Disseminated Microsporidiosis in a Pancreas/Kidney Transplant Recipient

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• Human microsporidiosis has been described most commonly in patients with acquired immunodeficiency syndrome and only rarely in those with other forms of immunosuppression. Only 11 cases of microsporidiosis have been reported previously in solid transplant recipients. To our knowledge, this is the first report to describe a case of microsporidiosis in a pancreas/kidney transplant recipient in whom multi–organ system dissemination was observed. This infection was not detected until postmortem examination of stained tissue sections revealed microsporidian spores that were identified as *Encephalitozoon* species by transmission electron microscopy. It is suspected that leakage from the duodenal anastomosis to the bladder may have contributed to the dissemination of this infection.

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Microsporidia are spore-forming, obligate, intracellular parasites that are ubiquitous and infect invertebrates as well as all classes of vertebrates.[1] Of the genera associated with human infections, predominantly reported in immunosuppressed patients with acquired immunodeficiency syndrome (AIDS), *Enterocytozoon* and *Encephalitozoon* are the most common. Human immunodeficiency virus (HIV) coinfection with microsporidia is characterized most commonly by symptoms of cachexia, chronic diarrhea, and cholangitis, caused by *Enterocytozoon bieneusi*.[1–3]

Despite a high frequency of microsporidiosis reported in patients infected with HIV, few cases have been described in those without HIV infection.[1,2] However, the increased susceptibility of persons with other forms of immunosuppression, particularly organ transplant recipients, has been noted recently, and 10 well-described cases of microsporidiosis in solid organ transplant recipients[4–12] and 1 case in a bone morrow transplant recipient[13] have been documented to date (Table). Infections with *E bieneusi* localized to the gastrointestinal tract were identified in 7 of these cases[6–11]; however, more invasive disease has been reported for *Encephalitozoon* species.[4,5] We report a case of microsporidiosis with multi–organ system dissemination in a pancreas/kidney transplant recipient.

REPORT OF A CASE

A 43-year-old man received a simultaneous, cadaveric, pancreas/kidney transplant with bladder drainage of the pancreas allograft. The patient, who was HIV seronegative, had a history of type 1 diabetes mellitus, complicated by end-stage renal disease. He received daclizumab preoperatively, followed by maintenance therapy with FK506, mycophenolate mofetil, and prednisone. His posttransplant course was complicated at day 40 by staphylococcal cystitis and a neurogenic bladder. The infection was successfully treated with antibiotics and bladder decompression. On day 57 posttransplant, he was admitted to the hospital with a fever of 37.9°C (100.3°F), left lower quadrant abdominal pain over the site of his renal allograft, anuria for 1 day, and diarrhea. Laboratory data included the following values: serum urea nitrogen, 45 mg/dL (16.0 mmol/L); creatinine, 3.3 mg/dL (292 μmol/L); amylase, 669 U/L; and alkaline phosphatase, 204 U/L. Urinalysis showed 6 to 12 white blood cells per high-power field. Computed tomographic scan revealed the presence of a fluid collection in the abdominal right lower quadrant. Yeast was reported from the Gram stain of this aspirate. Fluconazole was begun, but fungal cultures were negative. In addition, the concentration of amylase in this fluid was greater than 13 000 U/mL. Despite therapy, his fever and pain progressed, and an exploratory laparotomy was performed on posttransplant day 60 to investigate the possibility of fungal peritonitis. A small leak at the duodenal anastomosis to the bladder was repaired, but no evidence of intraperitoneal inflammation was observed, and cultures were negative for fungal or bacterial infection. Serum creatinine levels increased to 3.7 mg/dL (327.1 μmol/L) 1 week later, and renal allograft biopsy revealed evidence of FK506 toxicity only. Pancreatic biopsy was also performed for a progressive increase in serum amylase, but showed no evidence of acute rejection. To minimize FK506 toxicity, intravenous basiliximab was given, and the FK506 dose was reduced. Mycophenolate mofetil was replaced by azathioprine at the same time.

The patient's postlaparotomy course was complicated by pneumonia, urinary tract infection, and presumed chemical peritonitis. Chest radiography showed bilateral infiltrates and a large amount of pleural effusion. A sputum culture was positive for *Aspergillus fumigatus*, and urinary culture was positive for vancomycin-resistant *Enterococcus faecalis*. Despite extensive antimicrobial therapy, the patient remained febrile. He developed increasing dyspnea, requiring intubation and mechanical ventilation. In addition, upper gastrointestinal bleeding necessitated blood transfusions. Upper gastrointestinal endoscopy revealed 3

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Microsporidiosis in Transplant Recipients				
Year of Report	Age, y/ Sex	Transplant Type	Identification	Reference
2003	43/M	Pancreas/kidney	*Encephalitozoon* species	Present case
2002	43/F	Kidney	*Encephalitozoon cuniculi*	Mohindra et al[4]
2001	39/M	Kidney	*Encephalitozoon* species	Latib et al[5]
2001	36/F	Liver	*Enterocytozoon bieneusi*	Goetz et al[6]
2001	36/F	Liver	*E bieneusi*	Sing et al[7]
2000	38/F	Kidney	*E bieneusi*	Metge et al[8]
1999	48/M	Heart	*E bieneusi*	Gumbo et al[10]
1999	46/M	Kidney	*E bieneusi*	Guerard et al[9]
1999	24/M	Kidney	*E bieneusi*	Guerard et al[9]
1997	27/F	Bone marrow	Not determined	Kelkar et al[13]
1996	48/M	Heart/lung	*E bieneusi*	Rabodonirina et al[11]
1995	48/F	Liver	Not determined	Sax et al[12]

gastric ulcers without evidence of cytomegalovirus infection. The patient also required hemodialysis for acute renal failure. Although there was intermittent response to therapy and improvement of the patient's clinical condition, he eventually developed persistent hypotension and, shortly thereafter, acute liver failure with markedly elevated levels of aspartate aminotransferase (3008 U/L) and total bilirubin (31 mg/dL [530.1 μmol/L]). A clinical picture of multiorgan failure from presumed sepsis was apparent, and despite continued intensive care, the patient died on posttransplant day 81.

PATHOLOGIC EXAMINATION

Postmortem examination revealed marked chronic peritonitis, and tissue Gram stain (Accustain Gram Stain, Sigma, St Louis, Mo) revealed an extensive microsporidian infection (Figure 1). Identification of the microsporidia was accomplished by transmission electron microscopy of liver and omentum specimens, in which spores revealed a single row of 5 to 6 turns of the coiled polar tube, characteristic of *Encephalitozoon* species (Figure 2).[1–3] Presence of parasitophorous vacuoles, an identifying characteristic for *Encephalitozoon intestinalis* speciation, could not be determined because of postmortem autolysis. Multiple attempts at further identification using polymerase chain reaction with pan-microsporidian as well as *Encephalitozoon* species–specific primers with DNA extracted from paraffin-embedded tissues were negative.[2]

Dissemination of the organism was seen most prominently in the parenchyma of the liver, but also to a lesser extent in native kidneys and the transplant kidney, heart, brain, gastroesophageal junction, diaphragm, and omentum. The omentum and diaphragm showed chronic fibrous and acute and chronic inflammation in association with infection, whereas the small and large bowel tissues demonstrated acute and chronic serositis. Centriacinar necrosis occurred in the liver, and although no organisms were observed in the gallbladder, a chronic cholecystitis was detected. A large nonperforating ulcer was located at the prepyloric stomach with chronic inflammation, without evidence of microsporidian infection in the mucosa. A focal infection with *Aspergillus fumigatus* was noted in the right lower lobe of the lung. However, this infection was limited to this site and did not account for the patient's symptoms of sepsis.

In summary, the autopsy results indicated that the patient died of an opportunistic *Encephalitozoon* species infection that disseminated after infection of the peritoneal cavity, perhaps through leakage at his duodenal-vesicular anastomoses.

COMMENTS

Microsporidia have been reported with a wide range of clinical manifestations, including intestinal, pulmonary,

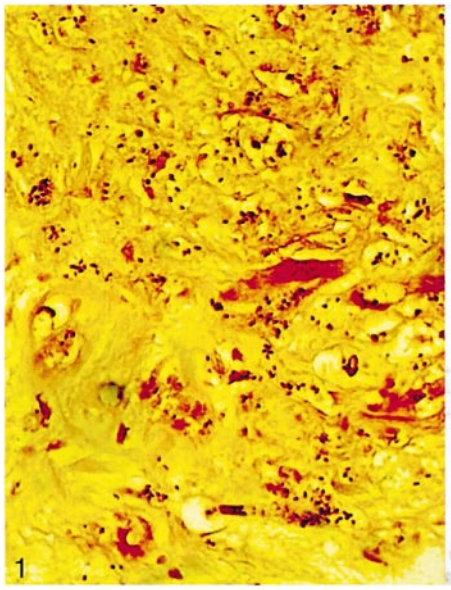

Figure 1. *Mature spores of* Encephalitozoon *species from liver tissue (Gram, original magnification ×125).*

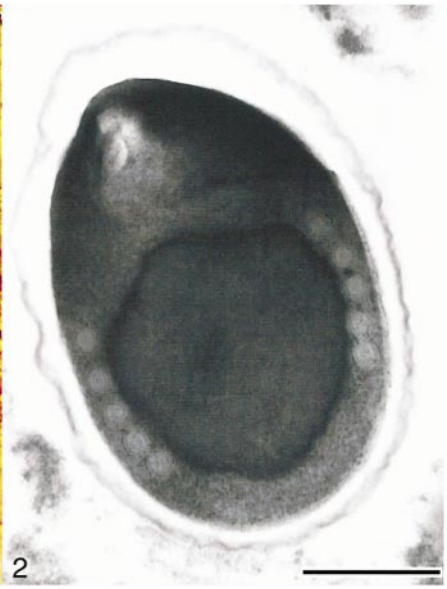

Figure 2. *Transmission electron micrograph of a mature spore of* Encephalitozoon *species from the liver. The 5 paired profiles of the coiled polar tube are clearly visible (bar = 0.5 μm; original magnification ×46 000).*

ocular, muscular, and renal disease.[1–3] In patients with advanced HIV and microsporidia coinfection, gastrointestinal symptoms are most common and 2 species, *E bieneusi* and *E intestinalis*, have been identified most frequently. *Encephalitozoon* species are less frequently identified in AIDS patients in comparison to the *Enterocytozoon* species, but are characterized by their potential to disseminate, in particular to the urinary tract, a feature that has not been seen with *E bieneusi* infections. The *Encephalitozoon* species have overlapping clinical symptoms including *E intestinalis*, which is often associated with chronic diarrhea and cholangitis, *E hellem*, which is associated with conjunctivitis, and *E cuniculi*, which demonstrates systemic dissemination.[14]

When the *Encephalitozoon* species have been encountered as pathogens in AIDS patients and transplant recipients, their characteristic tendency toward more invasive disease has been evident.[4,5,14] Since these cases are rarely reported, whether they signal the potential for more serious microsporidian infections in the population of transplant recipient patients remains to be determined. Their wide geographic distribution and the occurrence of self-limiting disease in immunocompetent individuals typify the microsporidia as pathogens poised for an emergence that is dependent on the immunological status of the host.

Detection of microsporidian infections is difficult. For example, because of unavailability of specimen or slides, it could not be determined whether in the present case the ''yeasts'' identified in the Gram stain of peritoneal fluid obtained at the time of exploratory laparotomy were, in fact, microsporidia. The much smaller size of microsporidia should have been an obvious identifying characteristic; however, in the absence of awareness for the potential of microsporidia to cause invasive disease in immunosuppressed transplant recipients, such an error would be more likely. Detection of microsporidia can be optimized by using a chromotrope-based stain, a modification of the trichrome stain, to search for microsporidian spores in clinical specimens (eg, stool or urine) and tissue Gram stain (eg, Brown-Brenn for paraffin-embedded tissues), as used in the present investigation.[1] Identification in human infections can be suggested by determining spore size by light microscopy; for example, *E bieneusi* measures 0.5 × 1.5 μm, whereas *Encephalitozoon* species measure 1.0 × 2.5 μm. However, transmission electron microscopy remains the gold standard.[2] Molecular diagnosis can also be used to rapidly detect and identify microsporidia.[2] As the present case demonstrated, however, the sensitivity of this method in autopsy tissue, including paraffin-embedded tissue, may be diminished, as previously reported.[15]

As awareness for the possibility of microsporidiosis increases, it is possible that more cases will be reported and the true prevalence of this opportunistic pathogen will be better defined.

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