Case Report

Rhinocerebral zygomycosis with pulmonary aspergillosis in a non-HIV-infected patient: an unusual case report from India

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Zygomycosis and aspergillosis are two serious opportunistic fungal infections that are commonly seen in immunocompromised patients. Since both these fungi invade vessels of the arterial system, an early and rapid diagnosis by direct examination of KOH mounts of the relevant clinical sample can confirm the diagnosis. Here, we present an unusual case of a diabetic patient who presented with nasal blockade and bleeding for 2 months, along with occasional haemoptysis for 15 days. On investigation, the patient was diagnosed with a case of rhinocerebral zygomycosis and was treated with amphotericin B ($1 \text{ mg kg}^{-1} \text{ day}^{-1}$), which was subsequently replaced with liposomal amphotericin B ($2 \text{ mg kg}^{-1} \text{ day}^{-1}$). However, the patient did not completely respond to therapy as haemoptysis continued. Further investigations revealed the presence of *Aspergillus flavus* in respiratory specimens. Thus, a final diagnosis of rhinocerebral zygomycosis with pulmonary aspergillosis in a non-HIV-infected patient was made, but due to infection of two vital sites by these fungi, the patient could not be saved.

Introduction

Zygomycosis is an opportunistic infection caused by saprophytic fungi, notably species of *Mucor*, *Rhizopus* and *Absidia* of the family *Mucoraceae*, order *Mucorales* and class *Zygomycetes* (Ellis, 2005). The major predisposing factors for acquisition of zygomycosis are uncontrolled diabetes mellitus, metabolic acidosis, debilitating diseases, immunosuppression and widespread use of broad-spectrum antibiotics and steroids, etc.

Rhinocerebral zygomycosis is the most serious and fulminant form of zygomycosis. The infection begins in superior turbinates and spreads to the paranasal sinuses, orbit and brain after inhalation of sporangiospores (Abedi *et al.*, 1984). These fungi have a predilection for invading vessels of the arterial system, causing embolization and subsequent necrosis of surrounding tissue. Hence, a rapid diagnosis by performing a direct KOH examination is extremely important for successful management and therapy (Rippon, 1988).

Aspergillus is a saprophytic fungus that is ubiquitous throughout the world (Bennett, 2005); it causes infection following inhalation of *Aspergillus* conidia or mycelial fragments on vegetation, decaying matter and soil. *Aspergillus* causes allergic bronchopulmonary aspergillosis, fungal ball,

invasive aspergillosis, paranasal granuloma and endocarditis. The invasive form of aspergillosis generally occurs in severely immunocompromised patients, where it may invade the lung tissue and disseminate to other organs.

Although dual fungal infections are known to occur in immunocompromised patients (Buenconsejo *et al.*, 2002; Stermer *et al.*, 1984), rhinocerebral mucormycosis with pulmonary aspergillosis has never been reported, to the best of our knowledge. Here, we present an unusual case report from a 48-year-old diabetic female diagnosed with a case of rhinocerebral zygomycosis with concomitant pulmonary aspergillosis.

Case report

A 48-year-old female was referred from a primary health care centre in Bihar to the ear, nose and throat department of a tertiary care hospital in Delhi, with chief complaints of nasal blockade, intermittent nasal bleeding with occasional blackish discharge, intermittent headache and pain around the left eye for the preceding 2 months, for which she was given some medication. She also complained of a productive cough with occasional haemoptysis for 15 days.

The patient was diagnosed with diabetes mellitus type II only 2 months prior to this and was on oral hypoglycaemic agents. She did not give any history of tuberculosis or asthma. No history pointing to the involvement of any

Abbreviations: CT, computed tomography; SDA, Sabouraud's dextrose agar.

other organ system could be elicited. The patient was a resident of a village in Bhojpur and had an agricultural background.

On examination, blood pressure was 160/90 mmHg while pulse rate and heart rate were normal. On examination of the respiratory system, bronchial breathing was heard on the left infrascapular area. Coarse crepitations were present in the left suprascapular, interscapular and infrascapular region along with the right infrascapular area. Local examination revealed the presence of necrotic debris in both of the nares, more so on the left side. Mild swelling was present on the left side of the face, along with superolateral proptosis and periorbital oedema. On ophthalmological examination, pupil and fundus were normal. Computed tomography (CT) of the paranasal sinuses revealed a nasal mass involving all sinuses on the left side and extending into the medial wall of the orbit. Chest X-ray showed an ill-defined, non-homogeneous opacification in the left, middle and lower lobes of the lung (Fig. 1). Endoscopy showed necrotic and charred nasal mucosa.

Haemogram revealed that haemoglobin was 9.8 g dl^{-1} ; total leukocyte count was $13\,000 \text{ mm}^{-3}$ and differential leukocyte count was 80 % polymorphs, 18 % lymphocytes, 2 % monocytes and 0 % eosinophils. Erythrocyte sedimentation rate was 39 mm in the first hour. Fasting blood sugar was 449 mg dl^{-1} and urinary sugar was $>1000 \text{ mg dl}^{-1}$, with an absence of albumin and ketone bodies. Blood urea and serum electrolytes were within normal limits. C-reactive protein levels were raised (2.4 mg dl^{-1}) and blood culture was negative. The patient was found to be non-reactive for HIV-1/2.

A pus swab containing secretions from the left middle meatus was received in the microbiology laboratory for fungal examination. The specimen was blood stained and

contained necrotic material. The sample was cultured on Sabouraud's dextrose agar (SDA) with and without cycloheximide and chloramphenicol, in duplicate (Emmons *et al.*, 1971); one set was incubated at 25°C and the other at 37°C . Analysis of 10 % KOH mounts of the specimens showed the presence of broad (10–15 μm) hyaline aseptate hyphae with irregular branching suggestive of zygomycetes (Fig. 2). However, culture on SDA revealed no growth. A provisional diagnosis of zygomycotic fungal pansinusitis was made and the patient was put on amphotericin B (initial dose $1 \text{ mg kg}^{-1} \text{ day}^{-1}$) on the same day, subsequently followed by liposomal amphotericin B ($2 \text{ mg kg}^{-1} \text{ day}^{-1}$). Surgical excision and debridement were performed, in which the nasal mass was completely removed along with the necrotic middle and inferior turbinates. Maxillary, ethmoid, sphenoid sinuses were exteriorized and the medial wall of the orbit, i.e. the lamina papyracea, was removed. Debridement of perforated and necrotic nasal septum was performed, and material was sent for histopathological examination. Para-amino salicylic acid-staining of the tissue showed broad, aseptate hyphae with irregular branching, which, along with the necrotic tissue, confirmed the diagnosis of zygomycosis.

After the initial surgical debridement, endoscopic debridement was done daily in the minor operation theatre for the initial week, followed by debridement on alternate days. The patient showed some signs of improvement; the proptosis regressed and the sinuses became healthier, but contrary to our presumption, the chest symptoms, including episodes of haemoptysis and breathlessness, continued. Also, CT of the chest showed no significant improvement. Therefore, a sputum sample was sent for microbiological examination and culture. The sample was processed for fungal examination, using the method described above, and also for bacterial culture. The specimen was inoculated

Fig. 1. Chest X-ray of the patient showing ill-defined non-homogeneous opacity in the left, middle and lower lobes of the lung.

Fig. 2. KOH mount of nasal secretion. Magnification, $\times 40$.

on 5 % sheep blood agar, MacConkey agar and brain heart infusion broth and incubated at 37 °C aerobically. A KOH mount of sputum revealed the presence of septate hyphae with dichotomous branching. Fungal culture on SDA without antibiotics showed rapidly growing white-coloured hyphae, which became green with a powdery appearance after 72 h incubation (at both 25 and 37 °C). A lactophenol cotton blue mount of these colonies showed hyaline branched septate hyphae corresponding to those seen on the KOH mount. The conidiophores arising from these hyphae were unbranched, terminating in swollen vesicles surrounded by biseriate phialides covering the entire vesicle. Based on this morphology (Richardson, 2005), the isolate was phenotypically identified as *Aspergillus flavus*. However, bacterial cultures did not reveal any

significant growth. A bronchoscopy was performed which again revealed black necrotic debris. The bronchoalveolar lavage fluid was sent for microbiological examination and processed in the same way as above. This also confirmed the presence of *A. flavus* (Fig. 3).

Thus, a final diagnosis of rhinocerebral mucormycosis with pulmonary aspergillosis was made; however, the patient deteriorated after bronchoscopy and developed massive haemoptysis despite therapy and succumbed to her illness.

Discussion

Rhinocerebral zygomycosis, which is the most common form of zygomycosis (Spellberg *et al.*, 2005), is a rare but

Fig. 3. KOH mount of bronchoalveolar lavage fluid obtained after bronchoscopy. Magnification, $\times 40$.

serious opportunistic infection of the sinuses and brain caused by saprophytic fungi (Earhart & Baugh, 2006). They thrive in acidic and glucose-rich media.

This particular patient had recently diagnosed diabetes mellitus when she reported to the hospital for nasal discharge. The patient had an agricultural background and, therefore, could have acquired this infection through inhalation of spores into the sinuses. As the patient was in a state of undiagnosed hyperglycaemia, which enhances fungal growth and impairs neutrophil chemotaxis (Earhart & Baugh, 2006), this saprophytic fungus must have thrived and led to the presented disease state. The patient had proptosis, periorbital oedema and left facial swelling at the time of presentation, which occurs due to erosion of the walls of the sinuses and the orbit.

A direct examination of the nasal discharge revealed hyaline broad aseptate irregular hyphae and confirmed the diagnosis of zygomycosis, as culture is positive only in 60% of zygomycosis cases (Dromer & McGinnis, 2003). These findings also support our diagnosis, as no zygomycetes could be isolated in culture on SDA. Moreover, culturing these organisms from a potentially infected site is rarely sufficient to establish the diagnosis of zygomycosis because the causative organism is ubiquitous, may colonize non-symptomatic people and is a relatively frequent laboratory contaminant (Spellberg *et al.*, 2005). Since the fungus can also be killed during processing of a specimen for culture (Waldorf *et al.*, 1982), a sterile culture does not rule out infection. Therefore, the diagnosis is made by biopsy of infected tissue; in this case, this also revealed ribbon-like aseptate hyphal elements with irregular branching.

The patient responded to amphotericin B initially, which is fungistatic for the agents of zygomycosis and is the only US Food and Drug Administration-approved drug for the initial therapy of invasive zygomycosis (Earhart & Baugh, 2006). However, bronchoscopy that was performed due to continued episodes of haemoptysis surprisingly identified *A. flavus*, which can colonize the respiratory tract (Bennett, 2005) but can also be a secondary opportunistic pathogen in patients with tuberculosis, sarcoidosis, carcinoma and other mycosis (Paganin *et al.*, 2003). This patient could have acquired *A. flavus* in the lungs due to predisposing factors in the form of rhinocerebral zygomycosis and immune suppression caused by diabetes mellitus.

The diagnosis of aspergillosis as a disease is based on the demonstration of characteristic hyphae in clinical specimens, along with repeated recovery of the same species of *Aspergillus* (Paganin *et al.*, 2003). In this case, characteristic hyphae of *Aspergillus* were also identified in a KOH mount and *A. flavus* was isolated from two consecutive samples from the same patient, thus confirming our diagnosis of pulmonary aspergillosis and ruling out any contamination. Moreover, the patient's chest X-ray also revealed ill-defined non-homogeneous opacification in the left, middle and lower lobes of the lungs, suggestive of aspergillosis (Maiorano *et al.*, 2005).

Although dual fungal infections like zygomycosis with candidiasis or aspergillosis with candidiasis have been reported from immunocompromised patients (Buenconsejo *et al.*, 2002; Stermer *et al.*, 1984), rhinocerebral zygomycosis with invasive pulmonary aspergillosis has never been reported in the literature to the best of our knowledge. Only one case of combined zygomycosis and aspergillosis at a single site, i.e. the oropharyngeal region, has been reported from a patient with Castleman's disease (Maiorano *et al.*, 2005).

Thus, this is the first case report of rhinocerebral zygomycosis with pulmonary aspergillosis in a patient with type II diabetes mellitus, from a tertiary hospital in India. This report emphasizes the importance of a simple direct KOH mount for preliminary identification of mycosis and a thorough work-up of the patient for other co-presenting conditions.

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