CEREBRAL PHAEOHYPHOMYCOSIS CAUSED BY FONSECAEA MONOPHORA: FIRST REPORT FROM INDIA

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ABSTRACT

We report a case of cerebral phaeohyphomycosis caused by a dematiaceous fungus, Fonsecaea monophora, in a patient with type 2 diabetes mellitus and decompensated chronic liver disease. CT brain revealed a 2x2cm hypodense cystic lesion in the right lentiform nucleus region with significant perilesional oedema. Stereotactic burr hole aspiration of the lesion with biopsy of the abscess wall was done, and the aspirated pus from the lesion showed branched, septate hyphae with light brown pigmentation. The culture of the pus grew a dematiaceous fungus, identified by morphological and molecular studies as Fonsecaea monophora. The isolate was susceptible to voriconazole (MIC, 0.004 µg/ml) but showed reduced susceptibility to amphotericin B (MIC, 4 µg/ml). The patient’s caregivers were not willing for a decompressive procedure and hence was treated medically with combined amphotericin B and voriconazole antifungal therapy. Ultimately, the patient expired due to raised intracranial tension and resultant brain-stem dysfunction. It is the first case of cerebral phaeohyphomycosis caused by Fonsecaea monophora reported from India.

KEYWORDS Brain Abscess; Cerebral phaeohyphomycosis; Dematiaceous fungus; Fonsecaea monophora;

Introduction

Intracranial abscesses (usually referred to as brain abscesses) are serious, life-threatening infections. Major advances such as newer imaging techniques for early detection, stereotactic neurosurgical procedures and availability of antibiotics against aerobic and anaerobic organisms that effectively cross the blood brain barrier have lead to a substantial reduction in the mortality.

Despite these advances, brain abscesses particularly those caused by fungi, remain a potentially fatal central nervous system (CNS) disease. Fungal brain infections are classically described in immunocompromised patients. Phaeohyphomycosis is a collective term used for infections caused by fungi with brown pigmented cell wall due to the presence of melanin (dematiaceous fungi) [1]. Cerebral phaeohyphomycosis is manifesting as brain abscess is mainly caused by Cladophialophora bantiana and Rhinocladiella (Ramichloridium) mackenziei, although several other dematiaceous fungi have also been implicated [1,2,3]. Cerebral phaeohyphomycosis can occur in immunocompromised as well as in immunocompetent hosts.
Here, we report a case of cerebral phaeohyphomycosis caused by a dematiaceous fungus *Fonsecaea monophora*.

**Case Report**

A 63-year-old Indian male with type 2 diabetes mellitus and decompensated chronic liver disease with a past history of endoscopic oesophageal variceal ligation, presented with headache and progressive left-sided weakness of 3 weeks duration. On examination, the patient was afebrile with a pulse rate of 88/minute and blood pressure of 130/80 mmHg. He was conscious and oriented. The pupils were equal and reactive to light. He had left hemiparesis with a power of grade 4.

Laboratory investigations of blood revealed haemoglobin: 16.3 g/%, white blood cells: 9300/mm³ with 88% polymorphs, 6% lymphocytes, 6% monocytes and platelets of 1,20,000/µL. Liver function revealed serum bilirubin 1.6 mg/dl (total) and 0.5 mg/dl (direct) with AST: 38 U/L and ALT: 26 U/L and alkaline phosphatase: 103 U/L. The patient had hypoalbuminemia (serum albumin: 2.5 g/dl). His blood urea was 42 mg/dl and serum creatinine was 0.6 mg/dl. The coagulation profile was deranged (prothrombin time: 17.9 seconds, international normalized ratio (INR): 1.37, index: 78.9%). He was sero-negative for human immunodeficiency virus (HIV) and hepatitis B virus (HbsAg). Abdominal Ultrasonography revealed cirrhotic liver (heterogenous coarse echotexture of liver with irregular outline) with bulky spleen and cholelithiasis.

Initial computed tomography (CT) brain (Figure: 1A) showed a 2x2 cm hypodense cystic lesion in the right lentiform nucleus region with significant perilesional oedema. After correction of the coagulation parameters with fresh frozen plasma transfusions and parenteral vitamin K, stereotactic burr hole aspiration

**Figure 1A:** CT Brain showing 2x2cm hypo-dense cystic lesion in the region of Lentiform nucleus with significant peri-lesional oedema.

**Figure 1B:** Post stereotactic aspiration CT Brain showing recurrence of the abscess in the right lentiform nucleus region with peri-lesional oedema.

**Figure 1C:** Repeat stereotactic aspiration CT Brain showing increased oedema in the right lentiform nucleus region even though the abscess itself had reduced in size.
of the right basal ganglionic lesion with biopsy of the abscess wall was done under general anaesthesia. Aspiration yielded about 10 ml of creamy pus, which was sent for microscopic examination and culture. The patient was empirically started on intravenous cefoperazone-sulbactam combination, metronidazole and amikacin after the aspiration.

Gram-stained smears of the aspirated pus showed many pus cells and branched septate fungal hyphae. Hence, the patient was initially started empirically on intravenous fluconazole therapy (200 mg, every 12 h) pending culture results. Since there was no improvement following one week of therapy, fluconazole was replaced with oral voriconazole (200 mg, twice daily).

Microscopic examination of the aspirated material with 10% potassium hydroxide (KOH) showed septate fungal elements with brown pigmentation of the cell wall (Figure 2). The dematiaceous nature of the fungus was subsequently confirmed by Fontana-Masson stain. Grocott’s methenamine silver (GMS), stain sections of the aspirated material, also showed branched fungal elements with beaded appearance (Figure 3). After seven days of incubation at 30°C, the Sabouraud dextrose agar (SDA) plates yielded olivaceous black colonies of a mold confirming the diagnosis as cerebral phaeohyphomycosis. The isolate was sent to Reference Laboratory for phenotypic and molecular identification.

Two weeks later, the neurological status of the patient deteriorated to a Glasgow Coma Score of 10/15 (E3M6V1). Anisocoria was noticed. Repeat CT-Brain (Figure: 1B) revealed recurrence of abscess in the right lentiform nucleus region with perilesional oedema and significant mass effect. On repeating the aspiration of the cerebral abscess, 6 ml of pus was drained. The direct smear of the repeated aspirate of pus also showed moderate pus cells and a large number of branched, septate melanized fungal hyphae. Since the patient’s condition was gradually worsening, amphotericin B therapy was started. A loading dose of 25 mg amphotericin B was given intravenously followed by 50 mg infusion daily diluted in 5% dextrose along with the combined voriconazole therapy.

Despite stepping up antifungal therapy the patient had progressive neurological worsening. He became unresponsive and comatose. Repeat CT scans (Figure: 1C) showed increasing oedema in the right lentiform nucleus region. The patient’s caregivers were not willing for a decompressive procedure and ultimately the patient expired due to raised intracranial tension and resultant brain-stem dysfunction.

Mycology
Colonies on SDA at 30°C were olivaceous to black in color, giving a velvety appearance. Microscopic examination carried out by slide culture technique on SDA revealed darkly pigmented septate hyphae and pale and olivaceous smooth-walled conidia on sympodially branched conidiophores (Figure 4).

Molecular identification
The genomic DNA from the patient’s isolate (In-8252/14) was prepared as described previously [4]. The internal transcribed spacer (ITS) region and the divergent (D1/D2) domains of rDNA were PCR amplified and sequenced [5].

GenBank basic local alignment search tool (BLAST) searches were performed for species identification. (http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE=WebPage = BlastHome).

Figure 2: KOH mount of the aspirate from the brain abscess showing branched septate fungal hyphae with brown pigmentation of the cell wall.

Figure 3: GMS stain on the cell block of the aspirate from the brain abscess showing fungal hyphae.

Figure 4: Slide culture of F. monophora isolate on Sabouraud dextrose agar at 30°C showing septate hyphae with sympodial conidiophores bearing conidia in short chains.
The entire ITS region sequence (649 nucleotides) of our isolate exhibited 100% identity with the corresponding sequence from Fonsecaea monophora strain CBS 123849 and 1 to 3 nucleotide differences with several other F. monophora strains (CBS 102238, CBS 117237, CBS 115830, CBS 117542, CBS 102248). The D1/D2 region sequence of our isolate also exhibited 100% identity with the corresponding sequence from F. monophora isolates CBS 102243 and ATCC MYA-4769. Based on previous observations that strains belonging to same species exhibit >99% nucleotide identity in the ITS region and D1/D2 domains of rDNA, the molecular identity of our isolate was determined as F. monophora [6,7].

The ITS and D1/D2 region sequences of our isolate have been deposited in EMBL under accession no. LN626652 and LN651287.

Susceptibility testing

The patient’s isolate was tested for its susceptibility to amphotericin B, 5-flucytosine, itraconazole, voriconazole, posaconazole, caspofungin and anidulafungin by Etest. To obtain enhanced sporulation, the isolate was grown on potato glucose agar and growth was harvested in distilled water by gently scraping colonies with the help of a cotton swab. Growth from several slants was used to obtain adequate inoculum. The larger hyphal fragments were allowed to settle, and the relatively homogenous conidial suspension was adjusted to 0.5 McFarland standard. All the antifungal agents were tested in RPMI 1640 with L-glutamine and 2% glucose and morpholinepropane sulfonic acid (MOPS) buffer at a concentration of 165 mM without sodium bicarbonate.

The plates were dried at ambient temperature for 15 min before applying the Etest strips. The minimum inhibitory concentration (MIC) values were read after 6 days of incubation at 35°C as follows: amphotericin B - 4 µg/ml, 5-flucytosine - >32 µg/ml, itraconazole - 0.19 µg/ml, voriconazole - 0.004 µg/ml, posaconazole - 0.032 µg/ml, caspofungin - 0.25 µg/ml and anidulafungin - 0.38µg/ml.

Discussion

This patient, a diabetic (without ketoacidosis) and a known case of alcoholic liver cirrhosis, developed brain abscess due to a melanized fungus, identified by microscopic characteristics and molecular methods as F. monophora. F. monophora is a dematiaceous (melanized) fungus belonging to the ascomyceteous family Herpotrichiellaceae of order Chaetothyriales. The genus Fonsecaea comprises three sibling species, namely F. pedrosoi, F. monophora, and F. nabica, with the ability to cause subcutaneous infection [8]. Fonsecaea monophora has recently been separated from F. pedrosoi as a distinct species by molecular studies [8,9]. In older literature, Fukushiro reported several cases of cerebral infections caused by F. pedrosoi [10]. Some of the isolates from these cases were reclassified recently by molecular methods as F. monophora [11]. In contrast to F. pedrosoi that is exclusively regarded as an agent of chromoblastomycosis, F. monophora can cause chromoblastomycosis, (subcutaneous mycosis) as well as cerebral phaeohyphomycosis [11,12,13]. Since morphologically both species are similar, molecular characterization by sequencing of the internal transcribed spacer (ITS) rDNA is required for unequivocal differentiation [11].

Cerebral phaeohyphomycosis due to F. monophora is a rare disease. So far seven cases have been reported in world literature (Table 1) [12,17,18,19,20,21,22]. The disease affects both sexes, mostly occurring in older age group with underlying predisposing conditions and carries a high mortality rate. Our patient had diabetes mellitus without ketoacidosis, and he was also diagnosed with chronic liver disease due to alcoholic cirrhosis with splenomegaly for the last 6 years.

Like other reported cases, the origin of F. monophora infection in our patient is not clear. There was no evidence of ostitis media, sinusitis or cutaneous lesions or pulmonary lesions. The probability of oesophageal variceal ligation carried out two years ago in our patient is unlikely to be the portal of entry of the fungus for haematogenous spread to the brain. Hence, our patient probably had a primary brain infection due to the neurotropic property of this fungus acquired from the environment. The natural habitat of members of the Genus Fonsecaea is soil and decaying plant materials, and they are distributed worldwide, particularly in tropical Asia, South America and Africa [11]. Since F. monophora species isolates obtained from environmental and clinical sources demonstrate considerable genetic similarity, it is quite probable that the infection is acquired from environmental sources [8,14].

The current recommendation for the management of cerebral phaeohyphomycosis is a combination of total surgical excision, followed by systemic antifungal therapy [15]. However, most important determinant of a successful outcome is the location of the lesion, the extent of parenchymal involvement and ability to remove it surgically. In our case, surgical removal of the lesion was not feasible since cerebral lesion involved lenticform nucleus (globus pallidus-putamen) region. Antifungal therapy alone is not sufficient to achieve survival. Our isolate showed reduced
susceptibility to amphotericin B, flucytosine and echinocandins, which is consistent with previous reports [13,14]. Amphotericin B, itraconazole, and voriconazole have been used with varying success. However because of the rarity of cases of cerebral phaeohyphomycosis due to F. monophora, it is hard to decide about the most effective antifungal drug for therapy. In our case, in the absence of definite etiology, we initially used fluconazole instead voriconazole, which is the recommended treatment for cerebral phaeohyphomycosis because it attains good levels in cerebrospinal fluid [16]. Among, triazoles, voriconazole, posaconazole and itraconazole are reported to have good in vitro antifungal activity against dematiaceous fungi especially Fonsecaea spp. 

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