

INTESTINAL MICROSPORIDIOSIS IN HIV-INFECTED CHILDREN WITH DIARRHEA

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Abstract. Intestinal microsporidiosis is a common opportunistic disease associated with diarrhea in adult AIDS patients in Thailand; the data regarding this infection in children are scarce. The present study was designed to investigate the prevalence and clinical features of intestinal microsporidiosis in hospitalized HIV-infected and uninfected (free of HIV) children with diarrhea. Of the 95 HIV-infected children and 87 uninfected children, 24 (25.3%) and 13 (14.9%) respectively were diagnosed with intestinal microsporidiosis. Species identification of microsporidia spores, by transmission electron microscopy, demonstrated *Enterocytozoon bieneusi* in 5 cases. *Cryptosporidium parvum* was a common coinfective parasite; pneumonia was the most frequent concurrent disease found in children with intestinal microsporidiosis. Malnutrition was commoner in the HIV-infected group (79.2% vs 23.1%; $p = 0.003$). This study indicates that intestinal microsporidiosis is an important disease in both HIV-infected and uninfected Thai children with diarrhea.

INTRODUCTION

Diarrhea is a principal symptom of AIDS. Diarrheal illness accounts for 30 to 60% of the morbidity seen in adults with AIDS living in industrialized countries and for up to 90% of that seen in adult AIDS patients living in Haiti or Africa (Kotloff *et al*, 1994; Framm and Soave, 1997). Specific pathogenic organisms can be detected in 44 to 85% of HIV infected patients with persistent diarrhea. Microsporidia are the commonest pathogens, found in 24 to 50% of patients with AIDS and chronic diarrhea (Framm and Soave, 1997). According to Kotloff *et al* (1994), the incidence of diarrhea is higher in HIV-infected infants than in uninfected infants, and episodes of persistent diarrhea are more common among HIV-infected patients. Despite the high prevalence of intestinal microsporidiosis in adult AIDS pa-

tients, information about microsporidia infection in children is scarce.

In Thailand, gastrointestinal infection is one of the leading causes of hospital admission among HIV-infected patients (Suwanagool *et al*, 1997). Many enteric organisms including parasites, bacteria, mycobacteria, and viruses have been identified as causes of diarrheal disease in AIDS patients (Manatsathit *et al*, 1996). Microsporidia were the most important parasites associated with chronic diarrhea in adult AIDS patients, intestinal microsporidiosis occurred in about one-third of cases and was more significant in HIV-infected patients than in uninfected patients (Wanachiwanawin *et al*, 1998; 1999). In HIV-infected children, diarrhea was a frequent symptom in 48% and was the cause of death in 46% of hospitalized cases (Chearskul *et al*, 1995; 1996). What is known about intestinal microsporidiosis in children in Thailand is limited and, therefore, we investigated the prevalence and clinical features of microsporidial infection among HIV-infected and uninfected Thai children with diarrhea.

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MATERIALS AND METHODS

The study population consisted of HIV-infected and uninfected (free of HIV) children who were hospitalized with diarrhea at Siriraj Hospital, Bangkok, Thailand, during the period 1996 to 2000. Acute diarrhea was defined as three or more loose or watery stools per day lasting for at least one day. Recurrent diarrhea referred to a new episode of diarrhea that began at least three consecutive diarrhea-free days after a previous episode. Chronic diarrhea was defined as an episode of diarrhea that lasted for more than 14 days. HIV infection was diagnosed if a child had persistently positive anti-HIV antibody at the age of 18 months or older, or at least 2 positive HIV-PCR results from separate blood specimens. On admission, HIV clinical and immunological stages were determined using the CDC classification system (1994); the Gomez classification system, modified for the growth curve of normal Thai children, was used to describe the children's nutritional status.

Multiple fecal specimens were obtained from 95 HIV-infected children and 87 uninfected children. Stool examination for parasites in-

cluded simple smear, formalin-ether concentration, iodine stain, iron-hematoxylin stain, and modified acid-fast stain. Microsporidia were detected using the modified trichrome staining method described by Weber *et al* (1992). At least three fecal smears were examined for the presence of microsporidia spores. Species identification was by transmission electron microscopy (TEM) (Weber *et al*, 1992).

Chi-square test, Mann-Whitney U test, and Fisher's exact test were used for comparative analyses. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The characteristics of all the hospitalized children with diarrhea in the study are outlined in Table 1. The underlying diseases or concurrent diagnoses of uninfected children are shown in Table 2. Microsporidia spores were detected in 24 (25.3%) of 95 HIV-infected children and in 13 (14.9%) of 87 uninfected children ($p = 0.122$). The clinical findings of children with intestinal microsporidiosis are shown in Table 3. Of the 24 HIV-infected children and 13

Table 1
Characteristics of the 182 hospitalized children with diarrhea recruited to the study.

Characteristic	HIV group n = 95 (%)	uninfected group n = 87 (%)
Mean age \pm SD (months)	15.9 \pm 16.0	16.3 \pm 15.0
Sex		
Male	60 (63.2)	49 (56.3)
Female	35 (36.8)	38 (43.7)
Fever	79 (83.2)	55 (63.2)
Course of diarrhea		
Acute	86 (90.5)	84 (96.6)
Chronic	7 (7.4)	3 (3.4)
Recurrent	2 (2.1)	0 (0)
Dehydration		
No	60 (63.1)	33 (37.9)
Mild	13 (13.7)	26 (29.9)
Moderate	13 (13.7)	21 (24.1)
Severe	9 (9.5)	7 (8.1)
Intestinal microsporidiosis	24 (25.3)	13 (14.9)

Table 2
Underlying disease or concurrent diagnosis
of uninfected children with diarrhea.

Underlying disease or concurrent diagnosis	No. of case
Pneumonia	10
Urinary tract infection	6
Congenital heart disease	4
Microcephaly/Hydrocephalus	4
Kidney disease	3
Bacteremia/Septicemia	3
Bronchitis	3
Liver disease	1
Hypothyroidism	1
Systemic lupus erythematosus	1
IgG2/IgG4 deficiency	1
Chronic otitis media	1
Meningitis	1
Gastroparesis	1

uninfected children with intestinal microsporidiosis, three (12.5%) cases and three (23.1%) cases respectively were co-infected with *Cryptosporidium parvum*. In both groups, the course of diarrhea was mainly acute and the stool was mainly watery. Fever was found to be an associated symptom in all HIV-infected cases with intestinal microsporidiosis and in 92.3% of children without HIV infection. Dehydration was present in 70.8% and 77.0% of HIV-infected and uninfected children respectively. Pneumonia was the most common concurrent diagnosis in children with intestinal microsporidiosis (Table 4). Among HIV-infected children with intestinal microsporidiosis, 29.2%, 45.8%, and 25.0% were classified in clinical category A, B, and C respectively and 29.2%, 33.3%, and 37.5% were categorized as immunological stages 1, 2, and 3 respectively. Malnutrition was more frequently found in the

Table 3
Clinical characteristics of HIV-infected and uninfected children with intestinal microsporidiosis.

Characteristic	HIV group n = 24 (%)	Uninfected group n = 13 (%)	p-value
Mean age \pm SD (months)	13.4 \pm 13.2	13.7 \pm 12.9	1.000
Sex, male : female	12 : 12	8 : 5	0.743
Fever	24 (100)	12 (92.3)	0.351
Course of diarrhea			0.538
Acute	21 (87.5)	13 (100)	
Chronic	2 (8.3)	0 (0)	
Recurrent	1 (4.2)	0 (0)	
Stool character			0.394
Watery	19 (79.2)	12 (92.3)	
Mucous	4 (16.7)	1 (7.7)	
Mucous-bloody	1 (4.1)	0 (0)	
Dehydration			1.000
No	7 (29.2)	3 (23.0)	
Mild	8 (33.3)	5 (38.5)	
Moderate	7 (29.2)	5 (38.5)	
Severe	2 (8.3)	0 (0)	
Nutritional status			0.003
0	5 (20.8)	10 (76.9)	
1	4 (16.7)	1 (7.7)	
2	9 (37.5)	1 (7.7)	
3	6 (25.0)	1 (7.7)	

Table 4
Concomitant diagnosis in children with
intestinal microsporidiosis.

Concomitant diagnosis	HIV group	Uninfected group
Pneumonia	3	3
Meningitis	1	1
Urinary tract infection	1	1
Chronic otitis media	1	0
Septicemia	1	0
Febrile convulsion	0	2
Microcephaly	0	1
Acute lymphoblastic leukemia	0	1

HIV-infected group than in the uninfected group (79.2% vs 23.1%; $p = 0.003$).

Transmission electron microscopy for the species identification of microsporidia spores revealed *Enterocytozoon bieneusi* in 5 cases.

DISCUSSION

Diarrheal illness is a major cause of childhood morbidity and mortality especially among HIV-infected children. Microsporidia are among the commonest opportunistic protozoa that cause enteritis. In the present study, microsporidia were frequently detected in hospitalized children, both HIV-infected and uninfected, with diarrhea. The microsporidia infection rate among HIV-seropositive children with diarrhea agreed with those given in previous reports about Thai adult AIDS patients, which showed that the infection rates ranged from 27 to 33% (Punpoowong *et al*, 1998; Wanachiwanawin *et al*, 1998; 1999). Whereas microsporidia infection was not detected in non-AIDS adults with chronic diarrhea (Wanachiwanawin *et al*, 1999), the present study found that intestinal microsporidiosis in children without HIV infection was not uncommon, the course of diarrhea in children with microsporidia infection was acute rather than persistent or chronic, as it is in adults with AIDS. Compared with the study performed by

Leelayoova *et al* (2001), the prevalence of intestinal microsporidiosis in HIV-seropositive children with diarrhea was higher in the present study (25.3% vs 10.8%). One possible explanation is the more severe HIV status of the children in our study. Microsporidia infection appears to be influenced by immunological status, it has been established that patients with the highest risk of intestinal microsporidiosis are those with CD4 counts of less than 100 cells/mm³. (Asmuth *et al*, 1994; Molina *et al*, 1993). Intestinal microsporidiosis has been infrequently reported in HIV-seronegative patients, as well as in patients with other forms of immunosuppression (Framm and Soave, 1997). Many HIV-free children with microsporidiosis have malnutrition or underlying disease, which may result in a higher prevalence of microsporidiosis than is found among normal children.

It has been documented that HIV-infected patients are susceptible to a variety of opportunistic pathogens. *Cryptosporidium parvum* was a common coinfective parasite, accompanying microsporidia in about one-quarter of adult AIDS patients (Garcia *et al*, 1994; Wanachiwanawin *et al*, 1998). Our data showed that *C. parvum* was a common coinfective pathogen in children with intestinal microsporidiosis regardless of HIV status.

The present study suggests that intestinal microsporidiosis is an important opportunistic disease in both HIV-infected and uninfected children with diarrhea in Thailand.

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