Letter to Editor

Post-COVID mucormycosis: Ascertainment of the pathological diagnostic approach

Sir,

COVID-19 pandemic continues to disrupt the human lives. Post-COVID mucormycosis is a serious late complication being observed in patients recuperating from COVID-19 infection. 14,000 cases have been reported from India alone, by the end of May 2021.[1] Popularly known as the "black fungus," it has been associated with diabetes and indiscriminate use of corticosteroids in COVID-19 patients. A recent meta-analysis on the worldwide case reporting of post-COVID mucormycosis indicates 81% of the cases to be reported from India with the most common affected sites being the nose and sinuses (followed by rhino-orbital).[2] With the mortality rate ranging from 40% to 80%[3] and India preparing for the onslaught of the third wave-oral pathologists should familiarize themselves with this angioinvasive infection and its critical differential diagnoses.

Clinically, Smith and Kirchner's (1958) criteria can be used for the identification of individuals with rhino-cerebral mucormycosis: Blood tinged nasal discharge with facial pain; black necrotic nasal turbinates (mistaken for dried blood); soft perinasal/periorbital pain with induration; ptosis of the lid and proptosis of the globe, dilatation and fixation of the pupil, limitation of globe mobility; progressive lethargy despite good diabetic response and loss of corneal reflex and onset of facial weakness [Table 1].[4] Primary mucormycosis of the oral cavity may present as multiple swellings in the gums, draining abcesses, mobile teeth in the affected region, oro-antral communications

Table 1: Clinical, radiological, microscopy and other investigations for the diagnosis of mucormycosis

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Diagnostic Criteria	**General findings**	**Specific findings**
Clinical findings	Nasal stuffiness	Oral manifestations
	Nasal discharge,	Multiple loose teeth
	Foul smell	Draining sinus
	Epistaxis	Palatal ulcer
	Unilateral facial edema	Most common site
	Proptosis	Ulcer over palate (ischemic necrosis of the mucoperiosteum with bony denudation)
	Palpebral fistula developing into necrosis	Other sites - Gingiva, lips, alveolar ridge, cheeks, tongue and mandible (ulcer)
Radiographic findings	Nodular thickening of the sinus necrosis	CT scan with contrast/MRI scan - erosion or destruction of the bone and may help to delineate the extent of the disease
	Sinus opacification without fluid level	
	Spotty destruction of paranasal sinuses	
Direct microscopy of the deep or endoscopy-guided nasal swab, paranasal sinus, or orbital tissue	KOH mount	
	Smear stained with H and E, PAS and GMS stains	
	Long-rapid identification	
	Fluorescent brighteners such as Blankophor and Calcofluor White together with KOH enhance the visualization	
Identification of organisms on culture	Advantage	Disadvantage
	Helps in genus and species identification	Low sensitivity
	Antifungal susceptibility testing	Can be falsely negative in up to 50% of mucormycosis cases
	Rapid growth of fluffy white, gray or brown cotton candy-like colonies can be seen The hyphae are coarse and dotted with brown or black sporangia	
Histological examination	H & E, PAS and GMS stains	
	Long	
	Broad branching	
	Nonseptate hyphae	
	Variable width of hyphae 6-25 μm	
Molecular-based methods	Not commercially available widely	
	Detection of DNA in serum as well as in other body fluids is very promising	
	75% sensitivity	

MRI: Magnetic resonance imaging, H & E: Hematoxylin and eosin, PAS: Periodic acid-Schiff, GMS: Grocott-gomori methenamine silver, KOH: potassium peroxide , CT: computed tomography

and exposed necrotic bone. In addition, a palatine ulcer should also be considered a red flag. However, clinical signs and symptoms have low sensitivity and specificity for establishing the diagnosis with other micro-organisms such as Pseudomonas showing similar features. In addition, the development of fungal infection in immunosuppressive patients receiving anti-fungal prophylaxis for *Aspergillus* (voriconazole) in immunosuppressive patients is suggestive of mucormycosis.[5]

The European Confederation of Medical Mycology and the Mycoses Study Group Education and Research Consortium have recently issued guidelines and a diagnostic algorithm for mucormycosis. Initial rapid diagnosis can be established through direct microscopy examination with potassium hydroxide. Preferable addition of fluorescent brighteners (Calcoflour, Blankophor) and subsequent examination under fluorescent microscopy enhance fungal visualization. However, for species identification and diagnostic confirmation-either histopathological examination of tissue sections stained with hematoxylin and eosin (H & E) stains, periodic acid Schiff or Grocott methenamine-silver or specimen culture can be performed. The presence of nonpigmented, pale, nonseptate/pauci-septate ribbon-like hyphae with width of 6–25μ and a haphazard pattern of branching (45°–90°); showing tissue invasion is essential for diagnosis of mucormycosis [Figure 1]. Focus on the wider and irregular nature of the branching of the Mucorales genera can prevent confusion arising due to tissue folding over itself (giving rise to artefactual septations). Aspergillosis should be excluded as it can mimic mucormycosis [Figure 2]. The identification of sporangia containing sporangiospores also aids in distinction of Mucorales from *Aspergillus* sp. which demonstrate conidia, popularly known as fruiting bodies. The lesional tissue also displays variable identifying features depending upon the duration of occurrence. In acute lesions: Widespread necrosis, angioinvasion, perineural invasion and neutrophilic infiltration is characteristic while the chronic lesions display pyogranulomatous inflammation, giant cells and Splendore–Hoeppli phenomenon.[3] Splendore–Hoeppli phenomenon refers to the intensely amorphous eosinophilic material arranged in star shaped or club-like configuration around the fungal hyphae.[6]

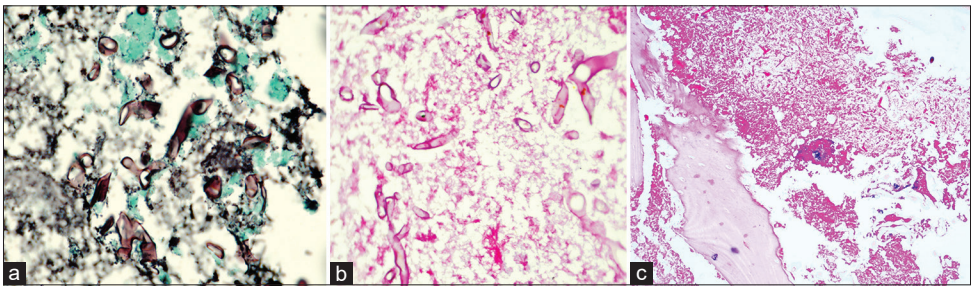

Figure 1: Histologically presenting as aseptate broad ribbon-like hyphae of mucormycosis with branching, as seen in Grocott methenamine-silver (a), periodic acid schiff (b) and Hematoxylin and eosin-stained tissues (c)

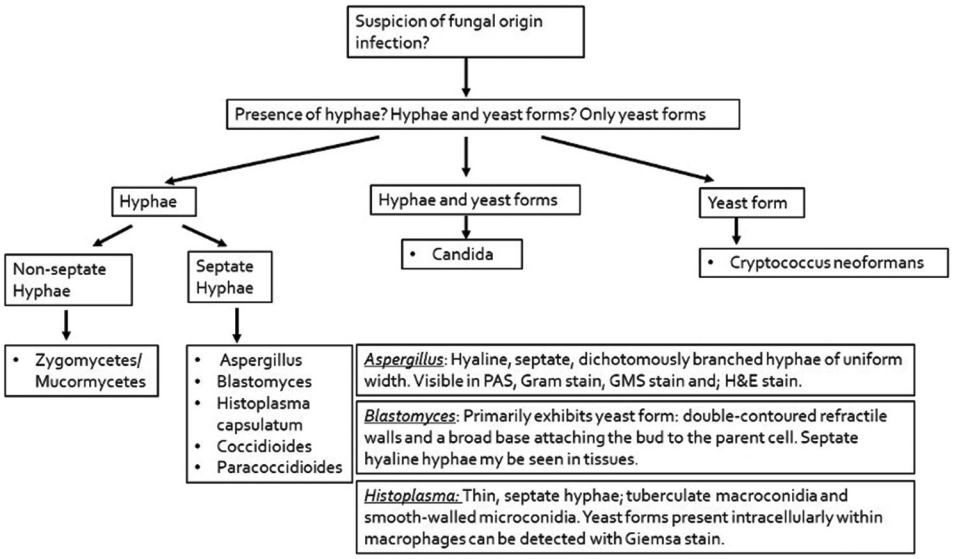

Figure 2: Flow diagram showing algorithm for ruling out other oral fungal lesions

Culture of the specimens is also recommended for determining sensitivity to antifungal therapeutic agents. Typical findings include cottony white or grayish black colonies in routine media at 30 and 37°. Mold identification can also be performed through Matrix-assisted laser desorption ionization-time of flight mass spectrometry, although it would require specific laboratory and computerized database set-up.[5]

Molecular methods are rapid diagnostic tests which can be applied to both fresh specimens and paraffin-embedded sections. In the recent years, several molecular methods have been investigated to facilitate the early diagnosis of mucormycosis: Lateral flow immunoassay, "internal transcribed spacer" region sequencing, molecular beacon probes based on ITS1 ribosomal DNA region, 28S ribosomal RNA gene and the CotH gene. A major limitation of these methods is their restricted identification of a few species of the Mucorales genera thus affecting the sensitivity of these tests based on the target species. Quantitative polymerase chain reaction (PCR) targeting using fungal primers (18S ribosomal RNA) for the detection of circulating Mucormycetes DNA in the serum or blood of the patients can be used for screening of high-risk patients as well as for monitoring the therapeutic effects of the medications. High sensitivity and specificity have been reported in particular for the serum-based PCR detection. PCR of the formalin-fixed paraffin-embedded (FFPE) tissue/fresh tissues can be performed in patients of proven histopathological tissue invasion but with negative culture reports or limited tissue specimen. While the PCR in the FFPE tissue has high specificity (100%), its sensitivity is affected due to the issues of technique precision, fungal fragmentation, low DNA load, environmental contamination and lack of standardization.[6]

Although numerous methods are now described in the literature for the diagnosis of mucormycosis, direct microscopy with histopathology remains the gold standard for the confirmatory diagnosis of mucormycosis. It is essential to be aware of the conditions which might mimic mucormycosis [Table 2]. Many of these entities are also part of spectrum of diseases related to post-COVID complications. Thus, it is imperative for an oral pathologist to understand thoroughly the nature of the fungus and act in appropriate direction for diagnosing it.

Table 2: Clinical differential diagnosis of post-COVID-19 mucormycosis

Corticosteroid-induced avascular necrosis
MRONJ
Squamous cell carcinoma (carcinoma cuniculatum)
Chronic granulomatous infection such as tuberculosis and tertiary syphilis
Midline lethal granuloma
Wegener's granulomatosis
Other deep fungal infection: Aspergillosis
Osteomyelitis

MRONJ: Medication-related osteonecrosis of jaws

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Conflicts of interest

There are no conflicts of interest.s

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