PROTOZOAN ENTERIC INFECTION IN AIDS RELATED DIARRHEA IN THAILAND

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Abstract. The aim of this study was to determine the prevalence of enteric protozoa and other pathogens in AIDS patients with diarrhea in Bangkok, Thailand. Of 288 consecutive patients screened in the 10 month period between November 1999 - August 2000 inclusive, 55 (19.2%) had *Cryptosporidium* spp, 13 (4.5%) had *Isospora* oocyst, 11 (3.8%) had *Giardia lamblia*, 3 (0.9%) had *Entamoeba histolytica*, and 1 (0.3%) had *Iodamoeba butschlii* infection. The prevalence of microsporidia was 11% in this study. Of 251 patients for whom stool culture for bacteria was performed, enteric bacterial pathogens isolated were *Campylobacter* spp in 18 (7.1%), *Salmonella* spp in 11 (4.3%), and *Shigella* spp in 1 (0.5%). Other pathogens found in these patients were *Clostridium difficile* in 16/102 (15.6%), *Mycobacterium* spp in 18/287 (6.2%), and *Strongyloides stercoralis* in 23/288 (8.0%). Overall, parasitic and bacterial pathogens were identified in 140 (48.6%) patients. These pathogens were identified by the routine simple wet smear technique in 32, formalin-ether concentration method in 46, culture for *S. stercoralis* in 5, and culture for bacteria in 30. Additional test, using modified Ziehl-Neelsen staining, identified cryptosporidial oocyst, isospora oocyst, and *Mycobacterium* spp in 72. The microsporidia, initially identified by modified trichrome blue staining, all were then determined to be *Enterocytozoon bieneusi* by thin sectioning electron microscopy. Protozoan and bacterial pathogens were confirmed to be important etiologic agents in diarrhea in AIDS in Thailand. They were all associated with increased mortality. Routine stool examination by simple wet smear detected only one-fourth of these pathogens. Therefore all diagnostic techniques for these organisms should be made more widely available in Thailand.

INTRODUCTION

The HIV pandemic continues to have a major impact on public health in Thailand. Patients with acquired immunodeficiency syndrome (AIDS) are prone to a wide range of opportunistic infections, including diarrheal diseases. A substantial number of patients suffer from chronic diarrhea and is associated with significant morbidity and mortality (Kelly *et al*, 1996; Foudraine *et al*, 1998). A variety of opportunistic and non-opportunistic pathogens may cause diarrhea in these patients (Goodgame 1996; Wittner *et al*, 1993). The accurate identification on the causes of diarrhea allows appropriate treatment and counselling about the prognosis, as well as entry into trials of new potential treatments. We have therefore prospectively studied a large cohort of patients with HIV infection to determine the spectrum of pathogens associated with diarrhea and to evaluate the diagnostic yield of various stool examination techniques currently available in Thailand.

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MATERIALS AND METHODS

All patients with history of diarrhea attending the out patient clinic at Siriraj Hospital, Bangkok and Bamrasnaradura Hospital, Nonthaburi, Thailand were asked to provide a stool sample, as part of a routine investigation for the cause of diarrhea. At the same time HIV infected patients without diarrhea, who were admitted to both hospitals with other conditions, were also asked to provide a stool sample.

Diarrhea was defined as at least twice daily watery or loose stools. Chronic diarrhea was defined as the continuous presence of diarrhea for more than 3 weeks.

A standard ova and parasite examination of the stool was performed at the Infectious Diseases and Tropical Medicine Laboratory, Department of Medicine, Faculty of Medicine Siriraj Hospital. *Entamoeba histolytica* was not differentiated from nonpathogenic *Entamoeba dispar.* Coccidial oocysts were detected in sediments obtained from the formalin-

ether concentrates and stained with the modified Ziehl-Neelsen staining. The Meriflour *Cryptosporidium-Giardia* monoclonal direct immunofluorescence detection kit (Meridian Diagnostics, Inc, Cincinnati, Ohio) was used according to the manufacturer's direction for the confirmation of *Cryptosporidium parvum* and *Giardia lamblia*. Microsporidial spores were detected by modified trichrome blue stained smear (Didier *et al*, 1995). An aliquot of feces thought to contain microsporidia was preserved in 10% formalin and sent to Manchester Public Health Laboratory, United Kingdom for further confirmation and species identification by immunofluorescence staining (Calcoflour staining method) and thin sectioning electron microscopy (Chioralia *et al*, 1998). Bacterial cultures included *Salmonella, Shigella, Vibrio* spp and *Campylobacter* spp, *Clostridium difficile* toxin A detection kit (Oxoid, Basingstoke, UK) was used, according to the manufacturer's instruction, to diagnose the *C. difficile* diarrhea. Detection of mycobacteria included examination of the modified Ziehl- Neelsen stained smear and cultures with the use of Lowenstein-Jensen media (Becton Dickinson, Franklin Lakes, NJ, USA). *Strongyloides* larvae were detected by microscopic stool examination or culture.

Demographic data, duration of diarrhea, CD4 count (if available) and results of all stool examinations were recorded in a standard form and then transferred to the database file.

RESULTS

Between November 1999 and August 2000, 394 fecal samples from 288 patients with HIV infection were examined. There were 248 patients (135 patients had history of diarrhea for less than 3 weeks and 113 patients had chronic diarrhea) in the diarrheal group and 16 patients who did not have diarrhea and provided stool samples as a control group. History of diarrhea could not be verified in 24 patients who were excluded from the comparison of the distribution of pathogens detected in each study group. Overall male: female ratio was 2:1 and the mean age was 32 (range 16 to 64) years. The CD4 count was documented in 90 patients and the median CD4 count was 32 (range 1-279) cell/mm^3 and 93.3% of them had CD4 count less than 200 cell/mm^3.

Parasitic causes

27 (9.6%) parasitic pathogens were detected in the simple smear of one stool. The second and third stools were obtained from 65 and 41 patients respectively. Pathogens were detected in 3 patients whom a parasite was not found in the first stool. Examination of formalin-ether concentrates revealed 42 (15%) and 46 (16.4%) parasitic pathogens in the first and second or third stool respectively.

Overall, cryptosporidial oocyst was the most common found protozoan and *S. stercoralis* (23 patients, 8.0%) was the most common helminthic parasite detected in this study. However, only 47 out of 55 cryptosporidial oocyst identified by the modified Ziehl- Neelsen stained smear reacted with the monoclonal antibody used in the Meriflour diagnostic kit. Twenty-two out of 28 stools initially thought to contain microsporidia were confirmed to be *Enterocytozoon bieneusi* by thin sectioning electron microscopy. One individual did not have the history of diarrhea. The median CD4 count, measured in 10 of these patients, was 15 (range 7-50) cell/mm^3.

Bacterial causes

Bacterial pathogens including *C. difficile*, *Salmonella*, *Shigella*, *Campylobacter* and mycobacteria were detected in 64 (22.2%) stools in this study. Overall, 18 patients had mycobacteria. Five of them did not provide enough fecal sample for the culture of mycobacteria. Mycobacteria culture revealed *Mycobacterium tuberculosis* in 3 patients, *Mycobacterium avium*-complex in 6 patients and nontuberculous mycobacteria in 4 patients.

Detailed list of pathogens and potential pathogens identified in this study and diagnostic yields of various examination techniques used in this study are shown in Tables 1 and 2 respectively.

The comparison between pathogens detected in patients with and without diarrhea

Overall, parasitic and bacterial pathogens were identified in 140 patients (48.6%). Dual and triple pathogens were found in 30 and 9 patients respectively. There were 3 patients (18.7%) without history of diarrhea, 55 (41.4%) patients with diarrhea for less than 3 weeks and 66 (58.9%) patients with chronic diarrhea had parasitic or enteric pathogens in their stools (p=0.001). The pathogen found in patients without history of diarrhea was microsporidia, *G. lamblia,* and *Campylobacter* in one each. Therefore the detection of *Cryptosporidia, Isospora, S. stercoralis, C. difficile,* and mycobacteria were significantly associated with diarrhea. However the distribution of pathogens detected was similar between patients with diarrhea of less than 3 weeks and chronic diarrhea of 3 weeks or more (Table 3).

DISCUSSION

The extensive investigation for the cause of diarrhea in AIDS patients was not a common practice,

Table 1
Pathogens and potential pathogens identified in the study group (288 patients).

	No. (%)
Parasitic causes	
C. parvum	55 (19.2)
E. bieneusi	22 (11.0)
S. stercoralis	23 (8.0)
Isospora oocyst	13 (3.8)
G. lamblia	11 (3.8)
E. histolytica	3 (0.7)
Blastocystis hominis	3 (1.0)
Opisthorchis viverrini	4 (1.4)
Hookworm	1 (0.3)
I. butschlii	1 (0.3)
Bacterial causes	
C. difficile	16 (15.6)
Mycobacteria	18 (12.9)
Salmonella spp	11 (4.4)
Shigella spp	1 (0.4)
Campylobacter spp	18 (7.1)

Table 2
Diagnostic yield of various investigations.

	Total number tested	Pathogen found No(%)
Simple wet smear		
One stool	288	27 (9.6)
2/ 3 stools	65/41	32 (11.3)
Formalin-ether concentration		
One stool	280	42 (15.0)
2/ 3 stool	64/40	46 (16.4)
Modified Z-N staining, overall	277	72 (25)
Cryptosporidial oocyst		55
Isospora oocyst		9
Mycobacteria		8
DFA for *C. parvum* [a]	288	47 (16.3)
Modified trichrome blue for microsporidia	200	28
EM confirmation[b]		22 (11)
Stool culture for enteric bacteria	251	30 (12.0)
C. difficile toxin A assay	102	16 (15.7)
Mycobacteria culture	152	13 (8.6)

[a] Direct immunofluorescence test (Meriflour test) for confirmation of *C. parvum.*
[b] Electron microscopy for confirmation and species identification of microsporidia.

especially in developing countries (Rabenec, 1993). Therefore routine stool examinations were usually limited to a simple stool examination and single stool culture for common bacterial pathogens. Examination of formalin-ether concentrates, modified Ziehl-Neelsen and modified trichrome blue stained smear were performed only on request.

Results of this study revealed that the diagnostic yield of single simple stool examination was very low

Table 3
The distribution of pathogens detected in patient with history of diarrhea.

	Patient with diarrhea	
	< 3 weeks (N=135)	> 3 weeks (N=113)
No pathogen	78 (58.6%)	46 (41.1%)
Pathogens found		
1 pathogen	38	50
2 pathogens	16	12
3 pathogens	3	5
Pathogens		
C. parvum	16	25
Microsporidia	8	11
Isospora	5	8
S. stercoralis	9	9
G. lamblia	4	7
E. histolytica	3	0
B. hominis	2	1
C. difficile	7	7
Campylobacter	6	8
Salmonella spp	8	2
Mycobacteria	9	4
Other bacteria	2	0

and stool examination from formalin-ether concentrates should be routinely performed. Examinations of two or three stools were neither convenient nor useful in increasing the diagnostic yield compared to single stool examination of formalin-ether concentrates. In addition cryptosporidial oocyst, *Isospora* oocyst and mycobacteria were easily identified by modified Ziehl-Neelsen stained smear, prepared from the formalin-ether concentrates. Modified trichrome blue stained smear was simple and could be routinely performed for the detection of microsporidia. Results of this study confirmed that protozoan and helminths, especially *S. stercoralis* were main pathogens found in HIV- related diarrhea. Therefore these techniques should be a routine in the investigation of diarrhea in such groups of patients.

Isoenzyme analysis and various methods of polymerase chain reaction studies indicated that two genotypes of *C. parvum* infection existed in human (McLauchlin *et al*, 1999). The direct immunofluorescence assay, using monoclonal antibody against *C. parvum* cell wall, was used to confirm the detection of cryptosporidial oocyst in this study. Only 85.5% of the cryptosporidial oocyst, initially identified by the modified Ziehl-Neelsen, reacted with the monoclonal antibody in this direct immunofluorescence test. This finding indicates that patients in this country were infected with two genotypes of *C. parvum* and only one genotype might be detected by the monoclonal antibody used in the Meriflour diagnostic test. Further study, using isoenzyme or appropriate PCR analysis, to confirm this hypothesis should be carried out.

Isosporiasis remained common despite widely use of co-trimoxazole chemoprophylaxis for *Pneumocystis carinii* pneumonia in Thailand. One patient in this study did not response to the standard high dose of co-trimoxazole treatment. Follow-up study in these patients to confirm the emergence of co-trimoxazole resistant *I. belli* is needed. Detailed prospective studies on this common spore-forming protozoan including cryptosporidia and microsporidia are in progress.

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