INTESTINAL MICROSPORIDIOSIS IN HIV INFECTED PATIENTS WITH CHRONIC DIARRHEA IN THAILAND

Darawan Wanachiwanawin[1], Sathaporn Manatsathit[2], Punpob Lertlaituan[1],
Kleophant Thakerngpol[3] and Parvinee Suwanagoo gool[3]

[1]Departments of Parasitology, [2]Medicine and [3]Pathology, Faculty of Medicine Siriraj Hospital,
Mahidol University, Bangkok 10700, Thailand

Abstract. Microsporidia have been recognized as emerging opportunistic agents affecting multiple organs. Intestinal microsporidiosis caused by *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* is a common disease which is associated with gastrointestinal symptoms, particularly in AIDS patients. So far, information on the frequency of this enteric disease in Thailand is not available. Therefore, the present study was undertaken to investigate the prevalence of intestinal microsporidiosis in HIV infected persons with chronic diarrhea. From 1995 to 1996, multiple diarrheal stool specimens were received and examined for the presence of the organism using Weber's modified trichrome staining method and transmission electron microscopy for confirmation. Twenty-two of 66 patients (33.3%) were positive for microsporidia which appeared as pink-red spores of 0.8-1.2 x 0.7-0.9 μm with the characteristic transverse or oblique band representing the coiled polar filament. Clinical features of these patients included chronic diarrhea (100%), weight loss (100%), abdominal pain (77%), fever (36%), vomiting (36%) and anorexia (18%). Transmission electron microscopic examination of fecal specimens from the 22 patients with positive staining results revealed *E. bieneusi* in 18 cases.

INTRODUCTION

Chronic diarrhea is a common problem in human immunodeficiency virus (HIV) infected patients and is often accompanied by severe weight loss. Intestinal microsporidiosis caused by microsporidia plays an important role in the etiology of about 10-50% of unexplained chronic diarrhea (Orenstein *et al*, 1990; Cali, 1991 ; Weber *et al*, 1992 ; Molina *et al*, 1993). The two species of microsporidia identified as enteric pathogens in human are *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*. The former, first documented by Modigliani *et al* (1985) and named by Desportes *et al* in 1985, produces a comparatively small spore (1.5 by 0.5 μm). The latter, formerly named *Septata intestinalis*, was described as a larger spore (2-2.5 by 1.0 μm) (Ryan *et al*, 1993). These organisms are small and difficult to identify, and hence the diagnosis has traditionally depended on endoscopic procedures to provide intestinal biopsy specimens for histopathologic examination and electron microscopic confirmation (Orenstein *et al*, 1990). Recently, a modified trichrome stain which was first applied to microsporidia by Weber *et al* (1992) and subsequently adapted by many investigators (Ryan *et al*, 1993 ; Kokoskin *et al*, 1994 ;

Didier *et al*, 1995 ; Wanachiwanawin *et al*, 1998) appears to be a favorable method for routine detection of the organisms in fecal specimens. Since *E. intestinalis* is somewhat more susceptible to treatment than *E. bieneusi* (Eeftinck Schattenkerk *et al*, 1991 ; Molina *et al*, 1993, 1995), a definitive method of species identification is required.

Despite the fact that infection of HIV in Thai people is becoming more prevalent (Suwanagool *et al*, 1997), the prevalence of microsporidial infection and species identification have not been determined. The purpose of this study was therefore to evaluate the prevalence of intestinal microsporidiosis in HIV infected patients with chronic diarrhea. The identification of the species of the organisms by transmission electron microscopy (TEM) was also carried out.

MATERIALS AND METHODS

This study was conducted to determine the prevalence of intestinal microsporidiosis in HIV-seropositive patients with chronic diarrhea from January 1995 to December 1996. Multiple diarrheal stool specimens were received from 66 acquired immunodeficiency syndrome (AIDS) patients who

either attended the outpatient diarrhea clinic or were hospitalized in Siriraj Hospital, Bangkok, and in Bamrasnaradura Infectious Diseases Hospital, Nonthaburi, Thailand. Chronic diarrhea was defined as three or more watery or loose stools per day for at least 4 weeks. Fecal specimens were collected and examined for ova and parasites, using the simple smear technique, the formalin-ether concentration method, the iodine stain for cysts of protozoa and the modified acid-fast stain for *Cryptosporidium* and *Cyclospora*. The stool samples were also investigated for *Salmonella, Shigella*, and *Mycobacterium*. Slides for light microscopic examination were prepared and stained using the modified trichrome staining method as described by Weber *et al* (1992). At least three fecal smears were used for the detection of microsporidia spores in each specimen. Each slide was scanned under a light microscope with a x100 oil-immersion lens for approximately 500 fields before being designated as negative. The criterion used for positivity was the identification of three or more pink-red spores of about 1 to 2 μm with characteristic diagonal or horizontal band representing the polar tubule (Weber *et al*, 1992 ; Garcia *et al*, 1994). Stool specimens for TEM were also processed as described by Weber *et al* (1992). Electron microscopic characteristics of microsporidia spores as described in previous reports were used as guidelines for identifying species of microsporidia (Canning and Hollister, 1990 ; Weber *et al*, 1992 ; Garcia *et al*, 1994).

Upper endoscopy and/or colonoscopy were performed to obtain tissue specimens from cases which multiple fecal examinations were negative. Several biopsy samples were examined for histopathology. TEM of biopsy specimens for the presence of microsporidia was also carried out.

RESULTS

Of the 66 HIV infected patients with chronic diarrhea, 22 (33.3%) were positive for microsporidia; 20 were male and 2 female. Age range was 27 to 59 years with a mean age of 35.7 years. Risk factors for acquisition of HIV infection were 19 heterosexual, 2 intravenous drug users and 1 bisexual. The mean CD4 count was 21 ± 9 cells/mm^3. Stool characteristics were more likely to be loose (18/22) than watery (4/22). There were no red

blood cells but a few leukocytes and mucus were present in 2 and 4 of the diarrheic stools respectively. The clinical features of the chronic diarrhea in the patients are shown in Table 1. Onset of symptoms was sudden with an intermittent course

Table 1

Clinical findings in 22 HIV infected patients with intestinal microsporidiosis.

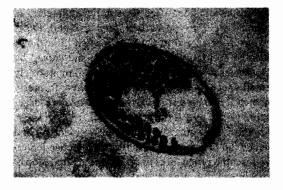| Clinical findings	No.	%
Duration of diarrhea (weeks)	4-104	(mean 30)
Frequency of stools/day	3-13	
Course		
Intermittent	12	(55%)
Persistent	10	(45%)
Onset		
Sudden	12	(55%)
Insidious	10	(45%)
Urgency	20	(91%)
Nocturnal diarrhea	20	(91%)
Incontinence	8	(36%)
Tenesmus	5	(23%)
Food aggravated diarrhea	3	(14%)
Associated symptoms		
Weight loss	22	(100%)
Abdominal pain	17	(77%)
Fever	8	(36%)
Vomiting	8	(36%)
Anorexia	4	(18%)

Fig 1-Transmission electron micrograph of *E. bieneusi* spore demonstrating characteristic cross sections of internal coiled polar tubule, an electron-lucent endospore layer and an electron-dense outer coat. x 52,500.

Table 2

Concurrent intestinal pathogens in the 22 AIDS patients with intestinal microsporidiosis.

Parasite	
Cryptosporidium parvum	5
Isospora belli	3
Giardia intestinalis	2
Strongyloides stercoralis	2
Hookworm	1
Bacteria	
Helicobacter pylori	3
Nontuberculous mycobacterium	1
Virus	
Cytomegalovirus	2
Herpes simplex virus	1

rather than an insidious onset and persistent diarrhea. All patients experienced weight loss. Urgency, nocturnal diarrhea and abdominal pain were major clinical findings. About one-third of the patients had fever, vomiting and incontinence. Less frequent symptoms were tenesmus and anorexia.

Microsporidia spores in fecal specimens were detected by modified trichrome stain which stained pink-red with a distinct belt-like stripe. They measured 0.8-1.2 x 0.7-0.9 μm. TEM for species identification of microsporidia spores in 22 patients revealed *E. bieneusi* in 18 cases. In the remaining 4 cases, the organisms were not detected by TEM. Electron micrographs of the spores displayed an electron-lucent endospore layer and a dense outer wall. Characteristic double rows of polar tube lay on either side of electron-lucent inclusion (Fig 1). The number of coils of polar filament varied between 4 and 8. Empty spores with intact membranes were also detected.

Upper endoscopy and colonoscopy were performed in 6 patients. Results of histopathology of stomach, duodenum and colon of 3 cases demonstrated chronic gastritis with *Helicobacter pylori* infection, chronic duodenitis and chronic colitis respectively. Of these patients, cytomegalovirus was detected in the esophageal biopsy specimen of one case. *Cryptosporidium* was demonstrated from the ileal section in one patient together with cytomegalovirus from the colonic specimen. In another one, nontuberculous mycobacterial infection was diagnosed from both ileal and colonic

biopsy specimens. Sigmoidoscopy in the other patient showed a large irregular-shaped anal ulcer with histological features of squamous papilloma which were compatible with herpes simplex virus infection. However, microsporidia were not identified by light and electron microscopic examinations in these intestinal biopsy specimens.

DISCUSSION

Microsporidia have been recognized as significant opportunistic pathogens in patients with AIDS. Intestinal microsporidiosis is the most commonly diagnosed microsporidia infection in man, which is responsible for diarrhea and other gastrointestinal symptoms (Canning and Hollister, 1990; Cali, 1991). *E. bieneusi* has been identified as a principal cause of enteric infection and has also been detected in the gallbladder and biliary tract resulting in cholecystitis and cholangitis (Beaugerie *et al*, 1992; Pol *et al*, 1993). In addition, the organism was reported in the respiratory tract causing pulmonary symptoms (Schwartz *et al*, 1994). *E. intestinalis* has also been observed to infect the intestines as well as other organs including the urinary tract and respiratory tract causing renal failure and rhinosinusitis respectively (Ryan *et al*, 1993; Schwartz *et al*, 1994; Molina *et al*, 1995). Although the two species accounted for similar gastrointestinal symptoms, the latter has a propensity for dissemination and has a more favorable reponse to therapy (Eeftinck Schattenkerk *et al*, 1991; Asmuth *et al*, 1994; Molina *et al*, 1995). Thus the organisms should be precisely identified. The diagnosis of microsporidia has primarily relied on light and electron microscopy. The development of a modified trichrome staining method was a significant improvement for detection of microsporidia spores in feces (Weber *et al*, 1992). Although this technic is practical for routine diagnosis, the species of the organisms cannot be detected. Electron microscopy remains an important method for species identification of microsporidia in biopsy or stool specimens (Molina *et al*, 1993; Asmuth *et al*, 1994).

Intestinal microsporidiosis has been recognized worldwide as a major cause of chronic diarrhea in HIV-seropositive patients. The infection rate ranges from 6.5 to 50% (Orenstein *et al*, 1990; Eeftinck Schattenkerk *et al*, 1991; Molina *et al*, 1993). In this study, the prevalence of intestinal micros-

poridiosis was found to be 33.3%. Of the 22 fecal specimens positive for microsporidia by the modified trichrome staining method, the species present in 18 was identified by TEM and all were diagnosed as *E. bieneusi*. Histopathologic and ultrastructural studies of intestinal biopsy specimens showed no microsporidia organisms. Fecal examination for the spores using the modified trichrome stain is the simplest method for diagnosing intestinal microsporidiosis (Schwartz *et al*, 1994). However in a case with light infection, serial fecal specimens should be obtained (Beauvais *et al*, 1993). Histopathologic study of intestinal biopsy tissue is usually sufficient to diagnose enteric microsporidiosis (Schwartz *et al*, 1994). In the present study, endoscopically-obtained biopsies could not demonstrate the organisms. This may be due to the restrictions on sampling specimens. Electron microscopy has been documented as a valuable technic for diagnostic confirmation of microsporidia infection, but it is a less sensitive method and the parasite can be missed detection in cases infected with few organisms (Schwartz *et al*, 1994; Didier *et al*, 1995).

The present study revealed that almost all patients were middle-aged males. The major clinical symptom was chronic diarrhea associated with weight loss. Other clinical features included abdominal pain, vomiting and anorexia. Similar clinical findings were obtained by Orenstein *et al* (1990) and Eeftinck Schattenkerk *et al* (1991). In addition, urgency and nocturnal diarrhea were found in a majority of cases which indicated organic causes of diarrhea. Incontinence and tenesmus were not prominent symptoms and may be related to colonic involvement of other organisms including cytomegalovirus, herpes simplex virus, and nontuberculous mycobacterium. Patients with intestinal microsporidiosis often had no fever unless other infections were present (Eeftinck Schattenkerk *et al*, 1991). In contrast to this study, only half of patients who had fever were coinfected with other pathogens such as *Salmonella* septicemia, pulmonary tuberculosis, cryptococcal meningitis, and intestinal nontuberculous mycobacterial infection. It has been documented that patients at highest risk for intestinal microsporidiosis are patients with CD4 counts less than 100 cells/mm^3 (Molina *et al*, 1993; Asmuth *et al*, 1994). Similar results were found in the current study. All patients had < 50 CD4 cells/mm^3, and other enteric opportunistic infections were frequently present. Five (22.7%) of the 22 patients were accompanied by intestinal

cryptosporidiosis. *Cryptosporidium parvum* was found to be the most common concurrent enteric parasite in AIDS patients with intestinal microsporidiosis. Our data agree with the report obtained by Garcia *et al* (1994) which indicated dual infections with *C. parvum* and microsporidia in 28% of patients. Although intestinal microsporidiosis and cryptospori- diosis are clinically indistinguishable, the drug-sensitivity patterns are quite different. Simultaneous infection should be precisely diagnosed to ensure proper management.

In summary, intestinal microsporidiosis is an important disease associated with chronic diarrhea in AIDS patients in Thailand. *E. bieneusi* is a significant cause of diarrheal illness in this population. Identification of the species of microsporidia may be beneficial in assessing the response to therapy and the risk of dissemination.

ACKNOWLEDGEMENTS

REFERENCES

Asmuth DM, DeGirolami PC, Federman M, *et al*. Clinical features of microsporidiosis in patients with AIDS. *Clin Infect Dis* 1994; 18: 819-25.

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

Beauvais B, Sarfati C, Molina JM, Lesourd A, Lariviere M, Derouin F. Comparative evaluation of five diagnostic methods for demonstrating microsporidia in stool and intestinal biopsy specimens. *Ann Trop Med Parasitol* 1993; 8: 99-102.

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

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

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

Didier ES, Orenstein JM, Aldras A, Bertucci D, Rogers LB, Janney FA. Comparison of three staining methods for detecting microsporidia in fluids. *J Clin Microbiol* 1995; 33: 3138-45.

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 microsporidial spores in fecal specimens from patients diagnosed with cryptosporidiosis. *J Clin Microbiol* 1994; 32: 1739-41.

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

Modigliani R, Bories C, le Charpentier Y, *et al.* Diarrhoea and malabsorption in acquired immune deficiency 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.

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.

Pol 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; 3: 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; 4: 101-9.

Suwanagool S, Ratanasuwan W, Techasathit W. The mounting medical care cost for adult AIDS patients at the Faculty of Medicine, Siriraj Hospital : consideration for management. *J Med Assoc Thai* 1997; 8: 431-9.

Wanachiwanawin D, Lertlaituan P, Mahakittikun V, Ongrotchanakun J. The rapid detection of microsporidia in stool using a new trichrome-methylene blue stain : a preliminary report. *Siriraj Hosp Gaz* 1998; 50: 79-85.

Weber R, Bryan RT, Owen RL, Wilcox CM, Gorelkin L, Visvesvara GS. Improved light-microscopical detection of microsporidia spores in stool and duodenal aspirates. *N Engl J Med* 1992; 326: 161-6.