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Intestinal Parasitic Infections in HIV and non-HIV Infected Patients with Chronic Diarrhea in Thailand

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Abstract : Chronic diarrhea is a common problem in AIDS patients, and enteric parasites are recognized as important causes. This study determined the prevalence of parasitic infections in HIV infected patients with chronic diarrhea. Ninety-one patients with AIDS who presented with chronic diarrhea and 103 patients who were HIV negative were enrolled in the study. Detection of stool parasites was made by microscopy of simple smear, formalin-ether concentration method, modified acid-fast and modified trichrome staining techniques. Species identification of microsporidia spores was made by transmission electron microscopy (TEM). Cases in which serial fecal examinations were negative were subjected to gastroduodenoscopy and/or colonoscopy. Parasitic infections were found in 51 (56%) AIDS patients with chronic diarrhea and in 18 (17%) non-HIV cases ($p < 0.001$). Microsporidia and *Cryptosporidium parvum* were the most common parasites found in HIV infected patients and were also significantly more prevalent than in non-HIV infected cases [29% vs 0% for microsporidia, ($p < 0.001$), and 25% vs 1% for *C. parvum*, ($p < 0.001$)]. Species identification of microsporidia spores in 26 patients using TEM revealed *Enterocytozoon bienersi* in 19 cases. This study indicated that microsporidia and *C. parvum* are important pathogenic causes of chronic diarrhea in AIDS patients in Thailand.

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เรื่องย่อ : การติดเชื้อปรสิตลำไส้ในผู้ป่วยที่ติดเชื้อเอชไอวีและที่ไม่ติดเชื้อเอชไอวีที่เป็นโรคท้องเสียเรื้อรัง. ดาราวรรณ วนะชีวานาวิน พ.บ.*, ศดาพร มานัสสทธิชัย พ.บ.***, พันธุ์พบ เลิศลายต่วน ค.บ.*, เกลิยวพันธ์ เถลิงพล วท.ม.***, ภาวิณี สุวรรณกุล พ.บ.***

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โรคท้องเสียเรื้อรังเป็นปัญหาที่สำคัญในผู้ป่วยที่ติดเชื้อเอชไอวี จากรายงานการตรวจหาสาเหตุของโรคท้องเสียเรื้อรังในผู้ป่วยเอดส์ในประเทศที่พัฒนาแล้วพบว่าสาเหตุส่วนใหญ่มาจากเชื้อปรสิต เนื่องจากข้อมูลของโรคท้องเสียเรื้อรังที่เกิดจากเชื้อปรสิตในผู้ป่วยในประเทศไทยนั้นมีจำนวนน้อยมาก คณะผู้วิจัยจึงได้ศึกษาเปรียบเทียบการติดเชื้อปรสิตลำไส้ในผู้ป่วยที่มีอาการอุจจาระร่วงเรื้อรังที่ติดเชื้อเอชไอวีจำนวน 91 ราย และที่ไม่ติดเชื้อเอชไอวีจำนวน 103 ราย ระหว่างเดือนมกราคม พ.ศ. 2537 ถึงเดือนมิถุนายน พ.ศ. 2540 พบอัตราการติดเชื้อปรสิตในผู้ป่วยเอดส์เป็นร้อยละ 56 และในผู้ป่วยที่ไม่ติดเชื้อเอชไอวีเป็นร้อยละ 17 ($p < 0.001$). *Microsporidia* และ *Cryptosporidium parvum* เป็นปรสิตที่เป็นสาเหตุของโรคท้องเสียเรื้อรังที่พบในผู้ป่วยที่ติดเชื้อเอชไอวีซึ่งมากกว่าที่พบในผู้ป่วยที่ไม่ติดเชื้อเอชไอวีอย่างมีนัยสำคัญทางสถิติ (ร้อยละ 29 และร้อยละ 0, $p < 0.001$, และร้อยละ 25 และร้อยละ 1, $p < 0.001$ ตามลำดับ) ส่วนการติดเชื้อปรสิตชนิดอื่นๆ ในผู้ป่วยทั้งสองกลุ่มไม่มีความแตกต่างกันทางสถิติ จากการตรวจสอบของเชื้อ *microsporidia* ด้วยวิธี transmission electron microscopy พบว่าเป็นชนิด *Enterocytozoon bieneusi* จำนวน 19 รายใน 26 ราย โดยสรุปผลการศึกษานี้พบว่าเชื้อปรสิต *microsporidia* ชนิด *E. bieneusi* และ *C. parvum* เป็นสาเหตุที่สำคัญของโรคท้องเสียเรื้อรังในผู้ป่วยเอดส์ในประเทศไทย

INTRODUCTION

Gastrointestinal disorders are among the major complications associated with human immunodeficiency virus (HIV) infection, and diarrhea is the most common enteric manifestation in acquired immunodeficiency syndrome (AIDS) patients. Diarrhea in these patients is usually chronic, and often results in significant morbidity and mortality. Definitive causes of chronic diarrhea can be identified in up to 85%-90% of cases, and multiple concurrent intestinal pathogens are commonly detected. According to western literature, the most frequently found organisms in stools are microsporidia, *Cryptosporidium* and *Mycobacterium avium* complex¹. *Giardia lamblia* and *Campylobacter* species are also common enteric agents reported in most studies². These reports suggest that parasites are the most important etiology of chronic diarrhea in AIDS.

However, there are very few reports of parasites causing diarrhea in AIDS and non-AIDS patients in Thailand^{3,4}. Hence, the purpose of this study was to determine the prevalence of parasitic infections in HIV infected patients with chronic diarrhea and to compare the findings in patients who were HIV negative.

MATERIALS AND METHODS

Patients and methods

Patients with chronic diarrhea attending the Outpatient diarrhea clinic or hospitalized either in Siriraj Hospital, Bangkok or Bamrasnaradura Infectious Diseases Hospital, Nonthaburi, Thailand between January 1994 and June 1997 were included in the study. There were 91 patients with AIDS and 103 patients with negative serology for HIV. Chronic diarrhea was defined as three or more loose or watery

bowel movements per day for at least 4 weeks. All patients had routine stool bacterial cultures and examinations for parasites. Methods for parasitic detection included simple smear, formalin-ether concentration, modified acid-fast and modified trichrome staining techniques. In all cases, multiple stool samples were examined. Species identification of microsporidia spores was done by using transmission electron microscopy (TEM). Gastroduodenoscopy and/or colonoscopy with biopsy were performed in cases where serial fecal examinations were negative, because some organisms may not be found in the stool but may be present in the intestinal mucosa.

Statistical analysis

Chi-square test with Yates' correction and Fisher's exact test were used for statistical analyses. A p value of less than 0.05 was considered significant.

RESULTS

The characteristics of all the patients in the study are outlined in Table 1. CD4 cell counts were available in 38 cases with AIDS. Intestinal parasites were found in fifty-one (56%) of 91 AIDS patients and 18 (17%) of 103 HIV seronegative patients with

chronic diarrhea (Table 2). Fourteen (15%) of the seropositive and four (4%) of the seronegative patients had more than one parasite in their stools. The prevalence of enteric parasitic infections was significantly greater in the HIV group than in the non-HIV group ($p < 0.001$). Multiple concomitant pathogenic parasites were more common in HIV infected patients than in HIV seronegative patients, but there was no statistical significance ($p = 0.763$). Intestinal parasitic causes of chronic diarrhea in both groups are shown in Table 3. Microsporidia and *Cryptosporidium parvum* were the two most common parasites found in the HIV group. They were significantly more prevalent in patients with AIDS than in non-HIV patients. TEM for species identification of microsporidia spores of 26 patients with intestinal microsporidiosis revealed *Enterocytozoon bieneusi* in 19 cases. In the remaining 7 cases, the parasites were not detected by TEM. Electron micrographs of the microsporidia spores demonstrated an electron-dense exospore layer and an electron-lucent inner wall. Each spore showed a characteristic double row of polar filament lying on either side of a posterior electron-lucent inclusion. The number of coils of polar tubule ranged from 4 to 8. All cases with cryptosporidiosis were diagnosed by modified acid-fast staining of stool, except for one made by histopathology.

Table 1. Characteristics of 91 HIV seropositive and 103 HIV seronegative patients with chronic diarrhea

	HIV group	non-HIV group
Age (mean)	31.1	40.6
Range (years)	16-66	17-81
Sex, male : female	80 : 11	59 : 44
CD4 count (cells/mm ³)	52±40	ND

ND = Not done

Table 2. Prevalence of pathogenic parasitic infection in HIV seropositive and HIV seronegative patients with chronic diarrhea

No. of kinds of parasites	HIV group n = 91	non-HIV group n = 103
1	37	14
2	11	2
3	2	2
4	1	0
Total	51 (56%)	18 (17%)

Table 3. Enteric parasitic causes of chronic diarrhea in HIV and non-HIV infected patients

Parasite	HIV group n = 91 (%)	non-HIV group n = 103 (%)	P
Microsporidia	26 (29)	0 (0)	< 0.001
<i>Cryptosporidium parvum</i>	23 (25)	1 (1)	< 0.001
<i>Isospora belli</i>	7 (8)	2 (2)	0.086
<i>Strongyloides stercoralis</i>	5 (5)	3 (3)	0.478
<i>Giardia lamblia</i>	5 (5)	1 (1)	0.101
<i>Blastocystis hominis</i>	2 (2)	7 (7)	0.177
Other nematodes			
<i>Trichuris trichiura</i>	2 (2)	0 (0)	0.219
Hookworm	1 (1)	2 (2)	1.000
<i>Capillaria philippinensis</i>	0 (0)	2 (2)	0.499
Flukes			
<i>Opisthorchis viverrini</i>	1 (1)	3 (3)	0.624
Small intestinal fluke	1 (1)	0 (0)	0.469
Other protozoa			
<i>Cyclospora cayentanensis</i>	1 (1)	0 (0)	0.469
<i>Entamoeba histolytica</i>	0 (0)	1 (1)	1.000
<i>Dientamoeba fragilis</i>	0 (0)	1 (1)	1.000

DISCUSSION

Since the start of the HIV epidemic, a number of opportunistic parasites have been increasingly recognized. Intestinal parasitic pathogens, such as microsporidia, *C. parvum* and *I. belli* are important causes of chronic diarrhea and are associated with weight loss in AIDS patients¹. The present study showed that parasitic infections were commonly found in HIV infected patients with persistent diarrhea, and accounted for 56% (51 out of 91) of the cases. Similarly, in AIDS patients with chronic diarrhea in Zaire and Zambia, the prevalence rates of enteric parasites were 59% (63 out of 106) and 57% (25 out of 44) respectively^{5,6}. In this study, the intestinal parasitic infection in non-AIDS patients with chronic diarrhea was 17% (18 out of 103), which was significantly less than that in the HIV seropositive group. In the group in Zaire, 15 out of 21 non-HIV infected cases (71%) were found to be positive for enteric parasites⁵, which was higher than the present report. This discrepancy may be explained by a difference in the number of subjects studied.

Microsporidia have been commonly detected in HIV infected patients. The frequency of intestinal microsporidiosis in AIDS patients with chronic diarrhea ranged from 24% to 50%. Few HIV seronegative patients with enteritis have been reported to be infected with microsporidia¹. In this study, the prevalence rate of intestinal microsporidia infection among HIV seropositive patients was 29%. In contrast, microsporidia were not identified in non-HIV infected patients with chronic diarrhea. Our finding is similar to previous reports showing that microsporidia are major parasitic causes in diarrheal disease associated with AIDS^{7,8}. Cases of intestinal microsporidiosis were generally diagnosed by fecal smears stained with modified trichrome stain⁹. Multiple stool examinations have improved the detection rate of microsporidia spores in cases which are infected with few organisms¹⁰. Although this method is simple and practical for most laboratories, species identification cannot be done using this method. TEM is essential for species identification. Most patients with microsporidial diarrhea were infected by *E. bienersi*. *Encephalitozoon intestinalis* was less commonly detected as a cause of diarrhea¹¹. The present study confirms that *E. bienersi* is the principal organism causing chronic diarrhea among

HIV infected patients. However, the species of microsporidia parasites was not identified in seven patients because of light infection and the insensitivity of TEM.

C. parvum infection has been documented as a cause of diarrhea in both immunocompromised and immunocompetent hosts, but their courses and prevalences differ substantially. Severe persistent enteritis with weight loss is seen in most patients with AIDS whereas self-limited diarrhea is commonly found in immunocompetent persons. *Cryptosporidium* accounts for 10% to 38% of the cases of diarrhea in HIV infected patients¹. In the non-AIDS group, the prevalence is lower than in AIDS patients, and the parasitic infection appears to be more common in children^{1,4}. Our data showed that *C. parvum* was the second most common parasite causing enteropathy in AIDS patients. The prevalence rate from cases of chronic diarrhea with HIV infection was significantly higher than that without AIDS (25% versus 1%). These results are in concordance with the study in Zaire, in which cryptosporidiosis was found in 22% and 0% of HIV seropositive and HIV seronegative cases with persistent diarrheal illness respectively⁵. The autoinfective capability of *C. parvum* results in overwhelming multiplication in the immunocompromised patient and this can produce severe and prolonged diarrhea. In addition, effective treatment for cryptosporidiosis in a susceptible host has not been established although various drugs have been studied in an attempt to eliminate the organism¹²⁻¹⁴.

As shown in Table 3, the parasites found to be more prevalent in the AIDS group than in the non-AIDS population included *I. belli*, *S. stercoralis* and *G. lamblia*. However, there were no statistical differences. This study is similar to other reports showing that isosporosis is less prevalent than cryptosporidiosis in AIDS patients associated with diarrhea^{5,15,16}. In contrast to *C. parvum*, *I. belli* is considered as a pathogen only in man and animal reservoir hosts are not recognized. Furthermore, there is no autoinfection in the *Isospora* life cycle, and isosporosis appears to be successfully treated with sulfamethoxazole-trimethoprim^{13,17}. *S. stercoralis* infection may contribute to serious problems because of the internal autoinfective portion of the life cycle, particularly in the immunosuppressed host. It has been documented that *G. lamblia* infection is more common in

children than in adults. Susceptibility to giardiasis has also been seen in compromised individuals. There was speculation that infections with *S. stercoralis* and *G. lamblia* might be influenced by a patient's HIV status. However, our findings and previous studies demonstrated no difference in prevalence rates of infection with these parasites in AIDS patients and the non-AIDS population^{1,13}.

In the present report, *B. hominis* was found to be the most common parasite associated with chronic diarrhea in immunocompetent patients. It appeared to be more prevalent in non-HIV than in HIV infected cases but this was not statistically different. Our data confirmed that the incidence of blastocystosis in AIDS patients did not differ from that in normal hosts associated with enteritis¹. In contrast to the study from Zaire, *B. hominis* infection was significantly higher in HIV seronegative than in HIV seropositive patients with persistent diarrhea⁵. This finding may be due to the small number of non-AIDS patients in the latter report.

CONCLUSION

We have shown that parasites are common pathogens associated with persistent diarrhea. The prevalence of intestinal parasitic infection is significantly higher in AIDS patients than in the non-AIDS group. Microsporidia and *C. parvum* are the organisms most frequently present in HIV infected cases with chronic diarrhea. The majority of patients with intestinal microsporidiosis are infected by *E. bienersi*.

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