Histopathological evaluation of a rare fulminant case of contemporaneous mucormycosis, aspergillosis and actinomycosis

Aadithya B Urs, Jeyaseelan Augustine, Shivani Singh
Department of Oral Pathology and Microbiology, Maulana Azad Institute of Dental Sciences, New Delhi, India

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INTRODUCTION

Fungal infections in humans occur as a result of defects in the immune system. Local compromising factors include decreased salivation, poor oral hygiene, wearing dentures among others while systemic factors include diabetes mellitus, nutritional deficiency, HIV infection/AIDS and others. Systemic mycoses is considered in cases where chronic oral ulcerations or unusual mouth lesions are observed particularly in the immunocompromised patient population.

CASE REPORT

A 76-year-old female patient presented to the department of oral pathology and microbiology with a chief complaint of difficulty in eating for the past 1 week. The patient also gave a history of pain in the upper right back tooth region for 8 months, following which extraction was performed due to the presence of periodontally compromised 16 and 17, after which the symptoms were temporarily relieved. On intraoral examination, a well-defined lesion approximately 2.5 cm × 1.5 cm in size with everted and slightly indurated margins was present on the edentulous alveolar ridge with respect to missing 16 and 17, which was extending to the vestibule. There were no symptoms of nasal regurgitation, but the patient presented with a nasal twang. The patient was diabetic and was under medication for the past 8 months after extraction was done. Bilateral submandibular lymph nodes were slightly enlarged and mobile. Orthopantomogram showed irregular alveolar bone loss. Water’s view depicted slight haziness in the right maxillary sinus and bone loss in the involved area. The patient complained of discomfort in the same region for the past 7 days and also submitted a piece of tissue which had been dislodged from the upper left back tooth region while eating. The tissue was immediately preserved, subjected to decalcification, processed and then histopathologically examined.

HISTOPATHOLOGY

The decalcified sections of the hard tissue bit received showed abundant bony trabeculae with empty osteocytic lacunae, absence of osteoblastic rimming and prominent resting and reversal lines. Numerous fungal hyphae and spores of varying morphology were seen in the marrow spaces. Some fungal hyphae were broad, aseptate and ribbon shaped, showing branching at obtuse angles representing Mucorales species, whereas others were thin and septate, showing branching at acute angles representing Aspergillus species [Figure 1]. Fungal spores of varying sizes were observed, exhibiting budding at some places along with fruiting bodies of Aspergillus [Figure 1]. These were easily identified as Aspergillus niger and Aspergillus flavus.

Numerous actinomycotic colonies were also noted along with other bacterial colonies, including both bacilli and...
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Figure 1: (a) Cross-sections of numerous *Aspergillus* and Mucorales hyphae. (b) Septate fungal hyphae of mucormycosis. (c) Fruiting bodies (conidiophores) of *Aspergillus*. (d) Actinomycotic colonies present with associated trabeculae of resorbing bone (H&E, ×40)

Figure 2: (a) Fungal hyphae of mucormycosis on GMS staining. (b) Septate aspergillus fungal hyphae on GMS staining. (×100 original magnification)

Figure 3: Abundant cross-sections of fungal hyphae for aspergillus (red arrow) and mucormycosis (yellow arrow) exhibiting difference in sizes on GMS staining. (×40 original magnification)

Figure 4: (a and b) Showing fruiting bodies closely associated with bony trabeculae on GMS staining. (×40 original magnification)

cocci arranged in clusters as well as individually in close association with peripheral bony trabeculae [Figure 1]. Focal areas showed chronic inflammatory cell infiltrate comprising lymphocytes. A few small blood vessels, extravasated RBCs, areas of necrosis and vegetable matter were observed. Grocott’s methenamine silver staining showed septate *Aspergillus hyphae*, aseptate Mucorales hyphae and fungal spores along with actinomycotic colonies [Figures 2-5]. The final diagnosis was given as chronic osteomyelitis associated with a combination of mucormycosis, aspergillosis and actinomycosis.

**DISCUSSION**

The case presented here depicted a coinfection of mucormycosis, aspergillosis and actinomycosis, within the right maxillary alveolar region extending into the maxillary sinus with latent diabetes mellitus. Many cases showing a simultaneous infection of mucormycosis and actinomycosis have been reported in the past.[1] Literature also reports cases where mucormycosis was present along with aspergillosis.[2,3] To our knowledge, a very few cases have been reported exhibiting a combination of mucormycosis, aspergillosis with actinomycosis and also where all the three coexisted.

The universally occurring *Aspergillus* species is acquired by inhalation of airborne infective conidial spores primarily involving the nasal cavity, sinuses and bronchi, producing allergic reactions, such as sinusitis and allergic bronchopulmonary aspergillosis. They may cause severe life-threatening infections, especially in immunocompromised hosts.[4]

Histologically, the *Aspergillus* species exhibit septate hyphae with branching at acute angles, giving a definitive diagnosis of invasive aspergillosis which should ideally be confirmed by a positive culture. Microscopically, the presence of conidial heads, also known as fruiting bodies, is a characteristic feature for designating the infection as aspergillosis. These conidial heads are capped by variable number of phialides which terminate as rows of conidia, which are virulent. The different morphologies of these phialides assist in the differentiation among different *Aspergillus* species, as *Aspergillus fumigatus* has one-third of the vesicle covered by a single layer of phialides, whereas
Aspergillus terreus has double layers on more than half of the surface. Preparation of Aspergillus for culture is tedious due to its ubiquitous nature. Cycloheximide is a standard constituent of culture media, usually added to inhibit the growth of rapidly propagating molds, to which Aspergillus also exhibits susceptibility. Thus, on culture mediums containing cycloheximide, the possibility of false negative results is elevated. In the current case, histopathological examination proved to be precise with characteristic fruiting bodies. Hence, further diagnostic investigations were not carried out.

Mucormycosis is an opportunistic hazardous infection affecting immunocompromised patients by invading into their arteries producing thrombosis, leading to necrosis of the involved site. It chiefly affects diabetic individuals, as was seen in the current case, as it has a propensity to grow in favorable environmental situations as created by the presence of ketone bodies. The increase in the levels of free iron ions favors fungal growth. Histopathologically, Mucor shows broad (diameter, 6–16 µm), nonseptate, ribbon-like hyphae, with right angled to obtuse angled branching in a tissue biopsy. The occurrence of such hyphal structures is mandatory for a definitive diagnosis, as these fungi grow in anaerobic, aerobic and microaerophilic conditions, though cultures often prove to be negative.

An increase in opportunistic fungal infection has been observed in the past decade, leading to the occurrence of more than one organism, leading to a poorer prognosis. The factors predisposing to the development of both actinomycosis and aspergillosis are dental treatment or trauma. Immunocompromised state of an individual due to cancer and transplant are among other predisposing factors. In the current case, the history of extraction could probably be considered as one of the causes for the growth of Aspergillus and actinomycosis.

An early definitive diagnosis is thus of paramount importance in cases with multiple infections. Specific antifungal therapy along with radical surgical debridement is necessary for steady recovery of the patient. Surgery plays a key role in fungal eradication, but it is only useful when certain other predisposing and supportive factors such as diabetes are brought under control. In conclusion, following a multimodality and multistep treatment, this case of chronic osteomyelitis associated with mucormycosis, aspergillosis and actinomycosis of the right maxillary alveolus and maxillary sinus was advised antifungal medications with adjunctive hyperbaric oxygen treatment and is on a regular follow-up.

Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest
There are no conflicts of interest.

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