identified by MALDI-TOF. However, improper tissue handling and prior use of antifungal therapy might provide false negative results in up to 50% of cases (Sen et al., 2021a,b). Cultivating and identifying the mold was successful in 80% of our cases.

Histopathology is a useful tool for confirming diagnosis. The presence of thick, non-septated, ribbon-like fungal hyphae invading tissue and causing necrosis and angioinvasion in tissue sections stained with hematoxylin-eosin, periodic acid-Schiff stain, and Grocott-Gomori's methenamine silver stain is useful for distinguishing true infection from colonization of tissue. The presence of an inflammatory reaction indicates that organism represents infection and not commensal (Cornely et al., 2019; Sen et al., 2021a,b).

Histopathology confirmed the diagnosis of angioinvasive mucormycosis in all 10 of our cases.

### Management

Early diagnosis of mucormycosis is the key for survival. A multidisciplinary team approach is required to optimize management of mucormycosis cases. Strictly controlling hyperglycemia and avoiding DKA play an important role in preventing progression of the disease.

High-dose steroids, especially in poorly controlled patients with DM, even if indicated in patients with severe COVID-19, should be used cautiously, and unjustified corticosteroid treatment of patients with non-hypoxemic COVID-19 should be avoided (Revannavar et al., 2021; Sen et al., 2021a,b).

AmBisome (the liposomal, less nephrotoxic form of amphotericin B) at 5–10 mg/kg/day is the antifungal therapy of choice. Posaconazole can be used as an alternative therapy or in combination with AmBisome in refractory cases, although there is no strong evidence of its superiority, and further studies are required. A step-down prolonged oral antifungal therapy for 3–6 months is required (Cornely et al., 2019; Sen et al., 2021a,b).

Endoscopic sinus surgery with or without orbital exenteration to control the spread of the infection and dissemination is essential (Sen et al., 2021a,b; Sharma et al., 2021).

Even with aggressive surgical debridement and systemic antifungal therapy, mortality ranges from 33.3–80% and up to 100% in disseminated disease with delayed intervention (Cornely et al., 2019; Sharma et al., 2021).

As in other reported cases, mucormycosis can occur even after recovery from COVID-19. Follow-up of these patients of up to 3 months and monitoring blood sugar might contribute to earlier diagnosis and prevention of devastating sequelae.

### Conclusion

Patients with poorly controlled DM and COVID-19 living in hot climates, which contribute to *Mucorales* proliferation, are at greater



risk of developing mucormycosis. Despite aggressive medical therapy with high-dose AmBisome and aggressive surgical interventions, those with poor blood sugar control still have high mortality rates and worse outcomes.

### Declarations of competing interest

The authors have no competing interests to declare.

### Funding

This work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

### Ethical approval

Ethical approval was not required as there were no active interventions done to the patients included in the study. Consent was obtained from all patients.

### References

- Al Shahawey MG, El-Housseiny GS, Elsayed NS, Alshahrani MY, El Wakeel LM, Aboshanab KM. New insights on mucormycosis and its association with the COVID-19 pandemic. *Future Sci OA* 2022;8:FSO772.
- Cornely OA, Alastruey-Izquierdo A, Arenz D, Chen SCA, Dannaoui E, Hochhegger Bet al. Global guideline for the diagnosis and management of mucormycosis: an initiative for the European Confederation of medical mycology in cooperation with the Mycoses Study Group Education and Research Consortium. *Lancet Infect Dis* 2019;19:E405–21.
- Garg D, Muthu V, Sehgal IS, Ramachandran R, Kaur H, Bhalla Aet al. Coronavirus disease (COVID-19) associated mucormycosis (CAM): case report and systematic review of literature. *Mycopathologia* 2021;186:289–98.
- Goddanti N, Reddy YM, Kumar MK, Rajesh M. Role of COVID-19 inflammatory markers in rhino-orbito-cerebral mucormycosis: A case study in predisposed patients at a designated nodal centre. *Indian J Otolaryngol Head Neck Surg* 2021(Nov 13);1–7.
- Gupta R, Kesavadev J, Krishnan G, Agarwal S, Saboo B, Shah Met al. COVID-19 associated mucormycosis: A descriptive Multisite Study from India. *Diabetes Metab Syndrome* 2021;15.
- John TM, Jacob CN, Kontoyiannis DP. When uncontrolled diabetes mellitus and severe COVID-19 converge: The perfect storm for mucormycosis. *J Fungi* 2021;7:298.
- Kumar M, Sarma DK, Shubham S, Kumawat M, Verma V, Singh B, Nagpal R, Tiwari RR. Mucormycosis in COVID-19 pandemic: Risk factors and linkages. *Curr Res Microb Sci* 2021;2:100057.
- Mahalaxmi I, Jayaramayya K, Venkatesan D, Subramaniam MD, Renu K, Vijayakumar P, Narayanasamy A, Gopalakrishnan AV, Kumar NS, Sivaprakash P, Rao K, RS S, Vellingiri B. Mucormycosis: An opportunistic pathogen during COVID-19. *Environ Res* 2021;201:111643.
- Perner A, Nielsen SE, Rask-Madsen J. High glucose impairs superoxide production from isolated blood neutrophils. *Intensive Care Med* 2003;29:642–5.
- Rao V US, Arakeri G, Madikeri G, Shah A, Oeppen RS, Brennan P A. COVID-19 associated mucormycosis (CAM) in India: a formidable challenge. *Br J Oral Maxillofac Surg* 2021;59:1095–8.
- Revannavar SM, Supriya PS, Samaga L, Vineeth VK. COVID-19 triggering mucormycosis in a susceptible patient: a new phenomenon in the developing world? *BMJ Case Rep* 2021;14.
- Richardson MD, R-autemaa-Richardson R. Biotic environments supporting the persistence of clinically relevant mucormycetes. *J Fungi* 2020;6:4.
- Sen M, Lahane S, Lahane TP, Parekh R, Honavar SG. Mucor in a viral land: A tale of two pathogens. *Indian J Ophthalmol* 2021a;69:244–52.
- Sen M, Honavar SG, Bansal R, Sengupta S, Rao R, Kim Uet al. Epidemiology, clinical profile, management and outcome of COVID-19 associated rhino-orbito-cerebral mucormycosis in 2826 patients in India – collaborative OPAL-IJO study on mucormycosis in COVID-19 (COSMIC), Report 1. *Indian J Ophthalmol* 2021b;69:1670–92.
- Sharma S, Grover M, Bhargava S, Samdani S, Kataria T. Post coronavirus disease mucormycosis: a deadly addition to the pandemic spectrum. *J Laryngol Otol* 2021;135:442–7.
- Singh AK, Singh R, Joshi SR, Misra A. Mucormycosis in COVID-19: A systematic review of cases reported worldwide and in India. *Diabetes Metab Syndr* 2021;15.