

CASE REPORT

ELECTRON MICROSCOPIC DIAGNOSIS OF INTESTINAL MICROSPORIDIOSIS IN THAILAND

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Microsporidia are unicellular parasites, which are classified in the phylum Microspora. The organisms are obligatory intracytoplasmic protozoa with spores containing unique coiled polar filament with extrusion apparatus for injecting infective sporoplasm into a host cell (Shadduck and Greeley, 1989). Intestinal microsporidiosis has been recognized as the most common form of microsporidia infection in human, especially in patients with acquired immunodeficiency syndrome (AIDS). The majority of cases were caused by *Enterocytozoon bieneusi* (Cali, 1991; Orenstein, 1991; Curry and Canning, 1993). The organism was first documented by Modigliani *et al* (1985) and subsequently reported by Dobbins and Weinstein in 1985. Electron microscopic study of the parasite was described and named by Desportes *et al* (1985) in the same year. The parasite can also infect extra-intestinal tissues including biliary tract, gall bladder, liver and pancreatic duct (Beaugerie *et al*, 1992; Pol *et al*, 1993; Schwartz *et al*, 1994). *Septata intestinalis*, another recently recognized microsporidia, is responsible for intestinal infection as well as disseminated microsporidiosis (Orenstein *et al*, 1992; Cali *et al*, 1993; Molina *et al*, 1995). Although the two species produce indistinguishable clinical presentations in terms of diarrhea and weight loss, their drug-susceptibility patterns are somewhat different. Metronidazole has been found to be beneficial in most cases of *E. bieneusi* (Eeftinck Schattenkerk *et al*, 1991) while albendazole has been more effective in eradicating *S. intestinalis* infection (Dore *et al*, 1995; Molina *et al*, 1995). Thus, the correct species identification is clinically essential. In Thailand, intestinal microsporidiosis was first described in an human immunodeficiency virus (HIV) infected patient, who presented with watery diarrhea (Pitisuttithum *et al*, 1995). Another case was detected by Morakote *et al* (1995). In both cases, the diagnoses were made only by staining methods,

no confirmatory electron microscopic study was available. To our knowledge, this is the first report in Thailand to detect and identify the species of microsporidia by using transmission electron microscopy (TEM).

Case 1

A 35-year-old Thai male, with a history of intravenous drug use and exposure to prostitute, was found to have positive serology for HIV infection. He presented with two-year history of episodic watery diarrhea and had been passing 5-6 times per day. He was cachexic and had lost 10 kg in weight. His illness was associated with abdominal pain and intermittent fever. Pruritic papular eruptions were observed on both extremities but other findings were unremarkable. Examinations of stools were negative for parasites except for microsporidia which was confirmed to be *E. bieneusi* by their characteristic internal coiled polar filament under transmission electron microscopic study (Fig 1). Sigmoidoscopy revealed a large irregular-shaped anal ulcer with histologic feature of squamous papilloma, this finding was compatible with herpes simplex virus infection. He was treated with metronidazole (1,200 mg daily) and norfloxacin (800 mg daily) orally for 7 days. Although acyclovir was prescribed as an additional drug, there was no clinical improvement.

Case 2

A 39-year-old Thai male, living in Bangkok, presented with watery diarrhea and mild abdominal pain. He had a past history of contacting prostitute and was later found to be HIV positive. During the past 2 months he experienced three to five bowel movements per day and lost 14 kg in weight. Physical examination disclosed right supraclavicular lymphadenopathy but no hepatosplenomegaly. Stool investigation for parasite showed *Cryptosporidium* oocysts.

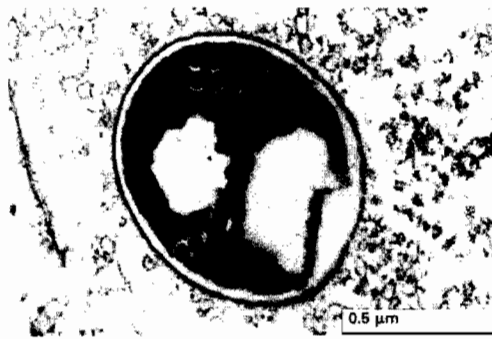


Fig 1 – Case 1. Transmission electron micrograph of *E. bienewsi* spore showing characteristic cross sections of internal coiled polar filament, an electron-lucent endospore layer and a dense outer coat.

sporidium parvum oocysts and microsporidia spores, stained by modified Ziehl-Neelsen and modified trichrome techniques respectively. The fecal specimen was processed for TEM to confirm and specify the microsporidia species which was found to be *E. bienewsi* (Fig 2). The pus aspirate of supraclavicular lymph node was positive for *Mycobacterium tuberculosis* which was properly treated with conventional antituberculous drugs. Oral metronidazole (1,200 mg daily) and norfloxacin (800 mg daily) were prescribed for diarrhea for 7 days without improvement. The patient was then treated with albendazole 800 mg per day for the subsequent 2 weeks. Partial improvement of diarrhea was observed 3 weeks later and he gained 2 kg in weight.

Intestinal microsporidiosis, an emerging opportunistic infection, has been recognized as the cause of chronic diarrhea in patients with AIDS (Shad-



Fig 2 – Case 2. Transmission electron micrograph of *E. bienewsi* spore demonstrating tangential sections of double rows of polar tubule.

duck and Greeley, 1989; Kotler *et al*, 1990; Greenson *et al*, 1991). The clinical presentations of patients with this disease are similar to those with intestinal cryptosporidiosis or isosporiosis (Orenstein *et al*, 1990). The patients often suffer from long-lasting diarrhea with weight loss. The diarrhea is usually persistent and may be 3-10 times a day. The stools are generally watery, not bloody or mucous, and several liters per day may be observed.

This can lead to dehydration, extreme wasting, malabsorption, malnutrition, and may be life-threatening. The other manifestations include nausea, vomiting, anorexia, abdominal pain and often no fever (Canning and Hollister, 1990; Orenstein *et al*, 1990; Eftinck Schattenkerk *et al*, 1991; Molina *et al*, 1993). There are many procedures for detecting enteric microsporidia infection. Light microscopic examination of microsporidia spores in fecal specimens using staining technique is non-invasive and practical for routine diagnosis (Weber *et al*, 1992; Ryan *et al*, 1993; Kokoskin *et al*, 1994). The organism appears to be oval in shape with diagnostic transverse or oblique band representing the polar filament. However, the parasite cannot be identified to the level of the genus and species by such staining method. TEM remains to be the essential method for species identification (Molina *et al*, 1993; DeGirolami *et al*, 1995).

Both cases with HIV infection had attended the Outpatients diarrhea clinic at Siriraj Hospital with the complaints of chronic diarrhea and weight loss. They had been diagnosed as intestinal microsporidiosis by the detection of stained spores under light microscopy. Multiple opportunistic infections are commonly detected in AIDS patients (Modigliani *et al*, 1985; Orenstein, 1991), as well as in these two cases. The first case had a coinfection with herpes simplex virus of anus and the second case was found to be coinfecting with intestinal *Cryptosporidium parvum* and tuberculosis of supraclavicular lymph nodes. Simultaneous intestinal infection of microsporidia and *C. parvum* was not uncommon and had been reported in approximately 30 % of HIV-infected patients (Weber *et al*, 1993; Garcia *et al*, 1994), thus the associated potential pathogens of diarrhea should be considered in these patients.

Transmission electron microscopic studies of fecal specimens in both cases confirmed the presence of microsporidia spores measuring 0.83-1.09 x 0.71-0.82 μ. The characteristic features included

electron-dense exospore, electron-lucent endospore, and cross section of four to seven coils of the polar tubule arranged in double rows. These electron microscopic findings were consistent with those described by van Gool *et al* (1990), Orenstein (1991), and Weber *et al* (1992) and were identified as *E. bienewisi*.

The two patients were treated orally with 1,200 mg per day of metronidazole and 800 mg per day of norfloxacin for 7 days without significant improvement. It may be that given dosages were probably too low to be effective for both microsporidiosis and cryptosporidiosis. The nature of the microsporidia spores and their intracellular habitat are usually rather resistant and difficult to be eradicated (Curry and Canning, 1993). In addition, oral therapy may not be suitable in the cases of enteric diseases with abnormal intestinal absorption (Molina *et al*, 1993). However, in the second case had been given high dose albendazole and the diarrhea had partially resolved. This may imply that albendazole might be potentially effective treatment to alleviate the diarrheal symptom.

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REFERENCES

Beaugerie L, Teilhac MF, Deluol AM, *et al*. Cholangiopathy associated with microsporidia infection of the common bile duct mucosa in a patient with HIV infection. *Ann Intern Med* 1992; 117: 401-2.

Cali A. General microsporidian features and recent findings on AIDS isolates. *J Protozool* 1991; 38: 625-30.

Cali A, Kotler DP, Orenstein JM. *Septata intestinalis* n. g., n. sp., an intestinal microsporidian associated with chronic diarrhea and dissemination. *J Euk Microbiol* 1993; 40: 101-12.

Canning EU, Hollister WS. *Enterocytozoon bienewisi* (Microspora): prevalence and pathogenicity in AIDS patients. *Trans R Soc Trop Med Hyg* 1990; 84: 181-6.

Curry A, Canning EU. Human microsporidiosis. *J Infect* 1993; 27: 229-36.

DeGirolami PC, Ezratty CR, Desai G, *et al*. Diagnosis of intestinal microsporidiosis by examination of stool and duodenal aspirate with Weber's modified trichrome and Uvitex 2B stains. *J Clin Microbiol* 1995; 33: 805-10.

Desportes I, le Charpentier Y, Galian A, *et al*. Occurrence of a new microsporidian: *Enterocytozoon bienewisi* n. g., n. sp., in the enterocytes of a human patient with AIDS. *J Protozool* 1985; 32: 250-4.

Dobbins III WO, Weinstein WM. Electron microscopy of the intestine and rectum in acquired immunodeficiency syndrome. *Gastroenterology* 1985; 88: 738-49.

Dore GJ, Marriott DJ, Hing MC, Harkness JL, Field AS. Disseminated microsporidiosis due to *Septata intestinalis* in nine patients infected with the human immunodeficiency virus: response to therapy with albendazole. *Clin Infect Dis* 1995; 21: 70-6.

Eeftinck Schattenkerk JKM, van Gool T, van Ketel RJ, *et al*. Clinical significance of small-intestinal microsporidiosis in HIV-1-infected individuals. *Lancet* 1991; 337: 895-8.

Garcia LS, Shimizu RY, Bruckner DA. Detection of microsporidian spores in fecal specimens from patients diagnosed with cryptosporidiosis. *J Clin Microbiol* 1994; 32: 1739-41.

Greenson JK, Belitsos PC, Yardley JH, Bartlett JG. AIDS enteropathy: occult enteric infections and duodenal mucosal alterations in chronic diarrhea. *Ann Intern Med* 1991; 114: 366-72.

Kokoskin E, Gyorkos TW, Camus A, Cedilotte L, Purtil T, Ward B. Modified technique for efficient detection of microsporidia. *J Clin Microbiol* 1994; 32: 1074-5.

Kotler DP, Francisco A, Clayton F, Scholes JV, Orenstein JM. Small intestinal injury and parasitic diseases in AIDS. *Ann Intern Med* 1990; 113: 444-9.

Modigliani R, Bories C, le Charpentier Y, *et al*. Diarrhoea and malabsorption in acquired immunodeficiency syndrome: a study of four cases with special emphasis on opportunistic protozoan infestations. *Gut* 1985; 26: 179-87.

Molina JM, Oksenhendler E, Beauvais B, *et al*. Disseminated microsporidiosis due to *Septata intestinalis* in patients with AIDS: clinical features and response to albendazole therapy. *J Infect Dis* 1995; 171: 245-9.

Molina JM, Sarfati C, Beauvais B, *et al*. Intestinal microsporidiosis in human immunodeficiency virus-infected patients with chronic unexplained diarrhea: prevalence and clinical and biologic features. *J Infect Dis* 1993; 167: 217-21.

- Morakote N, Siriprasert P, Piangjai S, Vitayasai P, Tookyang B, Uparanukraw P. *Microsporidium* and *Cyclospora* in human stools in Chiang Mai, Thailand. *Southeast Asian J Trop Med Public Health* 1995; 26: 799-800.
- Orenstein JM. Microsporidiosis in the acquired immunodeficiency syndrome. *J Parasitol* 1991; 77: 834-64.
- Orenstein JM, Chiang J, Steinberg W, Smith PD, Rotterdam H, Kotler DP. Intestinal microsporidiosis as a cause of diarrhea in human immunodeficiency virus-infected patients: a report of 20 cases. *Hum Pathol* 1990; 21: 475-81.
- Orenstein JM, Dieterich DT, Kotler DP. Systemic dissemination by a newly recognized intestinal microsporidia species in AIDS. *AIDS* 1992; 6: 1143-50.
- Pitisuttithum P, Phiboonnakit B, Chindanond D, Punpoonwong B, Leelasuphasri S, Vanijanond S. Intestinal microsporidiosis : first reported case in Thailand. *Southeast Asian J Trop Med Public Health* 1995; 26: 378-80.
- PoI S, Romana CA, Richard S, *et al.* Microsporidia infection in patients with the human immunodeficiency virus and unexplained cholangitis. *N Engl J Med* 1993; 328: 95-9.
- Ryan NJ, Sutherland G, Coughlan K, *et al.* A new trichrome-blue stain for detection of microsporidial species in urine, stool, and nasopharyngeal specimens. *J Clin Microbiol* 1993; 31: 3264-9.
- Schwartz DA, Bryan RT, Weber R, Visvesvara GS. Microsporidiosis in HIV positive patients : current methods for diagnosis using biopsy, cytologic, ultrastructural, immunological, and tissue culture techniques. *Folia Parasitologica* 1994; 41: 101-9.
- Shadduck JA, Greeley E. Microsporidia and human infections. *Clin Microbiol Rev* 1989; 2: 158-65.
- Van Gool T, Hollister WS, Eeftinck Schattenkerk J, *et al.* Diagnosis of *Enterocytozoon bieneusi* microsporidiosis in AIDS patients by recovery of spores from faeces. *Lancet* 1990; 336: 697-8.
- Weber R, Bryan RT, Owen RL, *et al.* Improved light-microscopical detection of microsporidia spores in stool and duodenal aspirates. *N Engl J Med* 1992; 326: 161-6.
- Weber R, Sauer B, Luthy R, Nadal D. Intestinal coinfection with *Enterocytozoon bieneusi* and *Cryptosporidium* in a human immunodeficiency virus-infected child with chronic diarrhea. *Clin Infect Dis* 1993; 17: 480-3.