INTESTINAL PARASITIC INFECTIONS AMONG HUMAN IMMUNODEFICIENCY VIRUS-INFECTED AND -UNINFECTED CHILDREN HOSPITALIZED WITH DIARRHEA IN BANGKOK, THAILAND

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Abstract. A prospective observational study was conducted to determine the prevalence and the clinical impact of intestinal parasitic infections in diarrheal illness among HIV-infected and HIV-uninfected children hospitalized with diarrhea in Bangkok, Thailand. Stool samples were examined for intestinal parasites using a simple smear method, a formalin-ether concentration method, a modified acid-fast stain and a modified trichrome stain. Intestinal parasites (IP) were identified in the stool specimens of 27 of 82 (33%) HIV-infected and 12 of 80 (15%) HIV-uninfected children (p=0.01). Microsporidia and *Cryptosporidium* were the most common IP found. Eighty-two percent of HIV-infected and 97% of HIV-uninfected groups presented with acute diarrhea and 76% of each group had watery diarrhea. Pneumonia was the most common concurrent illness, found in 22%. Clinical findings were unable to differentiate children infected with IP. Sixty-three percent of HIV-infected and 83% of HIV-uninfected children who had IP made a satisfactory recovery without specific anti-parasitic therapy. However, 9 children (7 HIV-infected and 2 HIV-uninfected) with persistent diarrhea who also had cryptosporidiosis and/or microsporidiosis did not respond to azithromycin and/or albendazole respectively. HIV-infected children with cryptosporidiosis were older and had more advanced HIV infection than those with microsporidiosis. Routine stool examination for IP should be considered due to the absence of clinical markers. The lack of effective therapy for the major IP found underscores the importance of preventive measures.

INTRODUCTION

Diarrhea is a common symptom of human immunodeficiency virus (HIV) infection in children. Compared with HIV-uninfected children, HIV-infected children have more than twice the incidence of acute diarrhea and are more likely to develop persistent diarrhea (Thea *et al*, 1993; Kotloff *et al*, 1994). In Thailand, diarrhea is a presenting symptom of HIV infection in 46-48% of HIV-infected children (Sirisanthana *et al*, 1993; Chearskul *et al*, 1995). Among the HIV-infected children who were hospitalized and died in Siriraj Hospital in Bangkok, 46% had diarrhea on presentation (Chearskul *et al*, 1996). Among the infectious causes of diarrhea, intestinal parasites (IP) are common, particularly in developing countries. These parasites may cause acute, chronic, prolonged or relapsing diarrhea leading to significant weight loss and wasting in HIV-infected patients, in contrast to the self-limiting diarrhea of HIV-uninfected people (Mac Kenzie *et al*, 1994; Pozio *et al*, 1997; Didier, 1998). In Zaire, the prevalence of intestinal parasitic infections (IPI) was similar among HIV-infected and HIV-uninfected children with acute diarrhea (Thea *et al*, 1993). In Thailand, however, IPI were found more often among HIV-infected adults with chronic diarrhea than among those HIV-uninfected (Wanachiwanawin

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et al, 1999). *Cryptosporidium* was found in 20-25% and microsporidia was found in one-third of HIV-infected Thai adults with chronic diarrhea (Manasathit *et al*, 1996; Wanachiwanawin *et al*, 1999). The data for Thai children are very limited.

We conducted a prospective study to determine the prevalence of IPI among HIV-infected and HIV-uninfected children hospitalized with diarrhea. We also evaluated the clinical findings and the outcomes associated with IPI.

MATERIALS AND METHODS

From January 1997 to April 1998, stool samples of children with and without HIV infection who were hospitalized with diarrhea at Siriraj Hospital, a 300 pediatric-bed tertiary care center in Bangkok, were collected for examination for intestinal parasites. The examinations included a simple smear method, a formalin-ether concentration method, a modified acid-fast stain and a modified trichrome stain, as described by Weber *et al* (1992) and Wanachiwanawin *et al* (1998). The stool specimens were also cultured for bacterial pathogens including *Salmonella*, *Shigella*, and *Cholera*. Medical records were retrospectively reviewed for nutritional status, clinical presentations, HIV clinical and immunological staging on admission using the CDC classification system (CDC, 1994), and the outcomes on discharge.

Fluid and electrolyte therapy was the standard treatment for each diarrheal episode. Antibiotics were prescribed empirically in cases of dysentery or suspected systemic infections. Children with cryptosporidiosis would receive azithromycin and those with microsporidiosis would receive albendazole unless the diarrhea had resolved by the time the result of the stool examination was known. The patients with other IPI were treated with specific anti-parasitic drugs regardless of symptoms.

Acute diarrhea was defined as a change in normal stool pattern with at least one day of increased frequency, liquidity, or presence of blood or mucus. Chronic diarrhea was defined as diarrhea that persisted for more than 14 days. The stool appearance was classified as watery, mucous, and bloody. The degree of malnutrition was classified using the Gomez classification system (Gomez, 1946) modified for the growth curve of normal Thai children.

A child was considered to be HIV-infected if he or she had persistent positive anti-HIV antibody at 18 months or older or at least 2 positive HIV-PCR-tested on separate blood samples. All the infants and children born to HIV-infected mothers were not breast-fed. The HIV-uninfected children were either HIV-seronegative or had at least 2 negative HIV-PCR: once at the age of 1 month or older and again at 4 months or older.

The outcome was defined as either the persistence or the cessation of diarrhea after fluid, electrolyte, and antibiotic therapies for at least 72 hours but before the administration of specific anti-parasitic treatment. The response to specific anti-parasitic treatment was described subsequently.

The comparative analysis used chi-square and Fisher's exact tests for categorized variables and Student *t*-test for continuous variables. All p-values are two-tailed.

RESULTS

Stool samples were collected from 82 HIV-infected children and 80 HIV-uninfected children admitted with diarrhea. The mean ages were 15.6 (SD 16.1) and 16.4 (SD 15.8) months respectively; 64% and 57% were boys in the HIV-infected and HIV-uninfected groups respectively. Malnutrition was found more frequently in the HIV-infected group: 71% *vs* 22%, $p<0.001$; malnutrition was 19% *vs* 6% first degree, 24% *vs* 10% second degree, and 28% *vs* 4% third degree respectively. Children with HIV infection had a higher prevalence of IPI than those without (32.9% *vs* 15%, p=0.01;

Table 1
Prevalence of intestinal parasites among children with and without HIV infection hospitalized with diarrhea.

	HIV-infected (n=82)	HIV-uninfected (n=80)	p-value
Cryptosporidium	5 (6.1%)	1 (1.2%)	0.21
Microsporidia	16 (19.5%)	7 (8.7%)	0.049
Cryptosporidium and microsporidia	3 (3.6%)	3 (3.7%)	1.00
Giardia lamblia	2 (2.4%)	0	0.50
Blastocystis hominis	1 (1.2%)	0	1.00
Entamoeba histolytica	0	1 (1.2%)	0.49
Total	27 (32.9%)	12 (15%)	0.01

Table 2
Clinical characteristics of children with and without intestinal parasitic infections (IPI) stratified by HIV status.

	HIV-infected (N=82)		HIV-uninfected (N=80)	
	With IPI (n=27)	Without IPI (n=55)	With IPI (n=12)	Without IPI (n=68)
Mean age ± SD (months)	19.3 ± 16.9	14.2 ± 15.6	14.5 ± 10.6	16.6 ± 15.8
Fever >37.5°C[a]	24 (89%)	44 (80%)	10 (83%)	45 (66%)
Mean temperature (°C)	38.4 ± 0.9	38.7 ± 1.0	38.3 ± 0.8	39.05 ± 0.6
Nature of diarrhea :				
Acute	22 (81%)	45 (82%)	11 (92%)	67 (98%)
Chronic[b]	5 (19%)	10 (17%)	1 (8%)	1 (2%)
Stool appearance :				
Watery	21 (78%)	41 (75%)	9 (75%)	52 (77%)
Mucous	4 (15%)	8 (14%)	3 (25%)	13 (19%)
Mucous bloody	2 (7%)	6 (11%)	-	3 (4%)
Other concurrent diagnosis:	7 (26%)[c]	26 (47%)[c]	3 (25%)[d]	28 (41%)[d]
Pneumonia	4	17	2	12
Sepsis	1	4	0	3
Meningitis	0	2	1	1
URI / Otitis media	1	3	0	3
Urinary tract infection	1	1	0	6
Shigellosis	0	0	0	2
Cholera	0	0	0	1
Outcome:				
Cessation of diarrhea[e]	17 (63%)	39 (71%)	10 (83%)	64 (94%)
Persistence of diarrhea	7 (26%)	11 (20%)	2 (17%)	3 (5%)
Death[f]	3 (11%)	5 (9%)	0	1 (1%)
Cause of death				
Sepsis	2	3	-	1
Pneumonia	1	2	-	-

[a]p=0.07 for HIV-infected *vs* HIV-uninfected; [b]p=0.001 for HIV-infected *vs* HIV-uninfected; [c]p=0.06; [d]p=0.35; [e]p=0.002 for HIV-infected *vs* HIV-uninfected; [f]p=0.03 for HIV-infected *vs* HIV-uninfected

Table 1). Microsporidia were the most common IP found, followed by *Cryptosporidium*, in both HIV-infected and HIV-uninfected groups.

Sixty-seven (82%) HIV-infected and 78 (97%) HIV-uninfected children had acute diarrhea (p=0.001). Seventy-six percent of each group had watery diarrhea. Fever was recorded in 82% of HIV-infected and 71% of HIV-uninfected children (p=0.07) and the mean temperatures were not different, regardless of the presence of IP in stool (Table 2).

Concurrent illness was recorded in 40% (33/82) and 39% (31/80) of HIV-infected and HIV-uninfected groups, respectively. Children without IPI in both groups tended to have other concurrent illnesses more often (47% *vs* 26% and 41% *vs* 25% in HIV-infected and HIV-uninfected groups, p=0.06 and 0.35 respectively). Pneumonia was the most common concurrent illess, found in 26% of HIV-infected and 17% of HIV-uninfected groups.

Among HIV-infected children with IPI, 20%, 40%, and 40% were in clinical category A, B, and C on admission respectively. However, 15% of HIV-infected children without IPI were asymptomatic (category N), and 19%, 37%, and 29% were in category A, B, and C respectively. The immunological staging was not different between those with and without IPI. Five (18%) and seven (13%) of children with and without IPI respectively were taking either dual nucleoside reverse transcriptase therapy or didanosine alone. No child received protease inhibitors.

After fluid, electrolyte, and antibiotic treatment, diarrhea had stopped in 69% (56/82) of HIV-infected and 92% (74/80) of HIV-uninfected children (p=0.002). Presence of IP in stool was not significantly associated with persistence of diarrhea in either group. There were 8 (9.7%) deaths among HIV-infected children from sepsis and pneumonia. There was one death (1.2%) from sepsis among the HIV-uninfected children (p=0.03), (Table 2).

The 9 children with IPI and persistent diarrhea (3 HIV-infected with cryptosporidiosis, 4 HIV-infected with microsporidiosis, and 2 HIV-uninfected with both cryptosporidiosis and microsporidiosis) continued to have diarrhea even with specific therapy using azithromycin for cryptosporidiosis and albendazole for microsporidiosis.

Among HIV-infected children, those with cryptosporidiosis were significantly older (mean age of 26.6±17.2 *vs* 12.8±13.2 months, p=0.02) than those with microsporidiosis. Seven (86%) HIV-infected children with cryptosporidiosis were in clinical category C and all were in immunological category 3 on admission compared with 4 (21%) and 10 (53%) in those with microsporidiosis (p=0.006 and 0.06 for category C and 3). However, the onset of diarrhea, stool appearance and outcome of diarrhea after standard treatment were similar in the two groups.

DISCUSSION

Many IP such as *Cryptosporidium*, *Isospora*, and microsporidia are opportunistic organisms causing significant morbidity in HIV infected people (Current and Garcia, 1991; Winter and Miller, 1994; Didier, 1998). Clinical features of these IPI are indistinguishable from one another, ranging from asymptomatic (Pettocllo-Mantovani *et al*, 1995; Didier, 1998) to severe, mostly watery diarrhea, fever, weight loss, and possibly extraintestinal manifestations (Current and Garcia, 1991; Pol *et al*, 1993; Vakil *et al*, 1996; Didier, 1998).

The majority of IP found in this study were *Cryptosporidium* and microsporidia: a similar finding to that of a study among HIV-infected adults with chronic diarrhea in our hospital, although the prevalence of each parasite among HIV-infected children in this study was lower than in adults (Wanachiwanawin *et al*, 1999). We did not find *Isospora* in our study, whereas it was found in the adult study. The prevalence of IPI among HIV-infected children was higher than among HIV-uninfected children. In fact, one-third of HIV-infected children who were hospitalized with diarrhea

were infected with IP.

In this study, diarrhea was a symptom of other systemic infections in a quarter of children with IPI and in about one half of those without IPI. However, clinical features alone could not identify children with IPI. Therefore, the routine examination for IP for HIV-infected children is warranted. IPI were more likely to cause illness among immunocompromised individuals (Current and Garcia 1991; Mac Kenzie *et al*,1994; Pettoello-Mantovani *et al*, 1995; Pozio *et al*, 1997; Didier, 1998). On the other hand, diarrhea among HIV-infected children could be the result of problems other than IPI, such as common viral and bacterial gastroenteritis, malabsorption, lactose intolerance, mycobacterial infection, other systemic infections, and HIV-infection itself. We found diarrhea in most IP-infected children resolved without specific anti-parasitic therapy. It was possible that these children were asymptomatic IP carriers who developed diarrhea of another cause (Pettoello-Mantovani *et al*, 1995; Sobottka *et al*, 1998).

Specific therapy for IPI may help to shorten the duration of diarrhea in some children, particularly those with microsporidiosis (Blanshard *et al*, 1992; Dieterich *et al*, 1994; Molina *et al*, 1998). However, many children in this cohort with microsporidiosis did not respond well to albendazole. This was probably because the majority of microsporidia identified in Thailand was *E.bieneusi* (Wanachiwanawin *et al*, 1999), which is more resistant to albendazole (Blanshard *et al*, 1992; Dieterich *et al*, 1994). No effective therapy is yet available for cryptosporidiosis.

An interesting finding in this study was that among the HIV-infected children, those with cryptosporidiosis were older and at a more advanced stage of HIV infection than those with microsporidiosis. This finding concurs with a study in adults in which 88% of patients with cryptosporidiosis had other AIDS-defining illnesses and all had CD4 counts <200/mm^3. Patients with cryptosporidiosis had a significantly shorter survival time after diagnosis than those without (Manabe *et al*, 1998). On the other hand, microsporidiosis was found in all stages of HIV-infection but the self-limiting diarrhea was observed in those with CD4 counts ≥200/mm^3. Chronic diarrhea with weight loss was common among those with CD4 counts <100/mm^3 (Didier, 1998).

This study has several limitations. We did not look for viral enteropathogens such as rotavirus and enterovirus, which are the major causes of diarrhea in children and may explain many episodes of diarrhea with or without IP co-infection. Information after hospital discharge was not available to allow analysis of the persistence of IPI or to enable us to determine whether those with IP had relapsed. The prevalence of IPI in this study might be overestimated due to selection bias of the patients in this study who tended to be sicker, or who may have some underlying problems that led them to be hospitalized in this referral center. Moreover, many HIV-uninfected children in this cohort were born to HIV-infected mothers and may be at higher risk for IPI from not being breast-fed and from possible exposure to pathogens from HIV-infected family members.

In conclusion, IPI were more common among HIV-infected than HIV-uninfected Thai children. Microsporidia and *Cryptosporidium* were the major IP found. Clinical presentation, HIV staging, history of antiretroviral therapy or response to standard therapy with fluid, electrolytes, and antibiotics were not different in children with and without IP. Cryptosporidiosis was associated with older age and more advanced HIV infection. The fact that effective specific therapy of the major causative organisms is not yet available underscores the need for good hygienic practices and the boiling of drinking water in order to prevent IPI.

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