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Case report

# A case of cerebral phaeohyphomycosis caused by *Fonsecaea monophora*, a neurotropic dematiaceous fungus, and a review of the literature

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## Summary

The *Fonsecaea* species, which are the leading causes of chromoblastomycosis, are not considered neurotropic fungal agents. *Fonsecaea pedrosoi* is the primary species in the genus and is usually isolated from chromoblastomycosis cases. However, the recently distinguished species *F. monophora* has been reported in a few cerebral phaeohyphomycosis cases. Here, a case of cerebral phaeohyphomycosis caused by *Fonsecaea monophora* is presented in a 71-year-old female subject with chronic diabetes mellitus and hypertension. The identification of *F. monophora* was made through mycological and molecular analysis, and an isolate was differentiated from the closely related *F. pedrosoi* by sequence data on key bases on the ribosomal internal transcribed spacer region. The case was successfully treated with surgical and medical approaches, and the patient has remained healthy and stable after a ten-month follow up. Given the increasing incidence of this type of infection of the central nervous system (CNS), this case provides further support for the consideration that *F. monophora* might represent a neurotropic agent.

**Key words:** Cerebral, phaeohyphomycosis, *Fonsecaea monophora*, neurotropic, dematiaceous.

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## Case report

The 71-year-old female patient was admitted to the emergency department with complaints of nausea, vomiting and headache. At admission, the patient was awake, alert and oriented with mild distress. The past



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medical history was significant for diabetes mellitus and chronic hypertension. On presentation, the blood pressure was 134/72 mmHg, and the body temperature was 36.4 °C During the physical examination, no nuchal rigidity or signs of meningeal irritation were noted. Neurological examination of the sensory, motor, and reflexive functions were within normal limits. The initial laboratory tests indicated 14.650 leucocyte/μl (with 88% neutrophils), glucose at 142 mg/dl, and C-reactive protein at 0.2 mg/l. The patients liver function tests were within normal limits and creatinine was 0.7 mg/dl. Non-contrast cranial computerised tomography results indicated possible tumorigenic lesion on the left temporal region, a 3 mm midline shift to the right side, vasogenic oedema in the left fronto-temporo-parietal area and compression in the lateral left ventricle. For a more detailed analysis, cranial magnetic resonance imaging was performed and the results confirmed the presence of a mass of 3 × 4 × 3 cm on the left temporal area (Fig. 1a). The patient was admitted to the hospital with an initial preliminary diagnosis of gliobastoma multiforme. A left temporoparietal craniectomy was performed and once opened a smooth contoured greyish-white coloured lesion was noticed. The lesion was not invasive to the parenchyma. The total resection of the mass was conducted and the excised material was sent for pathological and microbiological analysis. Histopathological examinations of the frozen sections indicated many fungal hyphae and inflammatory cells. No neoplastic findings were noted. On fixed sections, hematoxylin-eosin, periodic acid shift and Grocott's methamine silver staining were performed, and the reported findings were indicative of a cerebral fungal infection such as the presence of hyphal elements with thick septations, suppurative inflammatory changes, necrosis, foreign body giant cells and gliosis in peripheral parenchymal tissues.

The patient was given one dose of amphotericin B at 50 mg intravenously but developed apparent allergic reaction to amphotericin B and switched to liposomal amphotericin B at a dose of 350 mg $day^{-1}$. The patient was transferred from the intensive care unit to neurology service on day 3 of the post-operation (post-op) period. Compared to the pre-op analysis, a cranial CT with contrast material performed on day 12 of the post-op period indicated almost complete excision of the mass and the attenuation of the pressure previously exerted by the mass on the left lateral ventricle (Fig. 1b).

A nosocomial pneumonia developed during the post-op period with no microbiological confirmation, and it responded well to the broad spectrum antibiotic regimen (meropenem 3 × 1 g i.v. for 2 weeks). On day 40 of the post-op period, liposomal amphotericin B was replaced with voriconazole (2 × 200 mg i.v.) and the patient then remained on voriconazole i.v. for 3 weeks. On day 60 of the post-op period, the patient was discharged on the oral voriconazole tablet (2 × 200 mg/day). During follow-up examinations performed on 120th and 160th and 300th days of voriconazole treatment, the patient had no remarkable laboratory findings and remained healthy and stable. Control MRIs conducted on all three occasions indicated complete resolution of oedematous changes related to the craniectomy. The planned treatment will end after twelve months and a final MRI will be conducted to ensure complete resolution and no recurrence of the lesion.

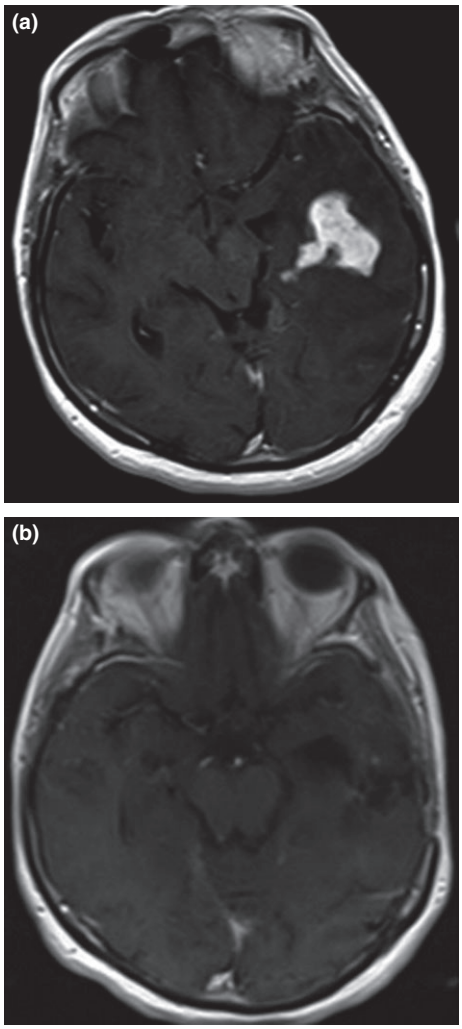


**Figure 1** Pre-op (a) and post-op (b) axial MRI of the brain performed with contrast material.

## Microbiological diagnosis

In the microbiology laboratory, the specimen from the excised mass was inoculated on both bacteriological and mycological media (Sabouraud Dextrose and Mycosel Agar, Salubris A.Ş., Istanbul, Turkey). No bacterial growth was noted. The growth of dematiaceous fungi was evident on the SDA media by the 1st week of the culture at 30 °C. The colonies grew at a moderate to slow speed and were velvety in texture with brown to black colour both in the front and the back (Fig. 2a). The initial morphological assessment on lactophenol cotton blue-stained fungal elements indicated that the fungus had an olive coloured septate hyphae with branches (Fig. 2b). The conidiophores with sympodial conidiation were reminiscent of *Fonsecaea* species (Fig. 2c). There were up to three and very rarely a small forth conidias formed on the tips of the conidiophores. The conidias were smooth and thin walled with differing sizes and with a loose connection to the bearing conidiophores. Because unequivocal differentiation of *F. monophora* from *F. pedrosoi* cannot reliably be performed by microscopy, a molecular analysis was conducted.[1,2]

The fungal DNA was extracted from 1-week-old colonies scraped from the growth on SDA media. The DNA extraction was performed with a commercial DNA extraction kit as suggested by the manufacturer (QIAamp DNA Mini Kit, Qiagen, Hilden, Germany). The amplification of the 18S rRNA-encoding region was performed through polymerase chain reaction using primers specific for internal transcribed spacer element ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and using the conditions described previously.[3,4] The amplified fragment was sequenced and the generated sequence was compared to the previously published data using the NCBI and mycobank data base (http://www.mycobank.org/) program. The blasting results indicated that the isolate belonged to the *Fonsecaea* species but did not make a definite unequivocal differentiation between *F. monophora* and *F. pedrosoi* species. Thus, a further analysis was conducted to determine the key bases.[2] Accordingly, the key bases in the ITS1 and ITS2 sequences help to differentiate between *F. pedrosoi* and *F. monophora* species. Thus, a sequence comparison for those key bases was performed to delineate the species identification more precisely. Accordingly, 9 out of 12 bases in ITS1 and ITS2 were identical to *F. monophora* compared to 3 out of 12 that were identical to *F. pedrosoi* (Table 1).

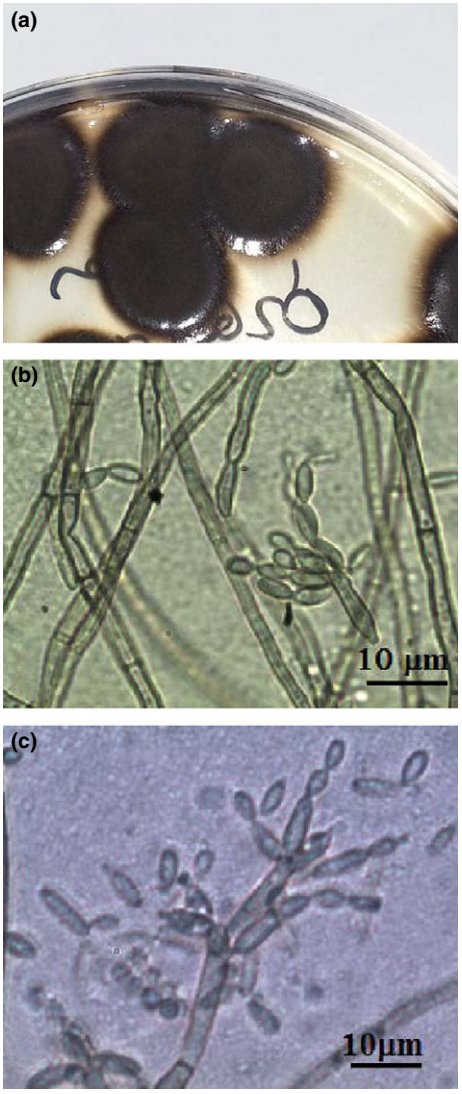


**Figure 2** (a) Black fungi on SDA. (b) Olive coloured septate hyphae with branches and conidias on the tips of the conidiophores. (c) Smooth and thin walled conidiophores with sympodial conidiation.

## Discussion and a review of the literature

Cerebral phaeohyphomycosis is an infection of the central nervous system (CNS) by dematiaceous fungi, and the mortality is reported to be as high as 100% in untreated and up to 65% even in surgically treated infections.[5–8] Infections of the CNS by melanised fungi largely manifest in the form of cerebral abscesses in male patients.[5,6] Immunosuppression does not seem to be the common underlying condition in the affected and there is no single risk factor identified.[5,6] Cerebral infections are rare occurrences compared to cases

**Table 1** The comparison of the key nucleotide positions in ITS1 and ITS2 genes in the test isolate used to differentiate between *Fonsecaea monophora* and *F. pedrosoi* species.

| | ITS1 (31..235, after ATCATTA) | | | | | | | | 5.8 S (236..395) | ITS2 (396..589) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nucleotide Positions[1] | 15 | 41 | 47 | 79 | 99 | 100 | 109 | 113 | 46 | 47 | 119 | 121 | 144 |
| *F. pedrosoi* | C | C | A | T | C | T | T | A | C | G | T | T | T |
| *F. monophora* | T/– | T | T | G | T | C | C | G | T | A | A | C | C |
| Test organism | **T** | **T** | A | T | **T** | T | **C** | **G** | C | **A** | **A** | **C** | **C** |

[1]The nucleotide positions were deduced from previously published sequences.[2]

where dematiaceous fungi are usually isolated.[7] The case definition of cerebral phaeohyphomycosis relies on three requirements; lesions indicative of CSN infection, isolation of the organism from brain tissue or cerebrospinal fluid, and lastly, mycological identification of the isolate as one of the agents of the phaeohyphomycosis[5]; our case is fully compliant with this definition criteria. Among the etiological organisms, *Cladophialophora bantiana*, *Exophiala dermatitidis* and *Rhinocladiella mackenziei* are the most common, and these fungi are classified as neurotropic organisms.[6]

However, *Fonsecaea* species are not among the leading organisms in cerebral phaeohyphomycosis and there are only a few cases where *Fonsecaea* species are identified as the etiological agent. In this case report, we documented a case of cerebral phaeohyphomycosis by *F. monophora*, where a successful surgical and medical treatment was attained.

*Fonsecaea* species are the most frequently isolated organisms from chromoblastomycosis cases and *F. pedrosoi* is the primary etiological agents of cutaneous and subcutaneous chromoblastomycosis infections.[7] It has been speculated that *F. pedrosoi* is a strict chromoblastomycosis agent and has not been isolated from any other cases.[1] In a 2004 study, *F. monophora* was designated as a separate species upon detailed molecular analysis and characterised as a general opportunist.[2] This re-classification of the genus brought doubts as to the identifications of previously published reports of *F. pedrosoi* cases from cerebral phaeohyphomycosis. Furthermore, detailed molecular examinations made it possible to re-classify the previously identified melanised fungi into different species. All of these studies made the assessment of the correct number of cerebral phaeohyphomycosis cases caused by *F. monophora* rather difficult. For example, in two cases where agents were previously reported as *F. pedrosoi*, molecular studies retrospectively identified those species as *F. monophora.*[2,9–11] In another case, Middle-Eastern isolates of *F. pedrosoi* cases were reclassified as *Rhinocladiella mackenziei.*[10,12] Furthermore, a previously identified *Cladophialophora bantiana* from cerebral infections was re-identified as *F. monophora* after molecular evaluations. In 2005, Surash *et al.* [10] concluded that a total of 4 cases of cerebral phaeohyphomycosis caused by *F. monophora* were reported in the literature. After these re-classifications, only a single case of *F. pedrosoi* induced cerebral phaeohyphomycosis was reported by Madhugiri and colleagues in 2011.[13] However, in that case, the identification of the insulting organism is determined via morphological observation made with lactophenol cotton blue staining, which might be insufficient to differentiate *F. monophora* from *F. pedrosoi.* Because two species are almost indistinguishable morphologically and in all molecularly studied cases *F. monophora* is identified as the causative organism, it is not un-reasonable to speculate that all cerebral phaeohyphomycosis cases with *Fonsecaea* isolations are indeed caused by *F. monophora.* Our report adds to the list of such cases and advances the notion that *F. monophora* might represent true neurotropic melanised fungi. Given the difficulty differentiating between *F. monophora* and *F. pedrosoi* and the very low number of reported cases, however, the future cases should still be studied in detail for the identification to rule in that indeed the isolate is *F. monophora*. In Table 2, we summarized the cerebral infections caused by *F. monophora* reported in the literature.

*Fonsecaea monophora* species isolated from environmental and clinical isolates demonstrated a very similar genetic make-up and thus, the source of clinical isolates are believed to be environmental organisms.[2] Because no vector is implicated, direct transmission from the environment to humans is the most probable route of infection.[2] The way in which the fungus reached the brain is questioned in most studies. However, the precise nature of transmission is usually lacking. Most *Fonsecaea* spp. infections manifest themselves as chromoblastomycosis occurring in various parts of the body (usually in the form of cutaneous or subcutaneous lesions). In fact, only 10% of the melanised

**Table 2** Some features of cerebral phaeohyphomycosis cases caused by *Fonsecaea* species.

| Organism | Age (year) | Sex | Risk factors | Outcome | References | Year reported |
|---|---|---|---|---|---|---|
| *Fonsecaea pedrosoi* | 49 | Male | Renal transplant | Death | 15 | 1988 |
| *Fonsecaea pedrosoi* | 15 | Male | None | Survival | 16 | 1995 |
| *Fonsecaea pedrosoi* (*monophora*) | 28 | Male | None | Death not related to *Fonsecaea* infection | 11 | 2003 |
| *Fonsecaea monophora* | 53 | Male | Diabetes mellitus | Survival | 10 | 2005 |
| *Fonsecaea monophora* | 62 | Female | Liver Transplant | Survival | 17 | 2007 |
| *Fonsecaea monophora* | 48 | Female | Renal Transplant | Survival | 9 | 2010 |
| *Fonsecaea pedrosoi* | 8 | Female | None | Survival | 13 | 2011 |
| *Fonsecaea monophora* | 71 | Female | Diabetes mellitus | Survival | Present study | 2014 |

fungi isolated from patients are considered true pathogens, and the rest of the isolates might represent the non-pathogenic contaminants.[7] Trauma to the skin is probably the primary means of infection. The CNS is usually not the target of such infections and CNS infections are considered secondary. In the present case, it was not possible to determine the source of infection. The patient did not present a history of trauma or an invasive fungal infection, both of which are known to introduce the fungal species to the cerebral tissues. In addition, no primary *F. monophora* infection in the rest of the body was detected. The location of the lesion was close to the sinuses and the temporal and orbital tissues, and direct spread from those sites could be a possibility. Microorganisms can also reach the brain through blood or lymphatic vessels, by directly spreading from adjacent tissues or lesions or by accidental inoculation.[6] Furthermore, fungi might gain access to the CNS through blood vessels, and fungal elements have been frequently observed in arterial walls. Pulmonary infection with the same agent might be the potential source for CSN infections and has been demonstrated and confirmed in experimental infections in animals. Exposure to soil or plant matter may be the source of respiratory infection. Additionally, the fungus might reach the brain through lymphatic vessels. In some cases, the fungus infects the CNS by directly spreading from adjacent organs such as the sinus and orbital tissues.[6]

Phaeohyphomycosis caused by *Cladophialophora bantiana* and *Rhinocladiella mackenziei* are endemic to specific regions of the world. Namely, *Cladophialophora bantiana* is usually reported from Indian cerebral phaeohyphomycosis cases and *Rhinocladiella mackenziei* from Middle Eastern countries with arid land and relatively scarce humidity.[5,7,14] Cerebral infections with *Fonsecaea* species are not associated with any specific region or land type.[2] There are only a few cases of CNS infection caused by *Fonsecaea* species reported in the English language literature. No regional distribution of these cases seems to be apparent in *F. monophora* induced cerebral infections.

Even though cerebral infections by melanised fungi are reported to have a high mortality even after surgical and medical interventions, the fatality rate in the cases caused by *Fonsecaea* species do not seem to support this conclusion (Table 2). This finding might be related to the factors inherent in the species and manifested as less neuropathogenic than those of other dematiaceous agents.

Voriconazole is reported to have an effective penetrating ability on the brain tissue and this is suggested by the recent joint ESCMID ECMM clinical guidelines.[14] Generally, amphotericin B was reported to have a poor outcome in CNS infections with melanised fungi. The susceptibility testing of the fungus to antifungals was not performed. The initial amphotericin B followed by voriconazole regimen provided a satisfactory medical treatment and the patient was healthy and stable after 8 months of follow up. No antifungal resistance nor an allergic reaction to the drugs was detected and consequently, the regimen was not altered,

In conclusion, our case represents one of the few confirmed *F. monophora* cerebral abscess cases where mycological and molecular approaches identified the agent. The report is important because there are a growing number of cerebral infections by *F. monophora*, and it supports the notion that *F. monophora* represents a true neurotropic dematiaceous agent.

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## Disclosure of conflict of interest

No conflict of interest is declared.

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