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Aspergillosis of Biliary Tract After Liver Transplantation: A Case Report

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Abstract We reported a case of aspergillosis presented as cholangitis in a patient after liver transplantation, even with prophylactic use of fluconazole. The patient had multiple predisposing factors, such as leukocytopenia, immunosuppressive drug therapy. He died 2 days after an exploratory laparotomy was carried out. The histopathologic finding of biopsy specimen from biliary tract was positive for *Aspergillus*. And the pathogen was identified as *Aspergillus flavus* by mycological culture and PCR. The patient was confirmed as a case of Aspergillosis of biliary tract that was responded poorly to fluconazole. This indicated that azole should have been switched to more effective antifungal agents at earlier stage when the patients responded poorly to the original treatment.

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Keywords Aspergillosis · Biliary tract · Organ transplantation · Leukocytopenia

Introduction

Aspergillus species have emerged as important causes of morbidity and mortality in immunocompromised patients [1–3]. *Aspergillus fumigatus* is the most common species recovered from cases of invasive aspergillosis [4]. The next most commonly recovered species are *Aspergillus flavus, Aspergillus niger,* and *Aspergillus terreus* [5]. Fungal infections of the biliary tract are uncommon and usually caused by *Candida* spp., which have been reported to cause biliary obstruction and cholangitis in the immunocompetent host [6–8]. Here, we report a case of Aspergillosis of biliary tract caused by *A. flavus* in a patient after liver transplantation.

Case Report

History

A 55-year-old man was admitted to the hospital with a 2-year history of recurrent episodes of debilitation, loss of appetite, epigastric discomfort, and dark urine and was diagnosed as chronic viral hepatitis type B. Two months prior to the admission, he developed hepatic encephalopathy and hepatorenal syndrome.

During the admission, his condition deteriorated because of gastric perforation. The patient recovered after supportive treatment and antibiotics. But the liver function worsened consequently. After 3 months admission, he was referred for liver transplantation. The operation was successful. He was continuously treated with mycophenolate moretil, prednisone, sulperazone, vancomycin, fluconazole as immunosuppressive and prophylactic drugs. The renal and hepatic function recovered gradually. After 24 days transplantation, the patient developed high fever, stomachache, abdominal tenderness abruptly. Diagnostic puncture showed bleeding in abdominal cavity. His conditions deteriorated quickly even after using a hemostatics and a transfusion treatment. An emergency exploratory laparotomy was then carried out. The patient died 2 days later.

Clinical Findings

The cavity was filled with about 700 ml bloody ascites. A large deposit of sludged blood appeared below the right lobe of liver with a slight odor. Transplanted liver was in situ with slight cholestasis. The bleeding was from porta hepatis that had a vomica with yellow staining after debriding the hematocele and adhesions. The common bile duct (2 cm upper and 1.5 cm lower anastomotic stoma) showed caseous necrosis, and the bile was leaking from the necrosis site. Small vessels around the biliary fistula showed active bleeding. The hepatic artery stoma, portal vein stoma, and caval vein stoma of transplantation operation were normal. A piece of common bile duct was biopsied for histopathological, mycological, and microbiological examinations. Ascites and bile were also sent for mycological and microbiological tests.

Investigations

Histopathologic Findings

There were 3–6 μm wide septate hyphae that typically branch at acute angles. A morphological yellowish mass around the hyphae were bile (Fig. 1).

Mycological Findings

Tissue from the bile was plated on Sabouraud dextrose agar with chloramphenicol and incubated at 25°. A filamentous fungus was recovered. Then, the colony was transferred to Czapek agar and malt extract agar (MEA), which was cultured at 25°. The strain showed good growth. Colonies on Czapek agar at 25° attained a diameter of 3 cm within 7 days and consisted of a dense felt of yellow–green conidiophores. Colonies on MEA grew faster otherwise, their characteristics were similar to those of colonies grown on Czapek agar.

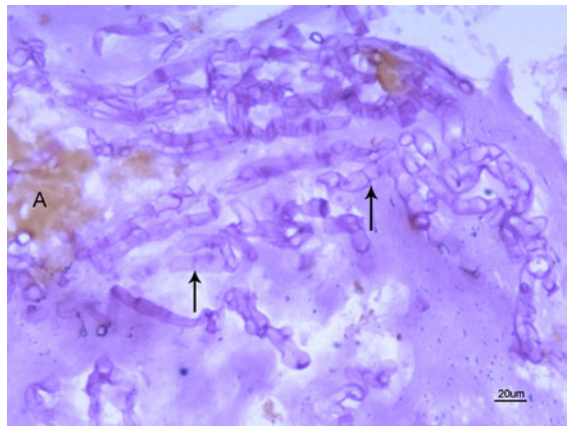

Fig. 1 Histopathology of the biopsy tissue. *A* bile juice; *arrows* showed the septate hyphae (PAS stain, ×400)

Identification of the Pathogen

Mycological Morphology The conidial heads typically radiated to nearly globose. There were two kinds of vesicles which were nearly globose, fertile over almost the entire surface. The big one was up to 22.5–50 μm in diameter with sterigmata in two series, and the small one with a single role of phialides, 12.5–35 μm in diameter. Conidia were globose to subglobose, 3.0–5.0 μm (Figs. 2, 3).

Sequence analysis Culture and collection of mycelium were carried out as previously described [9]. For sequence analysis, DNA was extracted from pathogen and identified using methods previously described [9, 10]. The 18S rDNA and β-tublin sequences were amplified by PCR with primer pair (18S rDNA forward primer: 5′-TCCGTAGGTG AACCTGCGG-3′ and reverse primer: 5′-TCCTC CGCTTATTGATATGC-3′; β-tublin gene forward primer: 5′-GGTAACCAAATCGGTGCTGCTTTC-3′

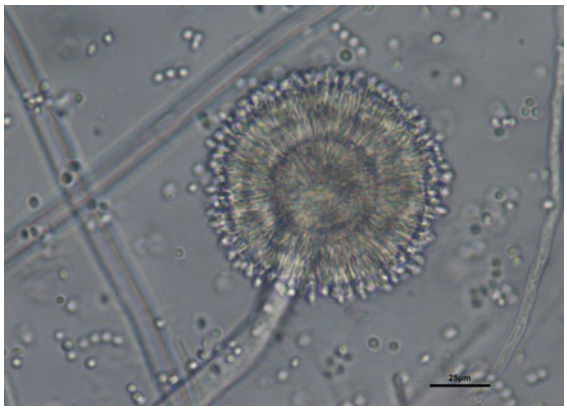

Fig. 2 The big conidia head, with sterigmata in two series (×400)

Fig. 3 The small one with a single role of phialides (×400)

and reverse primer: 5′-ACCCTCAGTGTAGTGAC CCTTGGC-3′). Sequencing of the PCR products were performed by the service of Sangon Company Ltd (Shanghai, China). The sequences obtained were submitted to GenBank for homology search with Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi). After homology searching against the GenBank or the proprietary fungal DNA databases, the sequences of pathogen were found to share 100% similarity with that of *Aspergillus flavus* in 595-bp 18S rDNA and 545-bpβ-tublin gene.

Micriobiological Findings

The Gram stanining and microbiological culture both were negative (biopsy tissue, ascites, and bile).

Laboratory Evaluation

The routine blood test showed that the white blood cell counts were significantly reduced(less than 2×10^9). The biochemistrical tests showed remarkable elevations of total and direct bilirubin (up to 715 and 368.5 μmol/l), total bile acids levels (more than 100 μmol/l) above baseline at day before transplantation.

Discussion

According to the clinical manifestation and mycological findings, the patient was diagnosed as a case of Aspergillosis of biliary tract caused by *Aspergillus flavus. A. flavus* is ubiquitous in nature and can cause a variety of aspergilloses, including central nervous system infections [11]. It is the second most common species, produces aflatoxin, may be less susceptible to polyenes [12]. But the biliary is seldom infected by this pathogen. Steven H reported a case of cholangitis caused by *Aspergillus terrus* [13]. It was the first report of *Aspergillus* cholangitis with liver failure. As *Aspergillus* species can be readily found in the environment, invasive aspergillosis is widely believed to occur as a consequence of exogenous acquisition of the conidia (spores) of the species [14]. The most common route of transmission of *Aspergillus* infection is the airborne route. *Aspergillus* conidia are resilient and may survive for long periods in fomites (any substance that can absorb, retain, and transport infectious species, e.g., woolen clothes or bedding) [15]. However, the sources of *Aspergillus* may be broader than have traditionally been thought, as waterborne transmission of *Aspergillus* conidia through contaminated aerosols has been suggested [16]. The infestation route in this case was unclear. It may possibly include contaminated operation procedure, drainage tube, or ascending pathogen from gastrointestinal tract.

Invasive aspergillosis is extremely uncommon in immunocompetent hosts, although infection in apparently normal hosts can occur. The frequency and severity of invasive fungal infections in immunocompromised patients have increased steadily over the past two decades with the growing population of patients undergoing transplantation and the persistent challenges in preventing, diagnosing, and treating

these infections [1]. Mortality due to documented invasive aspergillosis approaches 80% to 100% in high-risk patients, including those with underlying hematologic malignancy or bone marrow or solid organ transplantation and may be related to several factors, including diagnostic and therapeutic inadequacies [17–19]. And in our case, the leukocytopenia also was an important predisposing factor. The changes in epidemiology of invasive aspergillosis may also be the result of growing awareness of aspergillosis among clinicians, the introduction of noninvasive diagnostic tools, and improved microbiological laboratory techniques.

Although the most frequent fungal infections are caused by *Candida* spp., infections due to *Aspergillus* spp. have the worst prognosis [20, 21]. Invasive aspergillosis has been reported in 1.5–6.5% of liver transplant patients [22–24], although other authors have reported higher frequencies [25]. A nationwide study carried out by the Spanish Study Group for Infections in the Transplant Patient documented the development of invasive aspergillosis in 2.8% of 1,719 liver transplant recipients, with a case fatality rate of 86% [26].

In China, fluconazole is the most extensively used prophylactically to trea fungal infection, such as in our case. The agent is effective against *Candida* species, *Cryptococcus neoformans*, and *Coccidioides immitis* but appears to be ineffective against other molds, including *Aspergillus* species and *Zygomycetes* [12]. As to this opinion, fluconazole prophylaxis significantly reduces invasive fungal infections in liver transplant recipients in the early postoperative period [25]. But prevention of infections caused by invasive molds remains a great challenge.

Treatment failure with currently available antifungal medication in patients with invasive aspergillosis has been reported to be at least 50% [18, 19]. New antifungal therapies with activity against *Aspergillus* have been developed, including lipid formulations of amphotericin B, the broad-spectrum triazoles (voriconazole, posaconazole, and ravuconazole), and the echinocandins (caspofungin, micafungin, and anidulafungin). But Fortun et al. [27] believed that the administration of L-AmpB in high-risk patients is independently associated with a reduction of invasive fungal infection. The successful therapy depends not only an early diagnosis—which is often difficult to establish—but even more importantly, on reversal of underlying host immune defects, such as neutropenia or high-dose immunosuppressive therapy [17].

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