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Rhinocerebral Zygomycosis and Aspergillosis : First Case Report of Dual Infection

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Zygomycetes and Aspergillus are two opportunistic fungal pathogens which commonly infect para nasal sinuses and have predilection to invade blood vessels. We present here an unusual case of mixed rhino cerebral zygomycosis with aspergillosis from a diabetic patient who responded well to the timely treatment with amphotericin B and itraconazole with functional endoscopic surgery for sinuses.

Key words: Zygomycosis, Aspergillosis, Sinusitis.

A 55 year old male, resident of a village in Lucknow, India presented to Ear Nose & Throat department of Dr Ram Manohar Lohia Hospital & Post Graduate Institute of Medical Education & Research, New Delhi with chief complaint of loss of vision and pain around left eye for the past 9 months. The loss of vision was gradual and progressive. Patient also complained of headache for the past 11/2 year and running nose for past 6 months with occasional bleeding and blackish discharge from nose. Patient was a known case of *Diabetes mellitus* type II for the past 5 years and was only on dietary restriction. Hypertension was detected in the patient during his stay in the hospital. He was a non smoker, nonalcoholic. There was no history of tuberculosis, asthma etc. in the past. No history pointing towards involvement of any other organ system could be elicited. Patient had agricultural background and worked in sugarcane farms.

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On general physical examination, patient was conscious and well oriented. Vitals were stable and Blood pressure was 140/90 mm Hg. CVS & CNS examination was normal and chest was clear. Local ophthalmic examination showed complete loss of vision in left eye with only perception of light present. Vision in right eye was 3/6. Ocular movements were normal in both the eyes. Local nasal examination showed necrotic debris in left nostril. Endoscopy showed necrotic nasal mucosa on both sides.

CT scan showed fluid in both maxillary antrum with hypertrophy of inferior turbinates and thickened mucosa in sphenoid sinus. A soft tissue mass was present at the apex of left orbit with erosion of medical wall of orbit. (Fig. 1) MRI showed sphenoid sinusitis and significant thickening along the walls of bilateral posterior ethmoid air cells (L>R) with enhancing soft tissue mass extending up to left orbital apex and abutting left optic nerve.

Hemogram showed Hb 11.1gm%, TLC-8900/mm^3, DLC- $P_{53}L_{32}E_{15}M_0$, AEC-1200mm^3 Platelets were adequate & erythrocytic sedimentation rate was 21 mm in 1st hour (Westergren), serum electrolytes were within normal limits & LFT/KFT were normal. C-reactive proteins were raised (2.4 mg/dl) and rest of immunoglobulin

profile was within normal limits. Patient was found to be non reactive for HIV 1 & 2 antibodies and blood culture was negative.

Secretions from left and right nostril were received in the mycology lab for fungal examination. The secretions were grossly blood stained

The sample was cultured on Sabouraud's dextrose agar (SDA) with and without chloramphenicol and cyclohexamide in duplicate[1]; One set was incubated at 25ºC and the other at 37ºC. Examination of 10% KOH preparation of the specimen showed the presence of hyaline broad (9-13 µm) aseptate hyphae suggestive of zygomycete (Fig. 2). Based on above finding a provisional diagnosis of zygomycotic rhinocerebral sinusitis was made and functional endoscopic sinus surgery under local anaesthesia was performed on the patient and unhealthy tissue along with necrotic debris were removed from the sphenoid sinus. Posterioinferior part of middle turbinate were also removed. Periosteum at orbital apex was left intact while posterior ethmoid cells which were embedded by fungus were removed and all this material was sent for fungal examination.

The sample was processed as above. KOH mount of this sample surprisingly showed broad hyaline septate hyphae which showed typical branching at acute (45º) angles strongly suggestive of aspergillosis (Fig. 3). This finding put us in diagnostic dilemma but patient was put on amphoteicin B post operatively (1mg/kg/d) followed by liposomal Amphotericin B (2 mg/kg/d). Patient was also given insulin preoperatively followed by metformin 250 mg OD. Dexamethasone injection was also given 8 mg 8 hourly which was

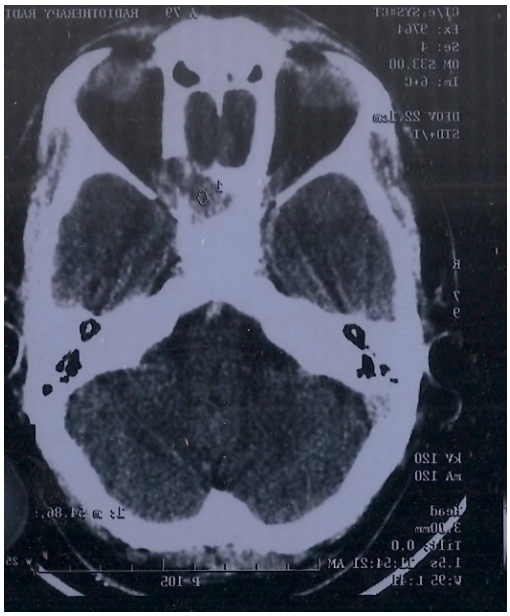

Fig. 1. CT scan of PNS showing soft tiiue mass at the apex of left orbit with erosion of its medial wall

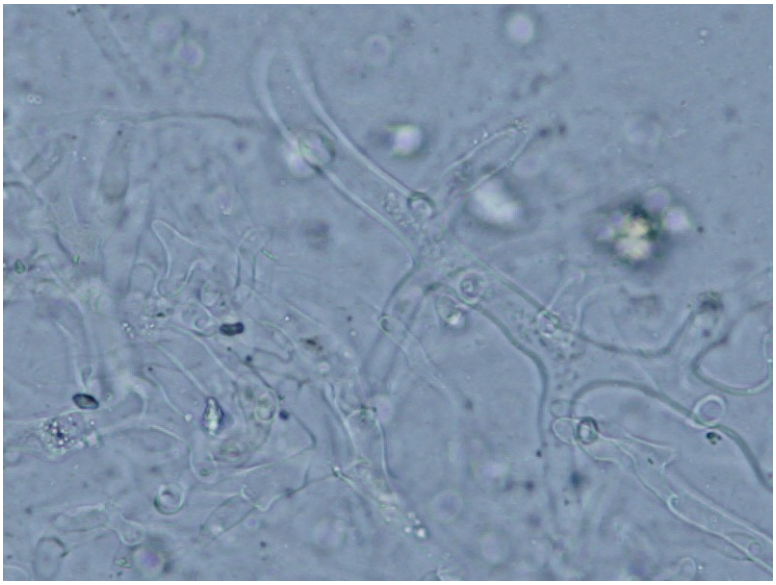

Fig. 2. KOH mount of nasal secretion (preoperated) suggestive of Zygomycetes

gradually tapered off over a period of one week postoperatively.

Patient improved symptomatically and endoscopic debridement was done daily in minor OT for initial one week followed by alternate day debridement and subsequently debridement every fortnight.

A rapid fluffy/cottony white growth was observed after 4-5 days of incubation at 25ºC and 37ºC on SDA without cyclohexamide and chloramphenicol from preoperative specimen. LPCB (lacto-phenol cotton blue) mount of this growth revealed wide (10-15µm) hyaline aseptate hyphae. Tufts of rhizoids were present and sporangiophores arising directly above this tufts were long and smooth walled. There was no branching of sporangiophores and they terminated in round grayish sporangium (100-150 µm) containing many sporangiospores (6-8 µm). Based on these factors the isolate was identified as rhizopus species.[2]

SDA slant of the post operative specimen also showed a rapid fluffy white coloured growth initially (within 4-5 days) which subsequently showed grayish to greenish powdery appearance along with persistent cottony growth in the background. LPCB mount of the powdery growth showed septate hyphae (2-4 µm wide) with characteristic dichotomous branching. The conidiophores were unpigmented coarse and bore

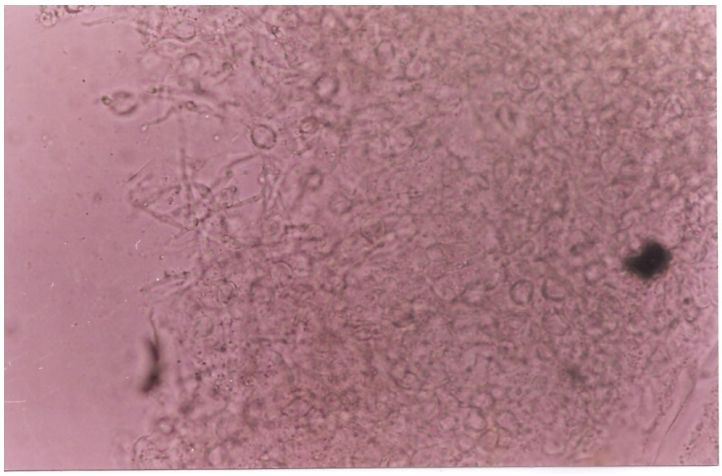

Fig. 3. KOH mount of nasal mass after FESS suggestive of Aspergillus

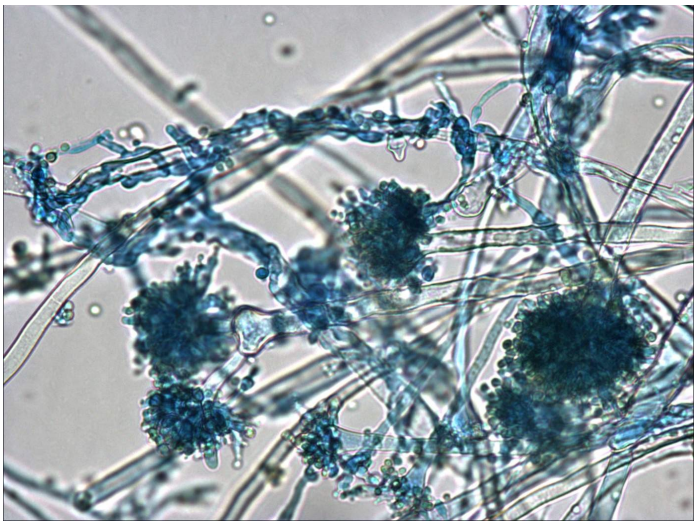

Fig. 4. LPCB mount of growth from SDA inoculated with postoperative specimen (nasal mass)

round vesicles (25-30 µ). The strigmata (6-10µm long) were present all around the vesicle and were biseriate. These bore chain of conidia which were 3-4µm in diameter. Based on this morphology growth was identified as aspergillus flavus[3] (fig 4) and patient's serum was sent to the VP Chest institute for detection of antibodies against Aspergillus to support our diagnosis. However the test was found to be negative. LPCB of the background fluffy growth was made but was completely covered with spores of Aspergillus and hence a subculture was made on SDA slant in duplicate without chloramphenicol and cyclohexamide and was incubated at 25º C and 37º C. This SDA slant showed initial cottony growth after 3-4 days of incubation and LPCB mount of this growth showed broad aseptate hyphae but identification could not be confirmed due to young growth and subsequent incubation of slant showed overgrowth of greenish Aspergillus spores all over it. Thus a diagnosis of combined fungal infection of zygomycosis and aspergillosis from the paranasal sinuses (sphenoid sinus predominantly) was made and patient was continued on liposomal amphotericin B along with itraconazole 400 mg BD and regular endoscopic debridement for 2 months. Repeat MRI scanning showed reduction in the size of the spenoidal mass and the patient is still on amphotericin –B and regular follow up.

DISCUSSION

Rhinocerebral zygomycosis is a rare but serious opportunistic infection of sinuses and brain caused by saprophytic fungi.

Our patient was a known case of *Diabetes mellitus* type II and was on dietary restriction only and blood glucose levels were not regularly maintained. Due to this patient must have acquired infection by Rhizopus spp. Patient also gave history of agricultural background. He worked in sugarcane field which is a known source of fungal spores. The series of events could have been initial infection with zygomycosis due to long standing uncontrolled diabetes mellitus which must have favoured growth of Rhizopus in PNS and it further involved the walls of orbit and during this course of disease there was secondary infection with Aspergillus. Direct examination of nasal secretion of preoperative specimen revealed non septate hyphae typically suggestive of zygomycosis which was later confirmed by culture also (Rhizopus spp.). Moreover presence of zygomycetes in KOH and culture from a symptomatic patient is in itself sufficient to establish the diagnosis of zygomycosis.

Diagnosis of aspergillosis as a disease is based on demonstration of characteristic hyphae in clinical specimen along with repeated recovery of same species of Aspergillus. In our case also we found septate dichotomously branched hyphae of aspergillus in the nasal mass specimen obtained after performing FESS and this was later confirmed by culture as aspergillus flavus from both the SDA slants incubated at 25ºC and 37ºC thus supporting our diagnosis of aspergillosis and ruling out any contamination. Moreover patients CT and MRI also had revealed nasal mass in sphenoid sinus eroding the walls of orbit strongly suggestive of a fungal sinusitis. However serology for Aspergillus antibodies was negative in the patient which could either be due to dual fungal infection or due to the effect of antifungal agents because this test was performed only after the isolation of Aspergillus flavus in the SDA slant & by this time patient was already on Amphoterecin B & ictraconozole.

Patient responded well to amphotericin B initially, which is USA FDA approved drug for invasive zygomycosis and is effective against both zygomycetes and aspergillus spp. To prevent further invasion by aspergillus, voriconazole which is an expensive antifungal agent recommended for invasive aspergillosis was planned for patient but due to restricted hospital funds and poor socioeconomic status of patient, he was put on itraconazole along with regular debridement in the postoperative period.

Although dual fungal infections like zygomycosis with candida or aspergillosis with candida[4] are known to occur in immuno-compromized patients, combined infection by zygomycetes and aspergillus from a non immunocompromized patient are rarely reported. Only one case report of rhinocerebral zygomycetes and pulmonary aspergillosis has been reported previously[5]. Mixed infection with zygomycetes and aspergillosis in orosininasal region from a patient with castleman disease has also been reported in past.[6] Another case report of mixed zygomycetes and aspergillus infection in renal and hepatic artery

from a combined liver and kidney transplant recipient has also been reported[7].

Thus we conclude that this is a rare case report of combined rhinocerebral zygomycosis and aspergillosis in a diabetic patient from a tertiary care hospital in India. The report signifies the rising incidence of fungal infections in non immunocompromized patients and emphasizes the need for thorough workup of all nasal mass patients for different kinds of fungi using simple KOH mount and culture.

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