



Case Report

Molecular Characterisation of *Cladophialophora* species Isolated from Brain Abscess in a Renal Transplant Recipient

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Abstract

Background: Though rare, primary central nervous system infection by melanized fungus *Cladophialophora*, is highly fatal inspite of surgical and medical intervention, posing a diagnostic dilemma, resulting in diagnostic delay, which adds to the poor outcome. *Cladophialophora*, being strikingly neurotropic, infects both immunocompetent and immunocompromised hosts, being more common in immunocompetent males. Among immunocompromised hosts, predominantly transplant recipients are affected. A case of brain abscess by *Cladophialophora* as an incidental finding in renal transplant recipient from western India is presented here. **Case presentation:** A 30 year old male, known case of chronic kidney disease, underwent renal transplantation. During post-transplantation period developed insidious onset headache and left-sided hemiparesis. CT and MRI imaging of head revealed presence of multiple coalesced abscesses and pus aspirate on direct microscopy revealed presence of septate filamentous fungi which was confirmed by fungal culture and a presumptive identification of *Cladophialophora bantiana* was made based on morphological characteristics. On definitive identification by ITS sequencing, microbe was found to be *Cladophialophora bantiana* strain IEC-CBM06 internal transcribed spacer 1; 5.8S ribosomal RNA gene. Patient responded well to appropriate surgical intervention and systemic antifungal therapy and is on regular follow up. **Discussion & Conclusion:** CNS infections by this neurotropic mould, which are usually fatal, should be taken into consideration for differential diagnosis of cerebral abscess in both immunocompetent and immunocompromised patients, as they are increasingly recognized, and timely diagnosis and prompt intervention is a good predictor of survival. Molecular identification plays a vital role in providing a timely and accurate laboratory diagnosis.

Keywords: *Cladophialophora bantiana*; Brain abscess; Renal transplant; Molecular characterization

Background

Primary cerebral phaeohyphomycosis is a rare infection caused by darkly pigmented fungi, called as dematiaceous moulds with melanized cell wall, which carry a high mortality rate up to 70% [1-3]. In a review of cases of primary central nervous system phaeohyphomycosis, the most frequently isolated species was *Cladophialophora bantiana* (48%) and the next most frequent isolate was *Ramichloridium mackenziei* (13%) [2,4]. *Cladophialophora bantiana* is strikingly neurotropic, ubiquitously present in soil, prefers warmer climate with high humidity and distributed widely (Asia, North and South America, Europe, Africa) [1,5,6]. Though its propensity for nervous tissue is not fully understood, a possible mechanism involves melanin production which interferes with microbial recognition, scavenges free radicals and prevents eradication of fungi from brain parenchymal tissue [7,8].

Brain abscesses caused by *C. bantiana* have been reported in both immunocompetent and immunocompromised patients, while more than half of the cases are seen in immunocompetent males [3,9]. It poses a diagnostic dilemma due to non specific clinical presentation, neuroimaging findings mimicking a space occupying lesion and relative rarity of this condition, causing a delay in diagnosis which results in fatal outcome inspite of aggressive surgical and antifungal therapy [9-11]. Laboratory-based investigations play a vital role in the diagnosis and management of such dreadful infections [11].

Here we present a case of cerebral *Cladophialophora* infection, an incidental finding in a renal transplant recipient.

Case presentation

A 30-year-old male with pre-existing chronic kidney disease since 2-3 years and dependent on regular dialysis, presented to our institution for a planned renal transplantation and underwent the same on 15th February 2023. During the post-operative period he was put on immunosuppressants. After an early uneventful post-operative period, on 17th post-operative day, he developed a mild headache to begin with which increased in severity over few hours, not associated with fever, nausea or vomiting. After few hours, patient became drowsy and developed left sided hemiparesis, for which neurosurgery consultation was called upon.

On physical examination, patient was afebrile, pupils were bilaterally reactive. Glasgow coma scale (GCS) score was reduced

at 10/15 (E3/V2/M5). Power in left upper and lower limb was reduced (3/5). Computed tomography (CT) scan of head was advised, which revealed presence of an ill-defined hypodense lesion with hypodense rim in right fronto-parietal lobe with severe perilesional edema, midline shift and mild obstructive hydrocephalus giving a picture of cerebritis with abscess formation (Figure 1). To know the extent of soft tissue lesion, magnetic resonance imaging (MRI) of head was performed, which revealed presence of multiple coalesced abscesses in right fronto-parietal lobe with abundant perilesional edema, causing a mass effect on ventricle. Complete blood count showed leukocytosis with raised polymorphonuclear leukocytes (91.8%).

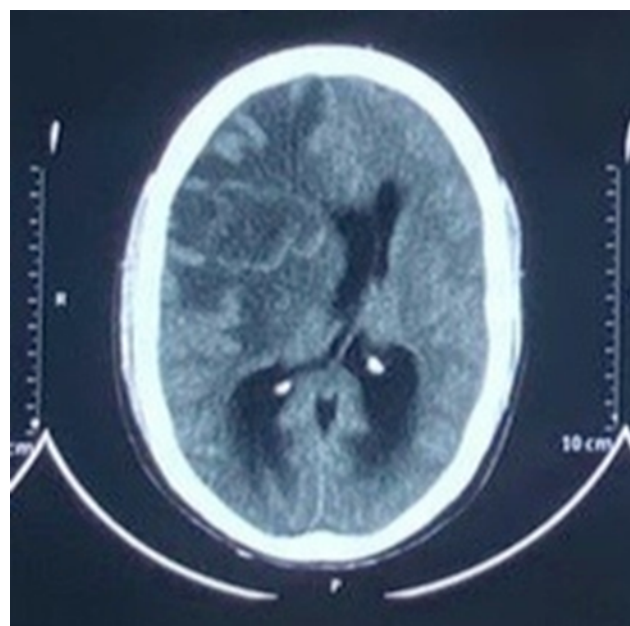


Figure 1: CT Head showing hypodense lesion in rt. fronto-parietal lobe.

Patient was started on empirical antibiotic coverage (Meropenem) and neuro-navigation guided aspiration of abscess was done yielding 15ml of purulent material which was sent for microbiological investigations. Gram stain showed presence of thin (6-8 μ m), septate, sparsely branched fungal hyphae, which was immediately conveyed to the attending clinician and patient was started on intravenous liposomal amphotericin B (Figure 2). No acid fast bacilli were observed on Zeihl Neelsen staining of aspirate and similar hyphal elements were observed as on gram stain. Potassium hydroxide (KOH) mount of abscess material revealed presence of thin, hyaline, septate, sparsely branched (Figure 3).

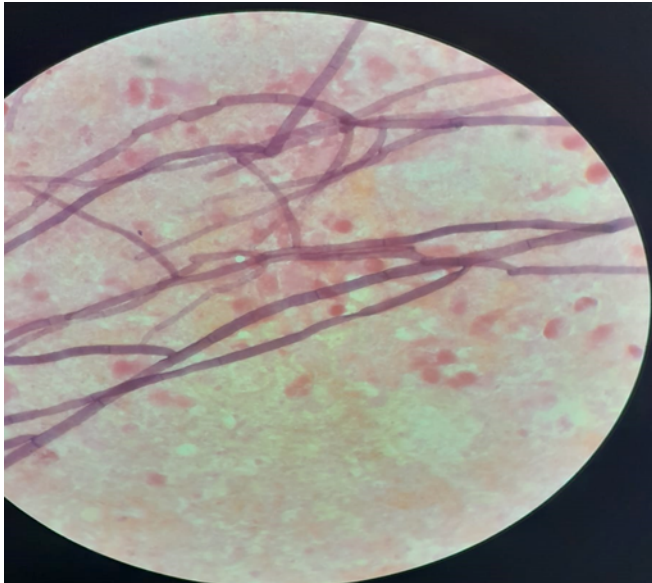


Figure 2: Gram stain of aspirate showing gram positive, thin, septate, sparsely branched fungal hyphae.



Figure 3: KOH mount of aspirate revealing thin, septate, sparsely branched hyphae.

Aerobic bacterial cultures were performed using Blood agar and MacConkey agar which remained sterile after 48 hours of incubation. Fungal culture was put on 4% Sabouraud's dextrose agar (SDA) with and without cycloheximide and incubated at

25°C and 37°C. Fungal growth appeared on SDA after 72 hours of incubation, initially small, only at the point of inoculation which was indicative of a phaeoid (pigmented) filamentous fungus and the same was communicated to clinician, following which voriconazole was added to the treatment regimen.

After 12 days of incubation, SDA showed an olivaceous greyish velvety fungal growth on obverse with a black reverse, both at 25°C and 37°C (Figure 4). Lactophenol cotton blue (LPCB) mount of the growth showed presence of brownish walled, oval to ellipsoidal single-celled conidia in chains, arising from undifferentiated rarely branching conidiophores (Figure 5). Depending upon macroscopic and microscopic morphological characteristics it was presumptively identified as *Cladophialophora bantiana*. It was tested for urea hydrolysis which turned out to be positive. For definitive species identification by ITS sequencing, isolate was sent to Biokart India Pvt. Ltd. It was performed using ITS forward primer 'TCCGTAGGTGAACCTGCGG' and ITS reverse primer 'TCCTCCGCTTATTGATATGC'. The Microbe was found to be *Cladophialophora bantiana* strain IEC-CBM06 internal transcribed spacer 1; 5.8S ribosomal RNA gene, which showed 100% match on analysis by basic local alignment search tool (BLAST) (Figure 6).



Figure 4: SDA showing olivaceous greyish velvety fungal growth on obverse (left tube) and a black reverse (right tube).

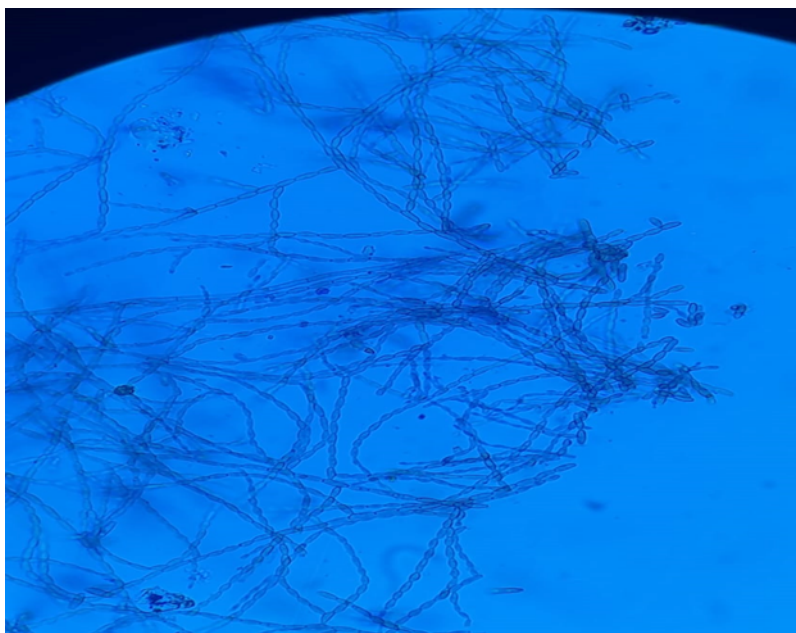


Figure 5: LPCB mount of the growth showing brownish walled, oval to ellipsoidal single-celled conidial chains and undifferentiated conidiophores.

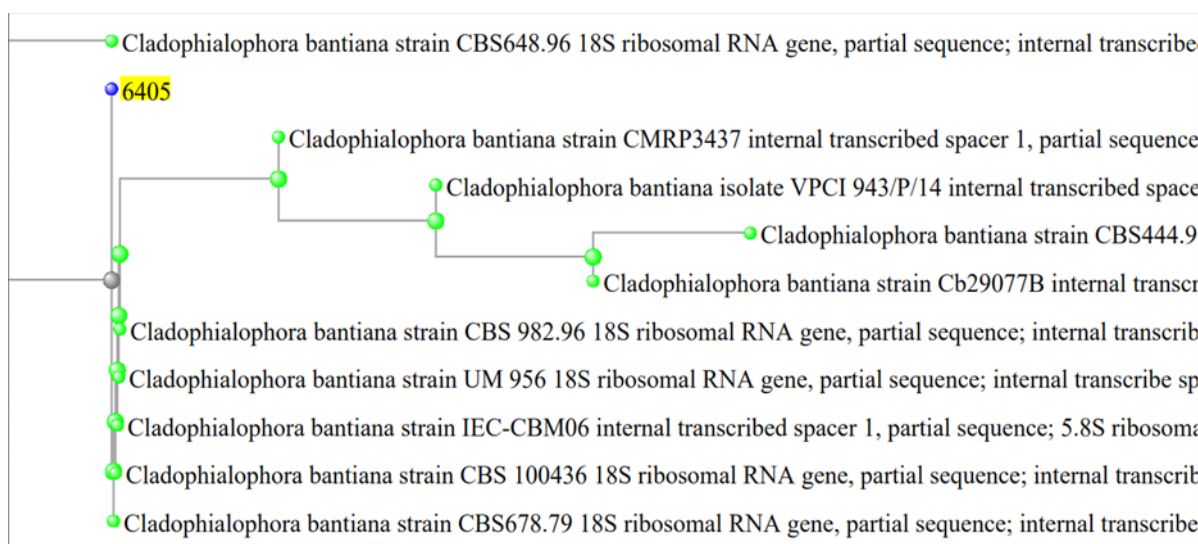


Figure 6: Phylogenetic tree of the fungal isolate obtained by ITS sequencing.

After 4 days of systemic antifungal therapy, GCS improved to normal (E4/V5/M6). Power in left upper and lower limb improved but did not regain completely (4/5). Patient was discharged on oral voriconazole and advised for regular follow up.

Discussion

Fungal brain abscess is a rare condition accounting for just 2% of the brain abscesses, mostly seen in immunocompromised hosts, while *Aspergillus*, *Cryptococcus*, *Zygomycetes* and *Candida* being the most common agents [10-12]. Nowadays they are increasingly recognized in immunocompetent hosts as well [13].

Cerebral phaeohyphomycosis caused by melanized fungi in particular *Cladophialophora bantiana*, though rare but highly fatal clinical entity, and majority of cases (57.3%) are being reported from Asian countries, mainly from India (50%), with an exponential rise in number of cases has been witnessed from 16 cases between 1950 and 2000, to 47 cases between 2001 and 2020 [9,11,14-16].

Cladophialophora is a true pathogen owing to its neurotropism and can cause primary central nervous system infections, whereas more than half of the patients are immunocompetent males [9,10]. Immunocompromised group include those with corticosteroid therapy, diabetes mellitus, neutropenia, intravenous drug abuse, bone marrow and solid organ transplantation [3,4,20]. In a review by Revankar et al of primary CNS phaeohyphomycosis, 15% patients were solid organ transplant recipients [4]. Case reports of cerebral Cladophialophora infection in renal transplant recipients have been earlier reported from India, though rarely, but none from Western India [14,18,19]. Here we report such a case of primary Cerebral Cladophialophora infection in a renal transplant recipient from this part of Western India.

The various postulated routes of seeding of this mould into the CNS include inhalation of airborne conidia followed by hematogenous dissemination, from a subcutaneous traumatic inoculation site and direct extension from infected paranasal sinuses, although primary focus is not apparent in many cases [3,9,11]. Likewise in our case, no history of sinusitis, trauma, intravenous drug abuse etc. was found. The patients typically have a clinical presentation suggestive of a space occupying lesion (SOL) includes headache, hemiparesis, seizures, altered sensorium, fever, vomiting, aphasia, dysarthria, and visual disturbance [9,11]. Our patient also presented with insidious onset headache, left sided hemiparesis and altered sensorium, however no fever was present which is not always apparent in these infections as reported by previous studies [3,17].

Laboratory diagnosis of this infection highly relies upon demonstration of pigmented septate hyphae on direct microscopic examination of aspirate or biopsy material and isolation in culture which is considered gold standard [9,11]. Culture can be time consuming and has safety concerns for these fungi requiring high levels of biosafety [11]. At the same time both of the above methods only provide presumptive identification of causative fungus. As many newer pathogenic species of Cladophialophora are increasingly being recognized, species identification becomes essential which has epidemiological importance too. Newer methods such as ITS1/ITS2 sequencing, rolling circle amplification and/or matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis can

only provide definitive identification to species level [21-23]. After initial identification by morphological features on microscopy and culture, final identification in our case was done by sequencing of ITS rDNA, and the isolate turned out to be *C. bantiana*, which confirmed our finding on morphological identification and no new species was detected.

Until recently, there was no consensus guideline for the treatment of invasive phaeohyphomycosis when in February 2021, the European Confederation of Medical Mycology together with the International Society for Human and Animal Mycology and the American Society for Microbiology proposed a set of comprehensive recommendations for diagnosis and management of rare mould infections including phaeohyphomycosis as a part of the One World-One Guideline initiative. According to this global guideline, complete surgical excision of abscess together with combination antifungal therapy should be the treatment of choice [9,21].

Antifungal susceptibility for these moulds is not routinely recommended as breakpoints have not yet been established by CLSI or EUCAST (European Committee on Antimicrobial Susceptibility Testing). Also, it is not frequently available and requires high levels of biosafety [9,11]. Antifungal susceptibility could not be done for our isolate. Traditional guidelines regarding the type of antifungal regimen, duration, type of optimal surgical intervention are still lacking for cerebral phaeohyphomycosis and choice of these interventions is largely made based upon experience in previous studies [11]. Most isolates of *C. bantiana* demonstrate low MICs against voriconazole, posaconazole and itraconazole, variable MIC against amphotericin B and high MICs against echinocandins [5,14,21]. Once considered therapy of choice, amphotericin B has shown treatment failure and development of resistance in few cases while voriconazole demonstrates good in-vitro activity against this mould and has good cerebrospinal fluid penetration as well [11]. Other promising azole is posaconazole which needs to be considered as an alternative in patients with pre-existing liver disease [3]. Our patient underwent neuro-navigation guided aspiration of abscess and was given amphotericin B followed by voriconazole on confirmation of phaeoid fungus, to which he responded well. He is currently on oral voriconazole and regular follow up.

Conclusion

Increased awareness, early recognition of clinical symptoms, radiological & mycological diagnosis helps in early initiation of specific intervention which can be lifesaving.

Conflicts of Interest: Nil

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