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Case Series

Fungal sinusitis post COVID 19 – A case series from a diagnostic microbiology laboratory

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ABSTRACT

Background: Fungal infections are on rise globally owing to use of immunosuppressant drugs for various diseases, increased use of antimicrobials and in-situ devices, long duration of stay in hospitalized patients. There was sharp rise in mucormycosis infections in patients post COVID 19 illness which were previously limited to patients of end stage diabetic ketoacidosis.

Materials and Methods: We studied the epidemiological, clinical, microbiological and radiological aspects for culture positive cases of fungal sinusitis from April 2021 to June 2021.

Results: The mean age of our patients was 55.4 years (range 38 to 70 years). M:F ratio was 1.5:1. All fungal infections occurred in laboratory and/or radiologically diagnosed cases of COVID 19**.** 70% patients (n=7) had diabetes mellitus as co-morbidity. 60% of the cases (n=6) grew Rhizopus arrhizus, 30% grew Aspergillus species (n=3) and 10% grew Penicillium species (n=1). 90% of the cases (n=9) improved after treatment whereas 10% (n=1) succumbed to the illness.

Conclusion: Along with rhino-orbito-cerebral mucormycosis infections, nasal aspergillosis was also noted as a prominent cause of fungal sinusitis in post COVID 19 illness. As the antifungal therapy for these two fungal infections differs significantly, caution must be adhered to while diagnosing and treating.

1. Introduction

SARS CoV-2 virus has spread worldwide and attacks respiratory tract in its progression. The virus causes mild, moderate and severe illness. Antivirals and corticosteroids form a main course of treatment to prevent progression to severe disease, especially in co-morbid conditions. Fungal sinusitis, post COVID 19 infection and corticosteroid therapy, are on rise. These mainly involve infections by Aspergillus species and Zygomycetes. Both fungi being angioinvasive in nature, have been implicated in progression of disease to involve rhino-orbito-cerebral area. Mucormycosis causing fungi are saprotrophic in environment and infections are seen in host whose immunological defense mechanisms are weakened. Neutrophils play a major role in defense against mucormycosis, aspergillosis, disseminated candidiasis. Also, macrophages kill the fungal spores by oxidative damage. Opportunistic fungal infections are seen in cases of severely ketoacidotic diabetes mellitus where monocyte/macrophages are dysfunctional, leukemia and other malignancies where granulocytopenia is present. Patients on immunosuppressive therapy such as long term intake of corticosteroids and also those on voriconazole prophylaxis have been reported to present with mucormycosis infections.[1] The primary reason that appears to be facilitating Mucorales spores to germinate in people with COVID-19 is an ideal environment of

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low oxygen (hypoxia), high glucose (diabetes, new onset hyperglycemia, steroid-induced hyperglycemia), acidic medium (metabolic acidosis, diabetic ketoacidosis [DKA]), high iron levels (increased ferritin) and decreased phagocytic activity of white blood cells (WBC) due to immunosuppression (SARS-CoV-2 mediated, steroid-mediated or background co-morbidities) coupled with several other shared risk factors including prolonged hospitalization with or without mechanical ventilation.[2]

2. Materials and Methods

A retrospective study of cases of suspected mucormycosis infections post COVID 19 were conducted in a Diagnostic Microbiology Laboratory in association with an ENT Hospital from April 2021 to July 2021. Presence of fungal growth on culture was an inclusion criteria of study. The cases were evaluated on the basis of history of COVID 19 infection, modalities of treatment used for COVID 19, onset and course of mucormycosis infection post COVID 19, radiological and microbiological diagnosis of mucormycosis, pre-existing immunosuppressive states or co-morbid conditions if any, management of mucormycosis infection and thus, patient outcome.

In Microbiology Laboratory, the specimen were immediately processed for KOH (Potassium Hydroxide) mount, Gram stain and Fungal Culture according to the requisition. For those specimen in which fungal culture request was awaited, the specimen were maintained at room temperature as refrigeration halts growth of Mucorales.

To prepare KOH mount, 10% Potassium hydroxide was used for pus specimen, 20% Potassium hydroxide for nasal and sinus tissues, 40% Potassium hydroxide for harder tissues. The tissues were lightly teased in sterile petri dish and the required strength potassium hydroxide reagent was added onto the teased specimen on the slide. The slides were left at room temperature inside a wet chamber for 20-40 minutes in order to allow for dissolution of tissue material and standing out of fungal hyphae. However, sometimes the specimen were left overnight at room temperature and reviewed, which has helped to detect even scanty hyphae and thus increased sensitivity of KOH mount.

The specimens were inoculated on two Sabouraud's Dextrose Agar (SDA) slants, one incubated at 37°C and another at room temperature. From a fungal growth observed, slide culture was prepared on SDA cube and incubated at room temperature in wet chamber, observing for sporulation on alternate days. Lactophenol Cotton Blue mount was prepared from the growth obtained and microscopic examination was done to diagnose and differentiate fungal species.

3. Results

Fungal infections post COVID 19 illness were seen mostly in age group of 35 years to 70 years. The treatment of COVID 19 illness invariably included steroids for all these cases. 90% of the cases (n=9) were treated with antiviral Remdesivir. One patient received Inj. Tocilizumab to combat cytokine storm whereas another received Inj. Baricitinib for the same. Oxygen supply was given to 90% of these cases (n=9). Duration between COVID positivity and appearance of symptoms of fungal sinusitis ranged from as less as 7 days to as long as 37 days. Symptoms of fungal sinusitis leading to deeper tissue invasion included headache, sinus pain, mucopurulent discharge through nose, ptosis, proptosis, vision loss and facial palsy.

70% cases (n=7) were having Diabetes mellitus as co-morbidity. However, remaining 30% cases (n=3) had impaired hemo glucose test attributed to steroid use. Magnetic Resonance Imaging showed muscular involvement in orbital area with bony erosions, fat stranding, abnormally enhancing soft tissue mass from sinuses.

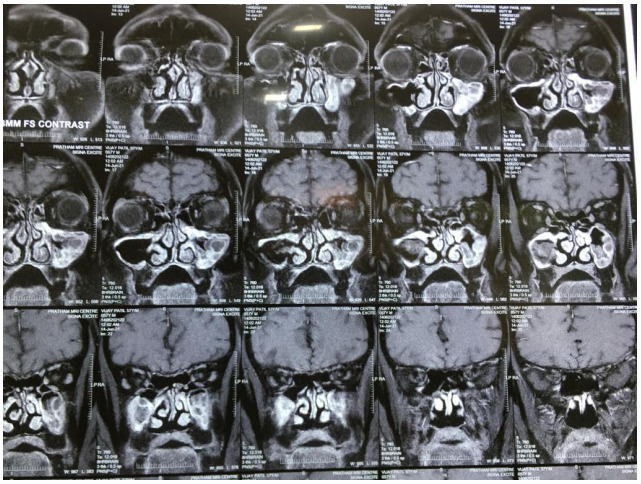

Fig. 1: MRI of paranasal sinuses suggestive of acute maxillary sinusitis of possible fungal etiology with left orbital involvement

60% of the cases (n=6) showed growth of Rhizopus arrhizus. Half (50%) of them grew at both room temperature as well as at 37°C. We found growth of A. flavus (n=1), A. fumigatus (n=1) and mixed growth of A. flavus and A. fumigatus (n=1). There was growth of Penicillium species from one case.

Surgical removal of infected tissue was done for all cases which included maxillectomy, ethmoidectomy, septectomy. Radical debridement was carried out wherever required and included orbital clearance for one patient. Surgical management was accompanied with Inj. Liposomal Amphoterecin B or Inj. Amphoterecin deoxycholate 5mg/kg for 3 to 4 weeks followed by Tab. Posaconazole

Table 1: Characteristics of COVID 19 illness and fungal sinusitis in suspected mucormycosis patients

S.No.	Age/ Sex	Covid RT PCR	Covid illness HRCT Chest score/ Chest X Ray findings	Treatment of COVID 19 illness	If oxygen-nation required	Duration between COVID 19 positivity and onset of symptoms of fungal sinusitis	Symptoms suggestive of fungal sinusitis/ mucormycosis in patients
Case 1	45 years/ Male	Positive	Covid pneumonia 18/25	Remdesivir, Steroids, Tocilizumab Antibiotics	Yes, 2 Litres/ minute	13 days	Proptosis and loss of vision in Right eye, Right sided Facial palsy, Foot drop
Case 2	57 years/ Male	Negative	Multifocal peripheral and angiocentric ground glass opacities in B/L Lung fields 15/25	Solumedrol, Barcitinib, remdesivir, Meropenem, Piptaz	Yes	16 days	Right sided ptosis and Right eye irritation, Nasal Blockage
Case 3	70 years/ Female	Positive	Covid pneumonia	Remdesivir, Methyl-prednisolone Piptaz	Yes	12 days	Right sided Headache Temporal region
Case 4	63 years/ Female	Positive	Bilateral Haziness	Remdesivir, Steroids	Yes	15 days	Left eye swelling and left facial pain f/b Diplopia in B/L Eye
Case 5	51 years/ Male	Positive	Covid Pneumonia	Remdesivir, Steroids	Yes	28 days	Right sided Facial Palsy, Loss of vision in Right eye, Headache, Nausea
Case 6	68 years/ Male	Positive	Covid Pneumonia 14/25	Remdesivir for 5 days, Solumedrol for 20 days	Yes, 1-2 litres/ minute for 5 days	16 days	Headache Right side
Case 7	59 years/ Female	Positive	Covid pneumonia	Remdesivir, Steroids	Yes	7 days	Bilateral Sinus pain
Case 8	62 years/ Male	Positive	Multiple atelectatic bands in resolving phase of Viral pneumonia 15/25	Steroids	Yes	37 days	Headache
Case 9	38 years/ Female	Positive	Covid pneumonia 8/25	Remdesivir, Antibiotic, Methylprednisolone 40mg BD for 7 days, Ivermectin, Azee, Post Discharge Prednisolone 8mg BD for 5 days f/b 8mg OD for 5 days	No	15 days	Right Orbital pain, Right Nasal and Maxillary Heaviness with Mucopurulent discharge
Case 10	41 years/ Male	Negative	Covid pneumonia 7/25	Remdesivir, Steroids, Antibiotics	Yes	20 days	Left side sinus pain

Table 2: Laboratory and investigations in post COVID 19 suspected cases of mucormycosis.

S. No.	Co-morbidity	HbA1C level	MRI PNS findings
Case 1	Diabetes mellitus	10.9	Mild ill-defined soft tissue in right intra-orbital extra-conal as well as intra-conal spaces along supero-medial aspect extending posteriorly in the region of orbital apex abutting optic nerve with mild retro-ocular fat stranding
Case 2	Diabetes mellitus	12.15	Soft tissue mass in Right Nasal Cavity
Case 3	Diabetes mellitus and Hypertension	8.3	Ill defined mildly enhancing soft tissue in right parapharyngeal region with bulky edematous right medial and lateral pterygoid muscles.
Case 4	Diabetes mellitus	7.5	Intraorbital extension with involvement of medial orbital wall
Case 5	Diabetes mellitus	8.5	Marked intraconal perifocal fat stranding, edema and post contrast enhancement around proximal half of right optic nerve. Mild thickening and heterogenous enhancement of proximal segments of medial, superior, lateral and inferior rectus muscles
Case 6	Diabetes mellitus	9.7	Pansinusitis
Case 7	Diabetes mellitus	9.2	Right sided soft tissue mass in nasal cavity
Case 8	Hypertension, Non-diabetic	Impaired HGT due to steroid use, Post prandial glucose – 288mg/dl	Abnormal enhancing polypoidal soft tissue thickening involving maxillary, ethmoid and sphenoid sinuses with subtle erosions of bony walls.
Case 9	Non-diabetic	Impaired HGT due to steroid use	An ill-defined non-enhancing T2 Hypointense areas in right maxillary, ethmoid and sphenoid sinuses, may represent inspissated secretions/ fungal infection.
Case 10	Non-diabetic	Impaired HGT due to steroid use	Masseteric/ Zygomatic inflammation treated with IV Antibiotics f/b Concretions in Maxillary sinus and Skull base osteomyelitis/ Clival osteomyelitis

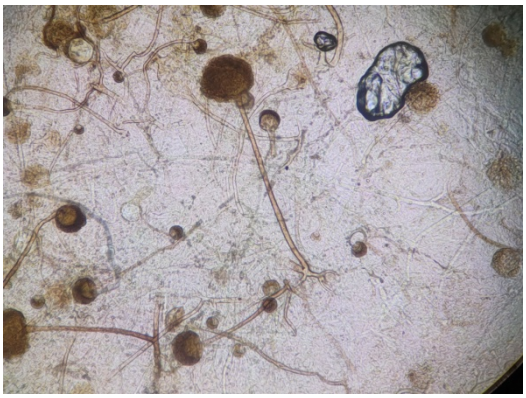

Fig. 2: Sporangia of Rhizopus arrhizus in KOH mount of nasal tissue

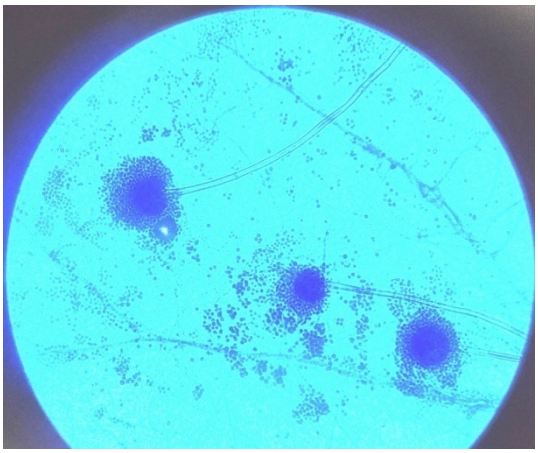

Fig. 3: Sporangia of Aspergillus fumigatus observed in LPCB Mount of slide culture

300 mg for 3 to 4 months. All of the cases improved on treatment except one patient succumbed. 20% of the patients (n=2) developed cerebral mucormycosis as evident on MRI are maintained on Tab. Posaconazole and improving.

4. Discussion

Immunosuppression and fungal infections go hand in hand. Candidiasis, Aspergillosis, Cryptococcosis and Zygomycosis are frequent opportunistic fungi of the compromised host. Rhinocerebral mucormycosis is acute and fulminant fungal infection in acidotic diabetic patient. The fungus Rhizopus arrhizus thrives in the medium of high glucose content. There is delayed cellular migration and mobilization, also diminished phagocytic function in ketoacidotic patient.[3]

The Colonization by wide variety of opportunistic pathogens by exposure to high concentration of spores in closed environment has been cited in animal infections.[4] There too was a speculation that oxygen masks and/or nasal canulae were not disinfected satisfactorily which lead to exposure of Mucormycetes spores directly to patient's

Table 3: Microbiological findings from different specimen sent from post COVID 19 suspected cases of mucormycosis

S.No.	Specimen received	KOH Mount	Gram staining	Sabouraud's Agar culture findings	LPCB Findings from Slide culture suggestive of Final Diagnosis
Case 1	Right Maxillary Sinus tissue	Hyaline, Broad, Sparsely Septate Hyphae seen	Broad, Sparsely septate hyphae	After 48 hours on both Room Temperature & 37°C, cotton wooly growth with black sporulation on obverse	Rhizopus arrhizus grown
Case 2	Nasal tissue	Broad, aseptate hyaline hyphae with sporangia of Rhizopus arrhizus seen	Broad, aseptate hyphae	Within 48 hours at Room Temperature & 37°C, cotton wooly growth with Black sporulation	Rhizopus arrhizus grown
Case 3	Right uncinate process and Maxillary sinus tissue	Narrow septate fungal hyphae seen.	Fungal hyphae not seen	After 4 days at Room Temperature, Greenish white Filamentous Growth	Aspergillus fumigatus grown
Case 4	Left Ethmoidal Sinus Tissue	Broad, Sparsely septate Fungal Hyphae	Broad Aseptate Hyaline Fungal Hyphae	After 48 hours at Room Temperature, White Cotton Wooly Growth	Rhizopus arrhizus grown
Case 5	Right Ethmoidal sinus & Right Orbit tissue	Septate and Aseptate Fungal Hyphae seen.	Septate Fungal Hyphae seen	Within 24 hours at 37°C and Room Temperature, Yellowish green Filamentous growth	Aspergillus flavus grown.
Case 6	Right Middle turbinate tissue	Broad, Aseptate, Hyaline, Collapsible Hypahe	Broad, Aseptate Hyphae	After 3 days at 37°C, Cotton wooly Growth with Black sporulation	Rhizopus arrhizus grown.
Case 7	Right Side Nasal crust	Septate Fungal Hyphae seen	Fungal hyphae not seen	After 6 days at Room Temperature, Khaki coloured powdery colony seen	Penicillium species grown.
Case 8	Bilateral Nasal Cavity Tissue	Broad sparsely septate hyphae seen.	Broad, Aseptate fungal hyphae seen	After 3 days at Room Temperature, Greyish white cotton wooly growth seen.	Rhizopus arrhizus grown.
Case 9	Nasal Tissue	Broad, Sparsely Septate, Hyaline, Collapsible Fungal Hyphae seen	Fungal Hyphae not seen.	Within 48 hours on both 37°C and Room Temperature, Greyish white cotton candy like growth seen.	Rhizopus arrhizus grown.
Case 10	Left maxillary sinus concretions	Narrow septate hyphae with dichotomous branching along with sporangia of A. flavus and A. fumigatus.	Septate fungal hyphae seen	Within 48 hours at 37°C – Smoky green and yellowish green white filamentous colony with yellowish brown reverse and at Room Temperature – white filamentous colony with yellowish brown reverse	Mixed growth of A. flavus and A. fumigatus.

Table 4: Management strategies for fungal sinusitis/mucormycosis cases post COVID 19 infection

S. No.	Surgical Management	Medical Management	Maintainance	Outcome
Case 1	Radical debridement by Modified Denker's approach Right side	Liposomal Amphoterecin B 5mg/kg for 3 weeks	Tab. Posaconazole 300mg BD on Day 1f/b 300 mg OD for 3 months	Developed cerebral involvement on maintainance, improving
Case 2	Left Medial Maxillectomy	Amphotrecin B 5mg/kg	Tab. Posaconazole 300mg BD on Day 1f/b 300 mg OD for 3 months	Improved
Case 3	Medial maxillectomy – Denkers approach	Amphotrecin B 5mg/kg	Tab. Voriconazole	Improved
Case 4	Left Modified Denker's approach, Septectomy done	Amphoterecin B 5mg/kg	Tab. Posaconazole 300mg BD on Day 1f/b 300 mg OD for 3 months	Improved
Case 5	Right Medial Orbital decompression with Orbital clearance	Tab. Voriconazole 400mg BD f/b Amphoterecin B 5mg/kg and	Tab. Posaconazole 300mg BD on Day 1f/b 300 mg OD for 1 and $\frac{1}{2}$ months	Deceased
Case 6	Right Medial Maxillectomy with Ethmoidectomy	Amphoterecin B 5mg/kg	Tab. Posaconazole 300mg BD on Day 1f/b 300 mg OD for 3 months	Improved
Case 7	Right nasal endoscopic resection	Itraconazole 200mg BD for 5 days f/b 100mg OD	Itraconazole 100 mg OD for 3-4 weeks	Improved
Case 8	Bilateral FESS with septectomy	Amphoterecin B 5mg/kg	Tab. Posaconazole 300mg BD on Day 1f/b 300 mg OD for 3 months	Developed cerebral involvement on maintainance, improving
Case 9	Radical debridement by Denker's approach	Amphoterecin B 5mg/kg	Tab. Posaconazole 300mg BD on Day 1f/b 300 mg OD for 3 months	Improving
Case 10	Removal of Maxillary concretions	Tab. Voriconazole 400 mg BD on Day 1	Tab. Voriconazole 200 mg BD twice a day for 3 months	Improving

sino-nasal area. In our study too, 90% of the cases (n=9) were given external oxygen supply to maintain saturation levels. Not only did the specimen from these patients grew Mucormycetes species but also Aspergillus and Penicillium.

Human infections are reported in variety of predisposing conditions eg. Trauma, Beta thalassemia, renal transplant, hyperglycemia, cirrhosis, burns, needle site injection as well as with no underlying conditions implicating that many factors are responsible for producing disease.[5–8]

Steroids are being used for treatment of various diseases since long but recent upsurge of mucormycosis infections in patients suffering from COVID 19 has alarmed all of us for a more controlled and cautious use of this wonder drug ensuing de-escalation as early as possible. Hyperglycemia is a known side effect of steroid drug and it is the side effect implicated in mucormycosis infections more along with pathophysiology of Sars-CoV-2 in current scenario. Also, diabetes mellitus is an established co-morbidity for Sars-CoV-2 virus infection. All cases (100%) in our study were prescribed with steroids (n=10). Also, 100% (n=10) either had history of diabetes mellitus with poor glycemic control (66.6%) or altered hemo-glucose test owing to steroid use (33.4%). Our findings were similar to those of Mishra N et al. who mentioned 80% of cases had pre-existing diabetes mellitus. However only 60% of patients from their study were treated with steroids.[6] Immune suppressants such as tocilizumab and baricitinib were also used in 20% (n=2) of our patients which added to suppression of T and B cells, in addition to lymphopenia caused by Sars-CoV-2 infection and inhibition of neutrophilic migration to site of inflammation due to inhibition of cytokines by steroids.

The mean age of our patients was 55.4 years (range 38 to 70 years) which is very similar to that of Mishra N et al.[9] Median duration for onset of symptoms of mucormycosis infection from onset of COVID 19 illness in our study was 15 days (range 7-37 days).

We came across one peculiar case wherein despite high index of clinical suspicion of mucormycosis, sinus tissue on KOH mount revealed septate hyphae and grew Aspergillus flavus on two occasions but inspite of surgical debridement and standard treatment with voriconazole, the patient continued to progressive disease involving orbit. Tissue from orbital decompression on KOH mount revealed scarce presence of aseptate hyphae suggestive of mucormycetes. The patient was again administered with Liposomal Amphoterecin B along with orbital clearance but succumbed to his illness. This patient also had homolateral facial palsy which is a feature of acute rhinocerebral mucormycosis.[5] Multiple cranial nerve involvement in mucormycosis infection has been reported by Paul S et al.[10]

In our study, two patients having mucormycosis infections developed facial palsy.

Also, aspergilloma of the nasal sinuses have been reported to eventually involve orbit of the eye.[11,12] Intracranial invasion has also been reported with invasive Aspergillus sinusitis due to its proximity. The outcome is usually fatal if not treated properly.[13,14]

5. Conclusion

Aspergillus species are increasingly getting isolated in tissues from fungal sinusitis cases along with Mucormycetes species post Sars-CoV-2 infections. Treatment of choice for mucormycosis infection is Amphoterecin B whereas for Aspergillus infections, voriconazole is drug of choice. Timely management is key to good prognosis in these patients. Hence, nasal aspergillosis too should be promptly reported and vigorously treated along with rhinocerebral mucormycosis infections.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.

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