Cerebral phaeohyphomycosis caused by *Fonsecaea pedrosoi* in Saudi Arabia

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A case of cerebral phaeohyphomycosis (CPM) in a 70-yr-old Saudi male was diagnosed recently at King Khalid University Hospital in Riyadh. Computerized tomography (CT) scans of the patient’s brain unveiled 2 abscesses in the left frontal and a 3rd abscess in the right frontal lobes. Aspirated pus from the abscesses contained branched, septate, brown hyphae diagnostic of CPM. Culturing of pus yielded a slow-growing, dematiaceous fungus which was identified as *Fonsecaea pedrosoi*. Combined therapy of amphotericin B and 5-fluorocytosine had little or no effect as the patient continued to have spiking fever and his condition remained more or less unchanged. Medical care of the patient was unfortunately discontinued as he was discharged, with a rather poor prognosis, at the insistence of his family and against medical advice. Prior to this case, work had been done on identifying the agents responsible for two previously diagnosed cases of CPM in Saudi patients with fatal outcome. The dematiaceous fungi that were isolated remained sterile for quite a time before we succeeded in inducing sporulation. Both these isolates were also identified as being *Fonsecaea pedrosoi*.

Key words: Cerebral phaeohyphomycosis; *Fonsecaea pedrosoi*; Saudi Arabia; fungus infection; brain.

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INTRODUCTION

Phaeohyphomycosis is a group of fungal infections in which the tissue form of the fungus is septate, brown hyphae. The group includes cutaneous, subcutaneous and systemic infections (1, 2, 11). This definition, as it was first introduced by Ajello et al. in 1974, differentiates it from the other dematiaceous fungal infection, chromoblastomycosis (chromomycosis), in which the tissue form of the agent is primarily dark, thick-walled muriform cells. It was not until recently that both terms were applied occasionally to the same infection. The aetiologic agents of chromoblastomycosis and phaeohyphomycosis are quite diverse dematiaceous fungi, with those of the latter disease being more heterogenous and newly discovered ones continually being identified (2, 3, 6, 7, 12, 15, 17, 18). Emmons et al. (1977) applied the term cerebral chromomycosis to the infection caused by any dematiaceous fungus which produces lesions in the brain. They cited *Cladosporium bantianum* (as *C. trichoides*) to be the most common agent of this infection with *Fonsecaea pedrosoi* (as *Phialophora pedrosoi*) and *Wangiella dermatitidis* (as *P. dermatitidis*) being rare aetiological agents. Cerebral chromomycosis was referred to later by Rippon (1982) and Gonzalez et al. (1984) as cerebral phaeohyphomycosis since the agents in tissue are in the form of septate, brown hyphae.

*Fonsecaea pedrosoi* is commonly considered as one of the agents of chromoblastomycosis (1, 4); however, it has been isolated on a few occasions from brain abscesses in cerebral phaeohyphomycosis (4, 18). In the brain, it produces long, branching, septate, brown hyphae.
that are difficult to differentiate from those of Cladosporium bantianum, the most common agent of cerebral phaeohyphomycosis (4, 18). A discussion between J. W. Rippon and L. Ajello has led to the understanding that F. pedrosoi may actually cause either phaeohyphomycosis or chromoblastomycosis (1).

A review of the literature failed to disclose any published report on cerebral phaeohyphomycosis or any fungal infection of the brain from Saudi Arabia. Therefore, it is the purpose of this paper to report a recent case of the infection from that country with reference to 2 cases diagnosed earlier. We also emphasize that Fonsecaea pedrosoi is an agent of cerebral phaeohyphomycosis since it was identified to be the fungus responsible for the 3 cases discussed here.

CASE REPORT

A 70-yr-old Saudi male was referred to King Khalid University Hospital (KKUH) on October 25, 1986 because of spiking fever, progressive loss of weight, and mental changes. The patient had had most of his small bowel, including half of the ascending colon, resected because of gangrenous bowels due to superior mesenteric artery embolism. Following that, he was re-admitted to the local hospital and diagnosed as having a short bowel syndrome and thus kept for about 4 wk on IV-fluids and multivitamins. His relatives noted mental and behavioural changes and, for 2 wk prior to admission to KKUH, he had been running spikes of fever (39°C and higher). Klebsiella and E. Coli were isolated from his blood and urine, respectively, and he was put on amikacin for 1 wk, but without effect. Upon examination, the patient was severely emaciated and dehydrated, his temperature was 37.4°C, chest and abdominal organs were normal. He was awake, but markedly slow in his responses. A mild right-sided papilloedema was present. He had a generalized increase of muscle tone with brisk, but equal, tendon reflexes. There was no focal deficit or cranial nerve dysfunction. His routine blood investigations disclosed only signs of normocytic, hypochromic anaemia and eosinophilia. On the 2nd d of admission the patient started spiking fever up to 39°C. Microbiological investigations of sputum, blood, and urine were negative. His CSF contained 150 WBC's with 75% lymphocytes and the rest PMN's, protein was 0.86 gm/l and sugar 6.8 mmol/l. Gram stain, Zeihl-Neelsen stain and India ink preparation of the CSF were all negative. Pre- and post-contrast tomography (CT) scans demonstrated two hypodense areas in the left frontal lobe and a third in the right frontal lobe with a regular ring-shaped enhancement. The 2 large cystic lesions were separated from the anterior horns of both lateral ventricles only by a thin membrane (Fig. 1A). In addition, there were signs of generalized brain atrophy.

By means of a frontal burr hole on each side, both abscesses were punctured, and 10–15 ml of thick greenish-yellow pus was aspirated from each of them. Gram and Giemsa stained pus smears as well as 10% potassium hydroxide (10% KOH) preparation revealed the presence of branched, septate brown fungal hyphae diagnostic of cerebral phaeohyphomycosis (Fig. 2C). Therefore the patient was immediately started on amphotericin B (1.5 mg/kg/d IV) and 5-fluorocytosine (1.6 gm, 6 hourly IV). Initially thereafter the patient seemed to be improving as he became mentally brighter. However, he continued to have fever between 37.5 and 38°C. A repeated CT scan 5 d later showed a collapsed right abscess with no changes in the left abscesses (Fig. 1B). Therefore, a re-tapping of the left abscess was done, but only turbid CSF was found. Subsequently, the patient's condition remained more or less unchanged and, despite 2 wk of antifungal therapy, he was still running spikes of fever. A further CT study revealed refilling of the right abscess and unchanged left abscesses (Fig. 1C). In spite of the patient's critical condition, and against medical advice, his family insisted on having him discharged on November 25, 1986 with a rather poor prognosis.

Brief clinical history of previous cases

The first case of cerebral phaeohyphomycosis in Saudi Arabia was diagnosed and managed at KKUH in Riyadh during the period from February 1, 1983 to January 21, 1984. It involved a 45-yr-old Saudi female who was found to have bilateral brain abscesses in the right parietal and left occipital regions. Examination of Gram-stained smears from aspirated thick cheesy pus revealed the presence of branched, septate fungal hyphae (Fig. 2A). Despite varied chemotherapy, including amphotericin B, 5-fluorocytosine and ketoconazole, the patient expired on January 21, 1984.

The second case involved 70-yr-old Saudi male who was seen between August 15, 1985 and September 22, 1985. CT scans of the patient's brain showed a cystic lesion deeply situated in the temporoparietal region of the right cerebral hemisphere. The abscess was punctured and thick yellow pus was aspirated. Gram-stained pus smears contained no bacteria, but branched, septate fungal hyphae (Fig. 2B). The patient was treated with amphotericin B with no success as he expired on September 22, 1985.

MATERIAL AND METHODS

Burr hole operations were performed on each of the patients and 9–20 ml of pus were aspirated from
each of the punctured brain abscesses. Potassium hydroxide (10% KOH) mounts as well as smears from pus were prepared for direct microscopic examination. The smears were stained by Gram or by Gram and Giemsa methods. Quantities of pus were cultured on various microbiological media including Sabouraud dextrose agar (SDA). In addition, pus from the latest case was cultured on neutral SDA (NSDA, Emmons modification of SDA), brain-heart infusion agar (BHI-A), and on potato dextrose agar (PDA). Inoculated media were incubated at 26 ± 1°C and some at 37°C, during which they were inspected periodically for growth of organisms.

Additional fungal media were used for sporulation induction experiments as the need arose. These included cornmeal agar (CMA), V-8 juice agar, and water agar (WA). Serial subculturing of the isolated fungi within and across the various media, as well as slide culture technique, were employed in these experiments. Furthermore, light exposure experiments (UV and fluorescent light) were also tried for inducing sporulation in the initially, poorly sporulating isolates. The isolated fungi were tested for their ability to liquefy gelatin, to grow at various incubation temperatures (26, 37, and 40°C) and to grow in the presence of cycloheximide (500 μg/ml medium).

*Fig. 1.* Postcontrast computerized tomography scans of the brain of a 70-yr-old Saudi male. A, preoperative scans showing large single abscess in the right frontal lobe (right photo) and 2 smaller abscesses in the left frontal lobe (left photo); B, 5 d following a bilateral tapping of the abscesses, the right abscess is almost entirely collapsed while the left ones remained unchanged; C, 18 d after re-tapping left abscess, showing still unchanged left abscesses and refilling of the right abscess.
RESULTS

Aspirated pus from each of the 3 cases contained branched, septate brown hyphae when examined microscopically. Hyphae in the pus specimens from the different patients were very similar morphologically (Fig. 2). Cultured pus from each of the cases yielded slow-growing dematiaceous fungal colonies on all inoculated media. The fungi isolated from the 1st and 2nd cases remained sterile for a considerable time as they were maintained on SDA medium, thus contributing to the delay in their identification. It was not until several months before admission of the 3rd patient that the fungi were induced to sporulate. This was achieved by continued subculturating of the organisms on various deficient media (PDA, CMA, V-8 agar, and WA). Exposure of the colonies to UV and fluorescent light did not help in inducing sporulation. Best spore production was found in PDA cultures (Fig. 3). No difficulty was encountered in inducing the isolate from the last case to produce spores as the primary colonies were subcultured to PDA and CMA media without delay.

The three isolates from the patients behaved similarly in culture. Colonies were first black in colour then turned grey-black as they grew older and became covered by short grey hyphae (Fig. 3). In PDA slide culture, each isolate at first produced one or two obovoid to elliptical conidia on denticles formed from short, lateral, hyphal outgrowths. As these young conidiophores extended gradually at their tips, more conidia were formed more or less freely on this upper portion, resulting in a Rhinocladiella-like sympodial sporulation. The fungi also produced conidia of another type in which the spores were formed in rather short chains similar to Cladosporium sporulation. In addition, some primary conidia giving rise to secondaries were observed. No phialides were seen in the processed PDA slide culture of the organisms. The predominant type of sporulation in all isolates was that of the Rhinocladiella-like conidiation (Fig. 3). The fungi grew well at 37 and 40°C. They did not liquefy gelatin nor were they inhibited by cycloheximide.

On the bases of the above-mentioned characteristics and upon consulting the literature (8, 10, 18) we identified our three isolates to be all within the dematiaceous species Fonsecaea pedrosoi (Brumpt) Negroni.

DISCUSSION

Ever since phaeohyphomycosis was clearly defined, the number of cases reported has increased dramatically, with variable aetiologies
Fig. 3. Six-week-old, potato dextrose agar plate and slide cultures of *Fonsecaea pedrosoi* (colonies and microscopic morphology) isolated from 3 cerebral phaeohyphomycosis Saudi patients; A, from a 45-yr-old female; B, from a 70-yr-old male; C, from another 70-yr-old male. Both *Rhinocladiella*- and *Cladosporium*-like sporulations can be seen in the microscopic photographs. x 500.

(2, 3, 6, 7, 12, 15, 16, 17, 19). Reports on cases with brain involvement, however, are limited (4, 6, 17, 19). The organisms causing the various phaeohyphomycosis infections are facultative parasitic dematiaceous fungi living in nature saprobically on decaying wood and vegetable debris in soil (5, 9, 10, 14, 18). The tissue form of the fungi is branched, septate brown hyphae as we have found in the cases discussed here (Fig. 2). We did not observe any thick-walled brown muriform cells in pus smears. Therefore, the diagnosis was made of cerebral phaeohyphomycosis. To our knowledge, this comprises the first report of the mycosis or any fungal brain infection from Saudi Arabia.

The genus *Fonsecaea* was first described by Negroni in 1936 to include all fungi that have two common types of conidiation, *Hormodendrum* (*Cladosporium*-like) and *Acrotheca* (*Rhinocladiella*-like) types (McGinnis 1980). Additionally, primary conidia in the sympodium may give rise to secondaries (10, 18). *Phialophora*-like sporulation is not considered as a major and stable characteristic of the genus *Fonsecaea*,...
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since it is rarely produced by the fungi or it is lacking in some strains (10, 18). It is primarily based on all above characteristics that *Fonsecaea* is differentiated from the genus *Rhinocladiella* and its closely related genus *Ramichloridium*. The latter two genera produce conidia sympodially and no blastoconidia are formed from the primaries. In addition, colonies of *Ramichloridium* are brown to olive green with shades of pink or orange colour on reverse, and the fungi produced dark-brown conidia sympodially (Rhinocladiella-like sporulation) or, less commonly, in a manner similar to *Cladosporium* sporulation. Some conidia in the spore heads were observed forming additions (Fig. 3). Therefore, the fungi isolated fit nicely in the genus *Fonsecaea*. As the spore heads were loose and conidia were obovoid to elliptical, the fungi were identified as being of the species *Fonsecaea pedrosoi*. We were unable to find phialides in the processed slide cultures, thus our isolates are perhaps of the strains that do not produce conidia by *Phialo­phora*-like sporulation (10, 13, 18). *Fonsecaea pedrosoi* (= *Phialophora pedrosoi*) is known to produce long branching, septate hyphae in the brain (4, 18) as we have seen in our cases. Therefore, this report is also added evidence to the few published cases of *F. pedrosoi* as an agent of cerebral phaeohyphomycosis.

The most commonly used antifungal drugs for treating phaeohyphomycosis have been amphotericin B, 5-fluorocytosine, ketoconazole, and thiobendazole. In subcutaneous phaeohyphomycosis successful treatments have been achieved by thorough excision alone of the subcutaneous mass (2, 16) or by excision plus amphotericin B, 5-fluorocytosine, and thiobendazole therapy (7). Prognosis of cerebral phaeohyphomycosis, however, has always been grave (4, 6). Emmons et al. (1977), discussing the published 35 cases of cerebral phaeohyphomycosis (=cerebral chromomycosis), stated that all patients expired within 1 yr of the recognition of symptoms, except for 2 patients who survived for 2 and 3 yr. The majority survived for less than 3 months. Combined treatment by amphotericin B and ketoconazole of a recent case of phaeohyphomycosis with brain involvement did not resolve the neurological impairment of the patient (17). Likewise, in our patients surgical drainage of brain abscesses and antimycotic therapy with amphotericin B, 5-fluorocytosine and, in 1 case, ketoconazole were not successful. As stated earlier, 2 patients have expired and we have lost contact with the 3rd who was discharged with a similar outcome expected. Concentrated effort from all involved in the field is needed to find a better treatment for cerebral phaeohyphomycosis.

REFERENCES


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