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Systemic phaeohyphomycosis due to *Exophiala (Wangiella)* in an immunocompetent child

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We report a rare case of systemic lymphadenitis and hepatic involvement due to *Exophiala (Wangiella) dermatitidis* in a pediatric patient. An 8-year-old immunocompetent boy with chronic fever was examined through the use of sonography and CT scan which demonstrated cervical and mesenteric lymph node enlargement and numerous small hepatic lesions. The etiologic agent was isolated by means of lymph node aspiration. The fungus was identified by its morphological characteristics and through DNA sequencing of the internal transcribed spacer region of rDNA. Despite initial amphotericin B and voriconazole therapy, the child's jaundice subsided and he died 7 months later. In addition to pathogenic aspects of *Exophiala dermatitidis*, the diagnostic approaches and relevant therapeutic strategies are discussed.

Keywords *Exophiala dermatitidis,* lymph nodes, liver granuloma, child, immunocompetent

Introduction

Exophiala (Wangiella) dermatitidis is a brown-pigmented, yeast-like fungus that has been isolated from several environmental sources. Although considered a saprobe, it has infrequently been identified as the cause of human infections, including those of the skin, brain, lung, eye, joints, and endocardium [1–3]. The case reported here represented invasive fungal infection associated with disseminated lymphadenitis and hepatic involvement due to *E. dermatitidis* in an obviously immunocompetent child.

Case report

An 8-year-old boy was admitted to our hospital for evaluation of fever, bilateral neck masses, and an abdominal mass. Four months prior to his hospitalization, the patient had developed a non-tender, firm, inflamed swelling in the left submandibular area, with following masses in the right submandibular area, posterior of the right auricula and left axillary region. In time, he had developed hepatomegaly. In addition, the patient had previously been hospitalized for 15 days for evaluation of lymphadenitis. Computed tomographic (CT) scan showed hypoattenuating areas in the liver with multiple abdominal and thoracic lymph nodes. Histopathologic findings associated with an excisional biopsy of the left axillary lymph node performed at the previous center, had indicated features of necrotizing suppurative lymphadenitis which were consistent with fungal infection. However, there was no knowledge of microbiologic investigation of the lymph node having been conducted during this earlier hospitalization. Five days before admission, a mild epigastric pain became constant and nausea developed, so the patient was transported to our hospital for evaluation and treatment.

He had been previously healthy and his growth and development had been normal. He lived in a rural area with his parents and had animal contact. The parents denied any preceding trauma, and the child had given no indication of pain around the lesion. There were children in his village who complained of similar

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findings, but these resolved spontaneously. There was no past history of tuberculosis or leishmaniasis.

He was febrile (38.5°C) at the time of admission. Physical examination revealed multiple lymph nodes between 1 and 5 cm in diameter on both sides of the neck (Fig. 1) and in both axillae. There was diffuse tenderness without rebound tenderness on his abdomen. A 4×5 cm mass was present in the epigastrial region with hepatomegaly. The remainder of the physical examination, including ears, nose, oral cavity and throat was normal.

The leukocyte count on admission was 18,000 cells per ml, with a differential of 68% neutrophils and 10% band forms. The peripheral blood smear showed no atypical lymphocytes. Hepatic enzyme levels were normal. Serologies were negative for human immunodeficiency virus (HIV), hepatitis B and hepatitis C, Epstein-Barr virus, cytomegalovirus, and brucella. Serologic tests for *Parvovirus* B19 (used NovaLisa Parvovirus B19 IgM ELISA kit, Novatec Immundiagnostica), and *Toxoplasma gondii* (used The AxSYM Toxo IgM assay, Abbott and Toxo IgG, ARCHITECT SYSTEM, Abbott) were negative. Mycobacteria was negative according to the Kinyoun (cold) acid-fast procedure and MGIT 960 system (Becton Dickinson) used for mycobacteria culture. Furthermore, the cryptococcal antigen test and visceral leishmaniasis antigen testi (rK39 ELISA) were negative. An underlying immunodeficiency was not found. Absolute lymphocyte count, CD4/CD8 ratio, the nitroblue tetrazolium (NBT) dye reduction by stimulated polymorphonuclear

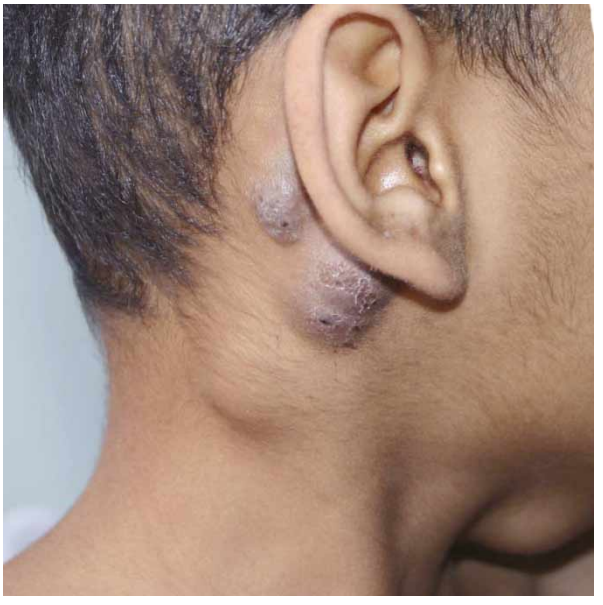

Fig. 1 Multiple lymph nodes between 1 and 5 cm in diameter on both sides of the neck.

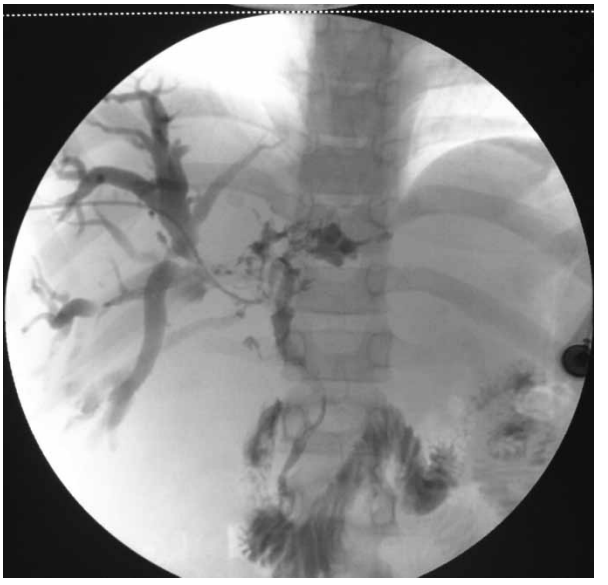

Fig. 2 Percutaneous transhepatic biliary drainage (PTBD) for preoperative relief of obstruction.

leukocytes and the mean concentrations of serum Ig G, Ig M and Ig A were normal. C3 and C4 levels, interferon-γ pathway were found to be normal.

On admission, after cultures of blood and left cervical lymph node aspiration were obtained, intravenous liposomal amphotericin B (L-AmB; 5 mg/kg/day) was started. Lymph node specimen was cultured for bacteria (anaerobic and aerobic) and fungi. Septate hyphae and neutrophils were observed in Gram and Giemsa stained left cervical lymph node aspirate samples from our patient which were sent to the microbiology laboratory at the Central Laboratory [Balcali Hospital, Cukurova University, Adana] (Fig. 3). Two days after incubation, wet, black-brown colonies were noted on Sabouraud's dextrose agar (room temperature and 37°C) and sheep blood agar (37°C) cultures (Fig. 4). The organism was slow growing and appeared upon prolonged incubation of up to 7 days. When the cultures were examined microscopically, the main structure was phaeoid (brown), budding, one-celled, round to oval yeast-like cells. Annelide production was not observed. Black yeasts were also recovered at 42°C. Histopathologic examination of hematoxylin and eosin (H&E) sections of the left axillary lymph node performed at the previous center revealed invasion by fungal hyphae. Irregular branching, septate hyphae and budding yeast-like cells were described.

The expanding lesions began to resolve within 10 days of starting of the patient on L-AmB alone. One month later, he again developed bilateral swelling and

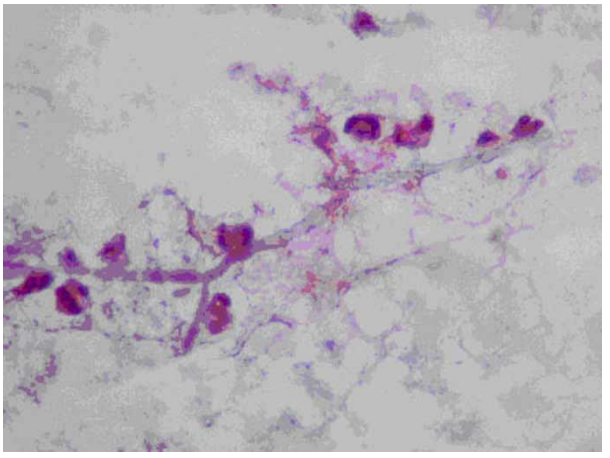

Fig. 3 Gram stained lymph node aspirate of our patient showing septate hyphae and neutrophils.

erythema at sides of his neck while still on L-AmB. Although cultures were negative, intravenous voriconazole (maintenance doses of 4 mg/kg/dose $\times 2$ dose) was added to L-AmB on day 33 for a possible recurrence of infection or the resistance of the etiologic agent. There was rapid clinical improvement, and therapy was continued for 1 month as both L-AmB (5 mg/kg/day) and voriconazole (4 mg/kg/dose $\times 2$ dose), with a following month as only intravenous voriconazole (4 mg/kg/dose $\times 2$ dose). At the time of discharge, 3 months after admisson, a repeat CT scan demonstrated a reduction in the size of lymph nodes and intrahepatic lessions. There were no neurological clues. The patient was discharged home on oral voriconazole (200 mg $\times 2$ dose) for 2 weeks.

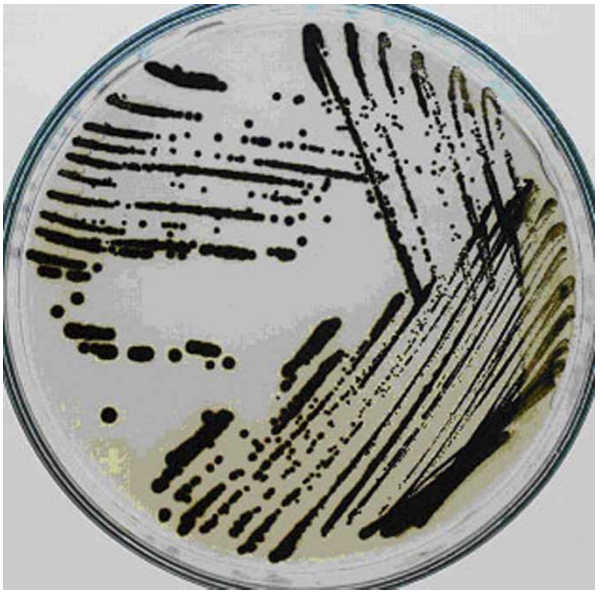

Fig. 4 *Exophiala dermatitidis* grown for 7 days on Sabouraud dextrose agar at 37 °C.

He remained well during 6 months of follow-up, until an episode of obstructive jaundice. Initial magnetic resonance (MR) disclosed a fist-sized inflammatory mass in the region of the head of pancreas, hepatic hilar, and obstructed biliary system. Peritonoscopy revealed multiple granulomas and plates over the liver. The hepatic biopsy revealed only a non-specific granulomatous hepatitis. There was no clinical, radiological or laboratory evidence of autoimmune liver disease, sarcoidosis, lymphoma or tuberculosis. The single biopsy sample failed to show the etiology of the granulomas. Percutaneous transhepatic biliary drainage (PTBD) with multiple drains was preferred for preoperative relief of obstruction (Fig. 2). The patient's family refused the laparotomy. The patient was treated palliatively with drains for biliary drainage. Although cultures of blood and PTBD fluid were negative, L-AmB and voriconazole were restarted for a possible recurrence of the fungal infection. In the following period the patient failed to respond to L-AmB and voriconazole and died 1 week later. His family refused an autopsy.

The *in vitro* antifungal susceptibility of our isolate of *E. dermatitidis* was determined using E test (AB Biodisk, Solna). It was found to be sensitive to amphotericin B and voriconazole, with minimal inhibitory concentration (MIC) 0.25 and 0.50 μg/ml, respectively, and resistant to fluconazole with a MIC of $=256$ μg/ml. All laboratory studies were performed under safety conditions (class II). For definitive identification of the isolated strain, molecular analysis of ribosomal DNA internal transcribed spacer (ITS) sequences were performed. The primers used for sequencing were ITS1 and ITS4 and the isolate was confirmed as *Exophiala dermatitidis*. Cycle sequencing was performed in Metis Biotechnology Laboratory (Ankara, Turkey) by using an Amersham dye-terminator sequencing kit in Visible Genetics automated DNA sequencing system (Toronto, Canada) [4]. Sequences were analyzed using GeneObjects Software v3.1. The obtained sequences were compared to all known sequences in the Genbank by use of BLAST (National Center for Biotechnology Information, Bethesda, MD [http://www.ncbi.nlm.nih.gov/BLAST/]). Aligning this partial sequence of the ITS1 with that of the ex type strain of *E. dermatitidis* CBS 149.90 (accession number AF 050268) revealed a 100% match except for an additional A at position 61, 301, 361 and 541. Of our fungal isolate, the Genbank accession number is EU 779950. The accession number assigned

to our strain by Centraalbureau voor Schimmelcultures (CBS) (Utrecht, the Netherlands) is CBS 123474.

Discussion

Since *Exophiala dermatitidis,*a dermatiaceous fungus, has been isolated from soil, water, wood, decomposing plant debris and human feces, its exact ecological niches remains unclear. In one recent case, the source of the infection could not be determined [5]. It was recently shown to be abundant in public steam baths and water reservoirs and a contaminated water supply has been described as the probable source of an *Exophiala* outbreak. A hypothesis of a route of human systemic infection through ingestion, namely translocation from the intestinal tract, has been put forward [6,7].

Infections caused by *E. dermatitidis* are called phaehyphomycosis due to the presence of dark pigmented hyphal elements in histopathological stained sections prepared from samples of lesions as in this case. Systemic phaeohyphomycosis caused by this fungus is rare and previous reports have emphasized the high mortality rate associated with such infections. A critical review of the literature of cases from 1960 to 1992 by Matsumoto *et al*. dealt with 37 cases that had appeared in the literature [1]. This report noted a uniformly poor prognosis, in that there was evidence of systemic disease in 20 cases, including 12 (60%) fatal disseminated infections, involving the skin, lymph nodes, central nervous system, and viscera. A review of the confirmed cases reported after 1992, suggested that the most common sites of *E. dermatitidis* infections of skin, subcutaneous tissue and the central nervous system [8] and less commonly, the eyes, lymph nodes, nails, peritoneum, lungs and internal organs [9–11]. We considered that the present case represented a systemic infection even though the agent was not isolated from blood, but only from the aspiration material. Cultures for *E. dermatitidis* may, however, be negative even when infection is present. The organism grows slowly on primary isolation media and as a result, its recovery might be missed if blood cultures are incubated for short periods. The incubation of cultures should be up to 4 weeks to ensure the recovery of *E. dermatitidis* [2]. Bakare *et al*. demonstrated that the amount of fungi isolated using antibiotic-containing culture media was much lower than that detected using fungal staining methods such as calcoflour [12].

Hepatic fungal infection is a clinical manifestation of the disemination of the etiologic agents in immunnocompromised patients or those with hematologic malignancies [6,13]. Most hepatic fungal microabscesses are caused by *Candida albicans* but this condition has been reported in *Cryptococcus* infections, histoplasmosis, and mucormycosis. The imaging features of all types of fungal disease are similar. Sporadic cases of liver infection by *E. dermatitidis* have also been reported, but to our knowledge an association with granulomatous hepatitis has never been described.

Necrotizing suppurative lymphadenitis caused by *E. dermatitidis* is very rare in humans [1,14,15]. The differential diagnosis of such infections is extensive, but important conditions to exclude are tuberculous adenitis, malignancies (especially lymphoma), infectious mononucleosis, toxoplasmosis, brucellosis, and other fungal diseases. In addition, necrotizing and suppurative lymphadenitis due to *E. dermatitidis* must be distinguished from an other cause of necrosis and suppuration such as visceral leishmaniasis, particularly in areas endemic for this disease, i.e., Turkey. The knowledge of similar diseases in the patient's village and his response to liposomal amphotericin B at the initial phase supports a diagnosis of visceral leishmaniasis. However, the rK39 test which is very sensitive and specific test for visceral leishmaniasis was negative and no extra/intracellular Leishman Donovan bodies on smear was found. In the present case, the definitive diagnosis of *E. dermatitidis* lymphadenitis depends upon isolation of the organism in culture and histological findings. The final identification of the causative organism was achieved by sequencing of rDNA ITS region of the fungus, since the morphological differentiation of *E. dermatitidis* from other species of *Exophiala* can be very difficult.

Alterations in the immune status do influence the progress of infectious diseases; immunocompromised and debilitated hosts are at increased risk of developing systemic phaeohyphomycosis secondary to *E. dermatitidis*. This organism rarely causes infection in immunocompetent persons. A considerable number of cases of cerebral phaeohyphomycosis even in immunocompetent individuals in eastern Asia, with no obvious risk factors have been reported [16,17]. Besides the patient's immunological status, the risk of infection may also depend on the kind of contact and the fungal mass. As other human opportunistic pathogens, fungi such as *E. dermatitidis* are known to colonize the respiratory tract of cystic fibrosis patients and may lead to infection if additional predisposing factors are present [2]. Malnutrition and genetic factors are established risk factor for the colonization of the respiratory tract by *E. dermatitidis*. In the present case, the patient developed invasive *Exophiala* infection despite the fact that he was not immunocompromised. Our patient had no underlying disease or predisposing conditions, and

immunologic tests such as immunglobulin subclasses and complement levels were normal, as well as the functional (nitroblue tetrazolium test).

The susceptibility of the etiologic agents of antifungal agents is another important factor affecting the development and outcome of *Exophiala* infection. Due to the rarity of the disease there have been no large-scale controlled analysis of the antifungal agents. Although there can be exceptions, currently available antifungal agents, including amphotericin B, flucytosine, itraconazole, voriconazole, and posaconazole have yielded clinical improvement in affected individuals [18–20]. The clinical outcome of deep-seated forms of phaeohyphomycosis is dismal, with long-term survival being reported only when complete surgical resection of discrete lesions is possible. Antifungal treatment alone may explain why we were unsuccessful in the treatment of our patient. Surgical excision or debridement with or without antifungal agents may offer the possibility of cure for phaeohyphomycosis due to *Exophiala* species. New antifungal agents and combination treatment may help to improve the management of these infections.

In conclusion, no case of liver and lymph node foci of *E. dermatitidis* in a child has been previously described. *E. dermatitidis* infection, despite cerebral infections are the commonest form of systemic phaeohyphomycosis and it should be suspected among differential diagnosis of lymphogranulomatous infections with hepatic involvement. This case represents one of the few reported instances of systemic infection of *E. dermatitidis*; a Pubmed literature search did not reveal any reports of the clinical isolation of this organism in Turkey.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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