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Case Report

Disseminated, fatal *Trichosporon asahii* infection in a bone marrow transplant recipient

Seyed Amir Ghiasian[1,2], Amir Hossein Maghsood[1,2], Seyed Hossain Mirhendi[2]

[1]Medical Parasitology and Mycology Department, School of Medicine, Hamedan University of Medical Sciences and Health Services, Hamedan; and [2]Medical Parasitology and Mycology Department, School of Health, Tehran University of Medical Sciences and Health Services, Tehran, Iran

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Trichosporon asahii is the most important species regularly isolated from systemic mycoses and shows a predilection for hematogenous dissemination. This report describes the first fatal case of disseminated trichosporonosis caused by *T. asahii* in a patient with familial aplastic anemia (AA). An 11-year-old girl with familial AA received chemoradiotherapy and immunosuppressive therapy for bone marrow transplantation. She was neutropenic and suffered from fever, cough, and severe mouth ulcers. *T. asahii* was repeatedly demonstrated by appropriate morphological and physiological characteristics, i.e., arthroconidium formation, urease activity, and assimilation of carbon and nitrogen compounds. *T. asahii* was found in samples of sputum, nose, and mouth ulcers by direct microscopy and culturing. Furthermore, postmortem histopathology study revealed vast tissue invasion of fungal hyphae characteristic of *Trichosporon* in the lung and liver. Disseminated trichosporonosis should be suspected in immunocompromised patients when a febrile condition does not improve after prolonged treatment with broad-spectrum parenteral antibiotics.

Key words: Bone marrow transplantation, fungemia, mycoses, Iran, *Trichosporon*

Introduction

Members of the genus *Trichosporon* Behrend are medically important agents of white piedra in immunocompetent hosts and disseminated infections in immunocompromised patients [1]. The organisms are found naturally in the soil and may also be a part of the normal human flora in stools or skin. Since Watson and Kallichurum [2] first reported disseminated trichosporonosis infection in a woman with cancer, there have been an increasing number of reports on trichosporonosis [3,4]. According to the new taxonomic revision of the genus *Trichosporon*, seven species, namely, *Trichosporon asahii* (formerly known as *Trichosporon beigelii*), *Trichosporon asteroides*, *Trichosporon cutaneum*, *Trichosporon inkin*, *Trichosporon mucoides*, *Trichosporon ovoides*, and *Trichosporon pullulans* are recognized as medically important species causing superficial, mucosa-associated deep-seated infections [5]. Among these, invasive disease caused by *T. asahii* appears to be increasing in frequency and remains an infection with a mortality rate in excess of 80% [6]. The source of human *Trichosporon* is believed to be the patient's own endogenous mycobiota. Mucosal colonization, which may be enhanced by empirical antibiotic therapy and subsequent seeding of the bloodstream through breaks in the integrity of the surface, is believed to be the early sequence in the pathophysiology of invasive disease. Trichosporonosis is most frequently reported in neutropenic patients, implying that neutrophils are the most important defense cells against this fungus [7].

Although several sporadic cases of disseminated trichosporonosis have been reported in the literature, none has involved disseminated trichosporonosis in Iran. In this communication, the first fatal case of disseminated infection caused by *T. asahii* in a patient

Corresponding author: Seyed Amir Ghiasian, Medical Parasitology and Mycology Department, School of Medicine, Hamedan University of Medical Sciences and Health Services, Hamedan 65155-518, Iran. E-mail: s.a.ghiasian@umsha.ac.ir

with familial aplastic anemia (AA) of the Fanconi type, who had received chemoradiotherapy and immunosuppressive therapy for bone marrow transplantation (BMT) in Iran is reported.

Case Report

The patient was an 11-year-old girl who had been diagnosed with familial AA. She was hospitalized in a BMT ward of Shariati Hospital, Tehran, Iran, and underwent allogeneic BMT after one week of admission. Upon physical examination, she was found to be anemic, severely ill, dyspnoeic at rest, and had persistent fever (39ºC) that was unresponsive to broad-spectrum antibiotic therapy. On graft day 3, blood cultures were positive for *Staphylococcus epidermidis*, but no fungus was recovered from the blood. Chemotherapy was initiated with amikacin, ceftazidime, vancomycin, and amphotericin B. Empirical cover with antibiotics for 1 week showed no beneficial effect, and she remained febrile.

On graft day 14, she showed mouth lesions followed by abscesses in the right jaw angle area. On graft day 27, a molar tooth was extracted and antibiotic therapy continued with nystatin, gentamicin, clindamycin, piperacillin, and rifampin. The lesions deteriorated as sensitive and painful necrotic ulcers with severe hypertrophic gum and her leukocyte count was 100/mm^3; platelet count, 16,000/mm^3; and absolute neutrophil count, 65/mm^3. On graft day 44, she had cough and her chest examination showed decreased breath sounds in the medial aspect of the right chest. On graft day 45, despite continued broad-spectrum antibiotic therapy, her persistent fever spiked to 40°C, her liver enlarged, and a disseminated fungal infection was suspected. Administration of amphotericin B was continued at a dose of 1 mg/kg/day and the patient was referred to the Department of Medical Mycology, School of Public Health, Tehran University of Medical Sciences, for investigation of a possible deep-seated fungal infection. After 1 week, the patient showed no improvement in mouth lesions, and she was managed conservatively on oral nystatin (100,000 U/mL, 5 mL 4 times daily), but her general condition deteriorated progressively and she died after 2 days.

Mycological investigations

Three swab specimens were collected for direct microscopy and cultured for fungus from mouth lesions. Then, three consecutive freshly expectorated sputum specimens were collected from the patient after she had thoroughly rinsed her oropharyngeal cavity with 0.5% Lugol's iodine solution. Direct microscopic examination of swab specimens and potassium hydroxide wet mounts of sputum samples showed mainly budding yeast cells and arthroconidia. The specimens were cultured on routine mycological media including Sabouraud dextrose agar (SDA) supplemented with chloramphenicol and on the same medium containing cycloheximide and incubated at 25°C and 37°C. Three sets of blood cultures were obtained at 37°C on biphasic brain heart infusion broth. All the sputum specimens inoculated on SDA media incubated at 25°C and 37°C yielded numerous white, glabrous, and yeast-like colonies in pure culture that was subcultured and eventually identified as *Trichosporon* sp. The same fungus was also isolated from sore lesions of the mouth on SDA media plates after 72 h of incubation. The blood cultures were all negative for fungus after 72 h of incubation. The species identification of *T. asahii* was based upon observation of prominent diagnostic morphological and physiological characteristics using standard techniques [5]. The isolate was tested for urease activity on Christensen's urea medium as well as carbohydrate and nitrogen assimilation profiles using the API 20C AUX (bioMérieux Vitek, Hazelwood, MO, USA) yeast identification system [8].

Description of the fungus

After 2 days of incubation, rapidly growing, yellowish white to cream colored fungal colonies were found on SDA. Slide culture on SDA revealed rectangular arthroconidia, blastoconidia, and pseudohyphae. The isolate hydrolyzed urea by urease enzyme production and was identified as *T. asahii* based on the above-mentioned characteristics and its carbohydrate assimilation profile obtained with the API 20C AUX yeast identification system [8].

Histologic findings

Histopathologic examination of the lung and liver demonstrated numerous centrally necrotic foci with minimal cellular inflammatory reaction, diffuse intra-alveolar and portal hemorrhage, and infarction. Presentation of blastoconidia, arthroconidia, and septate hyphae in a histologic section supported the diagnosis of invasive *Trichosporon* infection (Fig. 1). Both yeast and hyphal forms were located predominantly in the vessels.

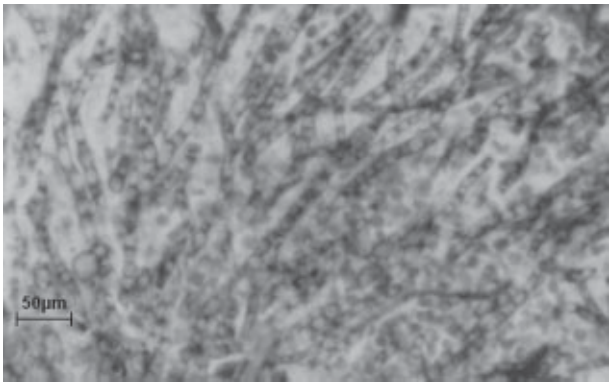

Fig. 1. Liver section demonstrating vast tissue invasion by *Trichosporon asahii* hyphae (hematoxylin and eosin).

Discussion

The clinical manifestations of our case were mouth lesions, fever, and pneumonia that did not respond to conventional antibiotic therapy. This is in accordance with most of the reported cases of trichosporonosis that consistently presented with persistent fever and development of neutropenia [9-11].

The macroscopic and microscopic morphology of *T. asahii* was compatible with the characteristics of the species. In order to further confirm the identity of the *T. asahii* isolate, it was examined for its ability to assimilate 20 different carbohydrates. The etiological role of *T. asahii* in this case was established by repeated demonstration of the fungus in sputum and mouth lesion specimens. However, the blood cultures were all negative for fungus. In clinical practice, *Trichosporon* cannot always be detected in blood samples, thus making it difficult to diagnose disseminated trichosporonosis. In addition, since it is histopathologically similar to disseminated candidiasis, it is often difficult to make a definite diagnosis [12,13]. The presence of arthroconidia is the major microscopic feature that differentiates *Trichosporon* and *Geotrichum* — the two genera that produce abundant arthroconidia — from *Candida*. Furthermore, *Trichosporon* differs from *Candida* in the absence of germ tube production, and from *Geotrichum* by the presence of blastospores [14,15]. According to Tashiro et al, of 203 autopsy patients with malignant disease, disseminated *Trichosporon* spp. infection was found in 7 (7.7%) cases and only two of these infections had been etiologically diagnosed before death occurred. The others had been misdiagnosed as candidiasis on the basis of clinical findings [16]. Girmenia et al, in a retrospective study in Italy, reported that around 50% of all invasive infections of *Trichosporon* spp. have been disseminated (defined as the involvement of two or more organs with or without fungemia) [14]. In this study, the postmortem histopathology revealed vast tissue invasion of *Trichosporon* in the lung and liver and was considered as disseminated infection.

The most favorable therapy for trichosporonosis has yet to be identified. Response to treatment with antifungals is frequently poor in patients with invasive *T. beigelii* infection due to profound immunosuppression and serious underlying conditions, presenting a therapeutic challenge [1]. In this study, despite prophylaxis and treatment with amphotericin B, the patient died. The lack of clinical improvement seen in the patient following administration of antifungals could possibly be attributed to impaired immune function.

In the reported cases, conventional amphotericin B alone or in combination with other antifungal agents was the drug most frequently used in the initial therapy of *Trichosporon* infections. The small number of cases treated with alternative antifungal regimens does not allow any comparative evaluation of the efficacy of these strategies. Several investigators have suggested dual-drug therapy with amphotericin B and flucytosine as an appropriate option for trichosporonosis [17-19]; however, there is no evidence that combination therapy was more effective than single drug regimens. In vitro amphotericin B resistance was detected in a number of *T. asahii* strains isolated from neutropenic patients who had disseminated trichosporonosis refractory to amphotericin B [20]. This explains the extremely high mortality of disseminated trichosporonosis in immunocompromised patients treated with amphotericin B. The results of treatment of disseminated trichosporonosis have been very poor, with about 80% mortality of patients with persistent neutropenia [6]. Although in recent studies, the new triazoles — voriconazole, posaconazole, and ravuconazole — have displayed potent in vitro activity against isolates of *T. asahii* and other *Trichosporon* spp. [21], the resolution of infection in patients with neutropenia is primarily dependent on recovery from granulocytopenia.

Better appreciation of *T. asahii* as a potential pathogen, especially in neutropenic patients, including BMT recipients, and greater familiarity with its laboratory diagnostic aspects will lead to more frequent recognition of disseminated trichosporonosis among clinicians. It is important to note that trichosporonosis may appear similar to disseminated candidiasis both in its clinical and histopathologic appearance and in

the type of patient infected. This case illustrates the need for clinical vigilance in situations unresponsive to conventional antifungal therapy and the importance of seeking laboratory evidence for rare mycotic agents in order to institute appropriate management strategies.

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