

HIGH PREVALENCE OF *MICROSPORIDIUM* INFECTION IN HIV-INFECTED PATIENTS

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Abstract. Sixty-four patients infected with human immunodeficiency virus (HIV) participated in a study to determine opportunistic enteric pathogens and compare them with the patients' clinical status. The most frequently found pathogens were microsporidium (81.2%), *Cryptosporidium parvum* (20.3%), *Candida albicans* (12.5%) and *Blastocystis hominis* (10.9%). Less frequently found pathogens were *Giardia intestinalis* (6.2%), *Cyclospora* (4.7%), *Opishorchis viverrini* ova (3.1%), *Strongyloides stercoralis* larvae (3.1%) and hookworm ova (1.6%). The presence of enteric pathogens was not significantly associated with sex, length of HIV seropositivity and diarrheal symptoms. A high prevalence of microsporidium, based on microscopic examination, was found in Thai HIV-infected patients. This confirms the importance of microsporidium in HIV-infected/AIDS patients and the necessity for stool evaluation in all HIV-infected patients.

INTRODUCTION

Diarrhea is a common clinical manifestation in human immunodeficiency virus (HIV) infection. The prevalence ranges from 33% (Pape *et al*, 1994) to 45% (Tarimo *et al*, 1996). Important protozoan pathogens causing diarrhea include cryptosporidium, microsporidium, *Isoospora belli* and cyclospora besides *Giardia intestinalis* and *Entamoeba histolytica* (Deodhar *et al*, 2000). The diarrhea, often associated with weight loss, may affect the nutritional state and quality of life in AIDS patients. It has been shown that patients with AIDS who presented with diarrhea have a greater degree of immunosup-

pression than those without diarrhea, predisposing the gastrointestinal tract to the action of protozoa, bacteria, viruses, and fungal pathogens which may cause morbidity and death (Manatsathit *et al*, 1996). Enteric protozoan parasites have become increasingly recognized as important and rapidly emerging human pathogens in immunocompromised individuals. They tend to aid disease progression and may be lethal. Manatsathit *et al* (1996) reported that cryptosporidium (20%) was the most common cause of chronic diarrhea in Thai HIV-infected patients. Other leading causes of morbidity include *Mycobacterium tuberculosis*, *Salmonella*, Cytomegalovirus and *Mycobacterium avium intracellulare* (Manatsathit *et al*, 1996). Microsporidium is also an important protozoan infecting HIV/AIDS patients. In general, the prevalence of microsporidium in HIV-infected patients ranges from 1-40% (Kotler and Orenstein, 1994). In Thailand,

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several studies have reported the prevalence to be from 1.67% to 33.3% (Punpoowong *et al*, 1998; Wanachiwanawin *et al*, 1998; Wiwanitkit, 2001). The prevalence of microsporidium infection varies greatly, and identification of the organism is often associated with diagnostic difficulties. Diagnosis of microsporidium is still dependent on morphological demonstration of the organisms. We studied the enteric pathogens in HIV-infected patients with a special emphasis on microsporidium, and we sought to determine possible clinical correlations.

MATERIALS AND METHODS

The study received ethical clearance from the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. We gathered stool samples from 64 Thai HIV-infected patients and examined for possible pathogens. Collected stool samples were prepared according to the study by Punpoowong *et al* (1998). They were fixed with 10% formalin, filtered with layers of gauze and the filtered product centrifuged. The supernatant was discarded, leaving the precipitate which was used for smear preparation. Each sample was stained with the following:

- 1) Modified hematoxylin and eosin stain to screen for possible organisms;
- 2) quick-hot Gram-chromotrope stain (Moura *et al*, 1997) to identify microsporidium;
- 3) modified acid-fast bacilli stain (DMSO method) to identify cryptosporidium and isospora;
- 4) Ziehl-Neelsen stain to identify cyclospora;
- and 5) modified Kinyoun's stain to identify acid-fast bacilli, cryptosporidium and cyclospora.

Information regarding clinical data was gathered by means of questionnaires with answers obtained on the day of sample collection. The latter collected information regarding age, sex, length of HIV seropositivity, risk factors for HIV infection, clinical

symptoms with special emphasis on diarrhea and associated symptoms.

Data analysis

Data gathered were described using descriptive statistics. Data were recorded and analyzed using chi-square for statistical significance.

RESULTS

The study documented the causes of diarrhea in HIV-infected patients. Of the 64 stool samples collected, infection with microsporidium (Fig 1) appeared to be most common with a prevalence of 81.2%. Mixed infections were identified in 26 out of 64 cases (40.6%), and 39.1% (25/64) had mixed infection with microsporidium. Microsporidium was identified as a co-infection with the following organisms: *Candida albicans* (5), cryptosporidium (4), cryptosporidium and *Giardia intestinalis* (1), cryptosporidium and *C. albicans* (1), cryptosporidium, cyclospora and *B. hominis* (1), *B. hominis* (5), *Opisthorchis viverrini* (3), *G. intestinalis* (2), *Strongyloides stercoralis*(2), and *Cyclospora* sp (1). Mixed infection without microsporidium was noted in only one case (cryptosporidium and cyclospora). Opportunistic protozoans were demonstrated in Fig 2(a-f). In three cases (4.7%) no organisms were found. The prevalence rates of non-opportunistic and opportunistic infections are shown in Table 1.

Forty-nine (76.6%) of the patients completed the questionnaire. There were 49 male patients (76.6%) and 15 female patients (23.4%). The length of HIV infection was more than 48 months in 28.6%, and 0-6 months in 20.4%. The most common risk factor for HIV infection in this study group was sexual transmission (93.9%). Only two cases (4.1%) acquired the infection by the use of intravenous drugs. There was one case (2.0%) of perinatal transmission.

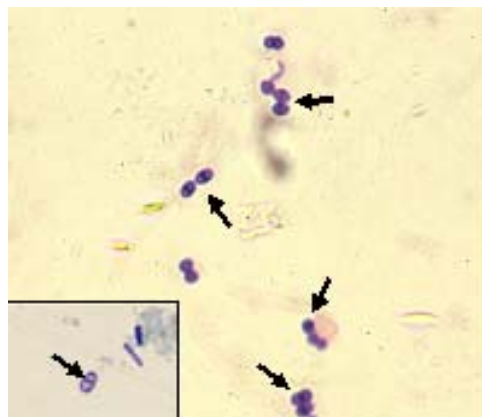


Fig 1—Stool sample of HIV-infected patient showing microsporidium. Note the important belt stripe which is usually centrally located (arrow) clearly seen in the inset. Gram chromotrope 1,000x.

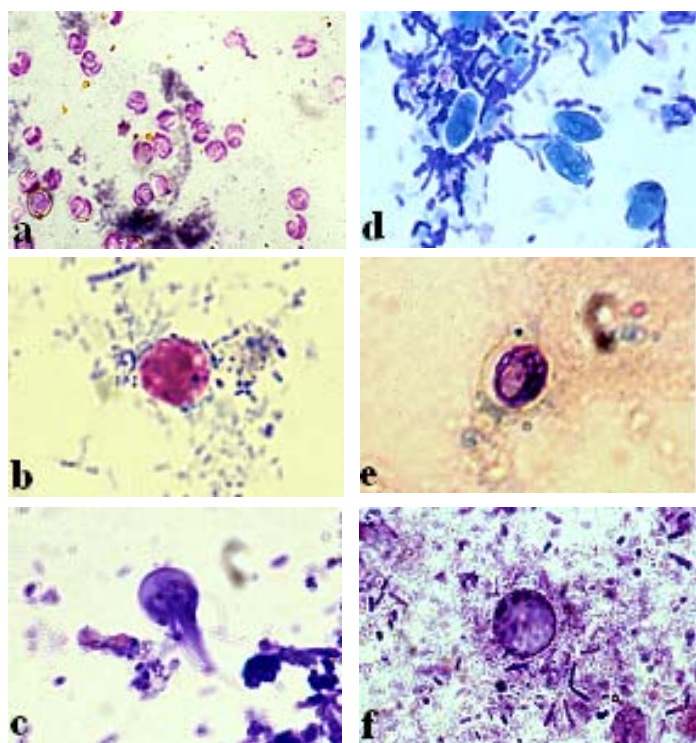


Fig 2—Opportunistic protozoa found in HIV-infected patients, showing cryptosporidium containing sporozoites, DMSO 1,000x (a); Cyclospora, Ziehl-Neelsen 1,000x (b); *Giardia intestinalis* trophozoite (c) and cysts (d), Gram chromotrope 1,000x; *Blastocystis hominis*, vacuolated form showing distinct glycogen vacuole, Ziehl-Neelsen 400x (e) and *B. hominis*, intermediate form, Gram chromotrope 1,000x (f).

The common presenting clinical symptom was fever (38.8%), followed by weight loss (32.7%) and diarrhea (30.6%). These cases usually (75.5%) occurred within one year of being diagnosed with HIV. At the time of interview, only 17 cases (34.7%) had diarrhea. The duration of diarrhea was 0-2 months in 52.9%, followed by 3-4 months in 29.4% and 5-9 months in 17.7%. Diarrhea was reported 1-2 times/day in 41.2% and 3-4 times/day in 35.3%. One case reported diarrhea of more than 10 times/day. The characteristics of the stools were watery or loose (100%), predominantly yellow (47.1%), followed by brown (17.7%), red (17.7%), green (11.8%) and black (5.9%). Mucus was present in the stool in 52.9%. Bloody diarrhea was present in 3 cases (17.6%). Associated abdominal pain was seen in 52.9%, tenesmus in 29.4%, nausea in 23.5% and vomiting in 17.6%.

Treatment

Thirty-two of 49 cases (65.3%) received treatment for opportunistic infections (Bactrim-28.6%, anti-fungal drugs-16.3% and anti-tuberculosis drugs-26.5%). Supportive drugs for diarrhea included oral rehydration solution (6.1%) and Lomotil (oral anti-diarrheal drug—a combination of diphenoxylate and atropine) (4.1%). The anti-retroviral drugs used by the patients

Table 1
Opportunistic infections in 64 HIV-infected patients.

Organism	Single infection n (%)	Mixed infection n (%)	Total n (%)
<i>Microsporidium</i> sp	26 (40.6)	26 (40.6)	52 (81.2)
<i>Cryptosporidium</i> sp	6 (9.4)	7 (10.9)	13 (20.3)
<i>Candida</i> sp	2 (3.1)	6 (9.4)	8 (12.5)
<i>Blastocystis hominis</i>	1 (1.6)	6 (9.4)	7 (10.9)
<i>Giardia intestinalis</i>	1 (1.6)	3 (4.7)	4 (6.2)
<i>Cyclospora</i> sp	1 (1.6)	2 (3.1)	3 (4.7)
<i>Opisthorchis viverrini</i> ova	0 (0)	2 (3.1)	2 (3.1)
<i>Strongyloides stercoralis</i> larvae	0 (0)	2 (3.1)	2 (3.1)
Hookworm ova	0 (0)	2 (3.1)	1 (1.6)

Table 2
Correlation between clinical data and presence of microsporidium and cryptosporidium infection in 49 cases of HIV-infected patients ($\alpha \leq 0.05$).

Demographic data	p-value	
	Microsporidium infection	Cryptosporidium infection
Sex (male/female)	0.7153	1.0000
Time being anti-HIV positive (0-24/>24 months)	0.4936	0.0600
Time being anti-HIV positive (0-48/>48 months)	0.7059	0.0960
Diarrheal manifestation (+/-)	0.4701	0.1097
History of diarrhea (+/-)	0.7250	0.4230
Length of diarrhea (0-2/3-3 months)	0.5764	1.0000
Stools/day (1-2/>3 times/day)	1.0000	1.0000
Color of diarrhea (yellow/red, green, brown and black)	0.0823	0.2941
Mucus diarrhea (+/-)	0.5764	1.0000
Bloody diarrhea (+/-)	1.0000	0.5411
Nausea (+/-)	0.5411	1.0000
Vomiting (+/-)	1.0000	0.5411
Stomachache (+/-)	0.2058	0.5764
Tenesmus (+/-)	1.0000	1.0000

were nucleoside reverse transcriptase inhibitors (NRTIs), which included zidovudine (2.0%), zidovudine and didanosine (4.1%), zidovudine and stavudine (6.1%). Seventeen cases (34.7%) had no treatment for opportunistic infections.

Analysis for possible correlations

There was no statistical significance between the demographic data and clinical symptoms collected and the detection of microsporidium in our study as shown in Table 2.

DISCUSSION

Microsporidia are a group of intracellular protozoans that measure 1-2 μm in size and have been described in HIV-infected patients (Desportes *et al*, 1985; Canning and Hollister, 1990; Kotler and Orenstein, 1994; Punpoowong *et al*, 1998; Wanachiwanawin *et al*, 1998). The first case of microsporidia infection was reported in 1959 (Matsubayashi *et al*, 1959). The commonest organisms in this group identified are *Enterocytozoon bieneusi* and *Septata intestinalis*. Although cryptosporidium is one of the most common causes of enteric infection, microsporidium sp (*E. bieneusi*) and cyclospora are emerging as important pathogens causing diarrhea in HIV-infected patients. Enteric protozoa are seen more commonly than other parasitic infections in HIV-infected patients.

The diagnosis of opportunistic infection in stool samples is difficult, especially the identification of microsporidia in routine stool samples. Our study utilized the Quick-hot-gram chromotrope stain (Moura *et al*, 1997) and found a high yield of microsporidium (81.2%) in this group of HIV-infected patients, which remained undetected on the original modified trichrome stain method used previously. The organism has been considered as a rare cause of diarrhea in AIDS patients and was previously reported in only 1.7% (Wiwanitkit, 2001) to 33.3% (Wanachiwanawin *et al*, 1998) of cases. Such a high prevalence in our study may be due to improved techniques for identification in the laboratory. Mixed infection was very common in our study group. Many were seen associated with *C. albicans* and cryptosporidium. Microsporidium was seen associated with a wide variety of pathogens.

We studied the clinical correlations with the occurrence of opportunistic intestinal infection. There were no significant associations between the clinical presentation and

the presence of microorganisms in the stool (Table 2). The clinical presentation does not provide a good basis for treatment, therefore, the correct identification of the microorganism is key to the most appropriate therapeutic approach.

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