Indian J Med Res 109, March 1999, pp 85-89

Enteric pathogens in southern Indian HIV-infected patients with & without diarrhoea

Ashis Mukhopadhya, B.S. Ramakrishna, Gagandeep Kang, Anna B. Pulimood, Minnie M. Mathan
Anand Zachariah* & Dilip C. Mathai*

*Wellcome Trust Research Laboratory, Department of Gastrointestinal Sciences & *Department of Medicine*
Christian Medical College Hospital, Vellore

Accepted February 8, 1999

This study was undertaken to determine the carriage rate of various enteric pathogens in southern Indian patients with HIV infection, both with and without diarrhoea. Stool from 111 consecutive HIV-positive patients (50 without and 61 with diarrhoea) was examined by microscopy and culture. Jejunal biopsy and fluid examination were carried out if diarrhoea persisted, with negative stool examination. Enteric pathogens were detected from stool in 57.4 per cent of diarrhoeal patients compared to 40 per cent of those without diarrhoea ($P > 0.05$). Jejunal biopsy and fluid examination provided 11 additional diagnoses. Protozoa accounted for 71.8 per cent of all pathogens isolated. *Isospora* was significantly more common in patients with (11/61) than in those without (2/50) diarrhoea ($P < 0.05$). Bacterial pathogens were isolated more commonly from patients with diarrhoea (12/61 compared to 2/50, $P < 0.05$). Isolation rate of pathogens was higher from patients with diarrhoea for more than 2 wk, compared to those with less than 2 wk duration. Remission of diarrhoea either spontaneously or with symptomatic therapy was observed in 22 patients with acute diarrhoea. A high enteric carriage of a number of pathogens was noted in HIV patients without diarrhoea, but *I. belli* and bacterial enteropathogens were more likely to be associated with diarrhoea.

Key words Acquired immunodeficiency syndrome - diarrhoea - enteric pathogens - human immunodeficiency virus

Diarrhoeal disease is a common complication of infection with the human immunodeficiency virus (HIV). Diarrhoea occurs in almost 90 per cent of patients with HIV in developing countries at some time during the clinical course[1], and is the presenting symptom of approximately a third of patients with HIV infection[2]. Chronic diarrhoea significantly reduces the quality of life in patients with HIV infection[3], and is an independent predictor of mortality in AIDS[4,5]. A variety of enteric pathogens have been isolated from AIDS patients with diarrhoea[6-9], but it is not clear that these enteric infections are necessarily associated with the presence of diarrhoea. There are significant geographic variations in the prevalence of individual enteric infections in HIV patients[6]. The present study was undertaken as a descriptive study of enteric pathogens in southern Indian patients with HIV infection and diarrhoea, and to determine whether there were significant differences in the overall and individual prevalence of enteric infection when compared to HIV patients without diarrhoea.

Material & Methods

The study was carried out in the Departments of Medicine and Gastrointestinal Sciences of the Christian Medical College Hospital, Vellore, between May

1995 and September 1996. A total of 111 consecutive patients who were HIV seropositive were included in the study. Of these 61 presented with diarrhoea (defined as three or more semi-liquid or liquid stools in the preceding 24 h). These patients had 65 episodes of diarrhoea that were investigated. Fifty HIV seropositive individuals did not have diarrhoea, and were included as a control group. HIV infection was defined as a reactive ELISA test with 2 different kits (VBI HIV - 1/2 EIA, United Biochemical Inc., USA, and Abbott HIV-1/HIV-2 third generation plus EIA, Abbott Laboratories, USA) or a positive Western blot test (DBL Western Blot Assay, HIV Blot 2.2, Genelabs Diagnostics, USA). AIDS was diagnosed on the basis of the 1987 case definition of AIDS established by the Centers for Disease Control and Prevention[10]. Symptomatic patients were categorised into two groups, acute (diarrhoea for less than 14 days) and chronic (diarrhoea lasting longer than two weeks).

All patients enrolled in the study underwent a complete physical examination, including rectal examination and proctoscopy. Three freshly voided specimens of stool were obtained from all subjects enrolled in the study, and were examined for parasites using saline and iodine preparations; formol-ether concentration; and staining with safranine-methylene blue and auramine[11,12]. One specimen of stool was cultured for enteric bacterial pathogens (excluding *Campylobacter* and *Clostridium difficile*).

In patients with diarrhoea, further testing was carried out if diarrhoea continued and faecal examination and culture did not reveal a pathogen. This consisted of peroral biopsy of the jejunal mucosa using a peroral biopsy capsule (Watson, UK), along with jejunal fluid aspiration. Jejunal fluid was examined by microscopy and culture. Biopsy specimens were bisected and one part fixed in 10 per cent formol-saline and taken through paraffin for staining with haematoxylin and eosin and examination by light microscopy. The remainder of the biopsy was fixed in glutaraldehyde and paraformaldehyde and sections processed for electron microscopy using a EM 201 electron microscope (Philips, UK). Jejunal intubation for biopsy or fluid aspiration was not performed in HIV patients without diarrhoea. The study protocol was approved by the Research Committee of the Christian Medical College, Vellore.

Statistical analysis was done using the Epi-Info software on a personal computer. The Student's 't' test or Chi-square test with Yates correction was used as appropriate to determine significance of differences.

Results

General patient characteristics : Thirty patients with acute diarrhoea and 31 patients with chronic diarrhoea were studied. Patients with acute diarrhoea had a mean (SD) age of 33.6 (7.4) yr, while patients with chronic diarrhoea were 33.3 (9.9) yr old. Mean duration of diarrhoeal illness was 8.9 days in patients with acute diarrhoea compared to 187.8 days in those with chronic diarrhoea. Stool frequency was comparable in the two groups (5.5 and 5.2 stools per day respectively). Diarrhoea was of small bowel type in the majority (87.9 and 90.6%). The mean interval between diagnosis of HIV infection and presentation with diarrhoea was 9.36 months in the acute group and 3.19 months in the chronic group ($P = 0.03$). Diarrhoea was the presenting symptom in seven of the acute group (23.3%) compared to 21 of the chronic group (67.7%). Sixteen patients with acute diarrhoea and 12 with chronic diarrhoea had clinical AIDS. Diagnosis of AIDS, which is based on the presence of certain opportunistic infections, was made on identification of enteric pathogens in 5 of the patients with chronic diarrhoea (4 *Isospora*, 1 *Cryptosporidia*).

Pathogens : Stool microscopy and culture revealed enteric pathogens in 35 (57.4%) patients with diarrhoea compared to 20 (40.0%) patients who did not have diarrhoea, the difference being non significant. Addition of jejunal fluid examination and biopsy increased the total yield of pathogens to 70.0 per cent in acute diarrhoea and 80.6 per cent in chronic diarrhoea (Table I). As spontaneous remission of diarrhoea was common

Table I. Utility of various tests in the etiological diagnosis of diarrhoea in HIV patients

Test	Acute diarrhoea	Chronic diarrhoea
Faeces parasites	14/30	11/31
Faeces culture	5/30	5/31
Jejunal biopsy	2/6	8/16
Jejunal culture	0/6	1/16

The denominator in each instance gives the number of patients in whom the investigation was performed. Jejunal biopsy and fluid culture were undertaken only when stool examination was negative and diarrhoea persisted despite symptomatic therapy

in patients with acute diarrhoea, jejunal biopsy and aspiration were carried out on only 6 patients with acute diarrhoea compared to 16 patients with chronic diarrhoea. More than one pathogen was sometimes detected in the same patient. Two co-infecting pathogens were isolated from 4 of 50 patients without diarrhoea, compared to 13 of 61 patients with diarrhoea.

Table II lists the individual pathogens isolated. Protozoal infections were common in all HIV patients, accounting for 21 of 25 pathogens isolated from non-diarrhoeal patients, 20 of 31 pathogens in the acute diarrhoea group, and 21 of 30 infections in the chronic diarrhoea group. *Giardia intestinalis* infection was the most common parasitic infection in non-diarrhoeal patients (8 of 25 isolates, 32%). Prevalence of *Giardia* was lower though not significantly in patients with diarrhoea (9 of 61 isolates, 14.9%). *I. belli* was more common in patients with diarrhoea (11 of 61 isolates, 18.0%) compared to patients without diarrhoea (2/25, 8%) ($P < 0.05$).

Table II. Pathogens isolated from HIV patients

Pathogen	No diarrhoea (50)	Acute diarrhoea (30)	Chronic diarrhoea (31)
Protozoa			
Isospora belli	2	3	8
Giardia intestinalis	8	4	5
Cryptosporidium parvum	3	5	1
Cyclospora cayatenensis	1	2	2
Microsporidia spp.	2	2	2
Blastocystis hominis	2	1	2
Balantidium coli	0	1	0
Entamoeba histolytica	0	1	0
Dientamoeba fragilis	3	1	1
Nematodes :			
Strongyloides stercoralis	2	3	2
Ascaris lumbricoides	0	1	0
Cestodes :			
Hymenolepis nana	0	1	0
Bacteria :			
Shigella spp.	1	1	2
Aeromonas spp.	0	2	2
Vibrio spp.	0	2	1
Salmonella spp.	1	0	1
Enterobacter spp.	0	0	1
Virus :			
Cytomegalovirus	0	1	0

Course : Diarrhoea settled spontaneously or with symptomatic therapy (anti-diarrhoeal agents, oral hydration) in 22 patients with acute diarrhoea and in 9 with chronic diarrhoea. Specific therapy included co-trimoxazole, albendazole, metronidazole, ampicillin, tetracycline and ciprofloxacin. Three patients with acute diarrhoea had recurrence after subsidence of the original symptoms. In each instance a new organism was isolated. Two patients with chronic diarrhoea (*Isospora* 1 and *Strongyloides* 1) continued to have infection and diarrhoea despite therapy, and both died during the study period. Seven patients were lost to follow up.

Discussion

The present report documents that infection with enteric pathogens is common in southern Indian HIV patients, regardless of the presence of diarrhoea. The overall isolation of enteric pathogens from stool was similar in patients without or with diarrhoea. A similar observation in the past cast doubts on the relevance of infective agents isolated from the gastrointestinal tract of HIV patients[7]. However, specific pathogens were clearly associated with diarrhoea. These included *I. belli*, found in 11 of 61 diarrhoeal patients compared to 2 of 50 non-diarrhoeal controls, and bacterial enteropathogens isolated from 12 diarrhoeal patients compared to 2 controls. *G. intestinalis*, *Cyclospora* and *Microsporidia* were not related to the presence of diarrhoea. It is not clear that diarrhoea in HIV patients can be ascribed to these pathogens. Enterocyte or neural dysfunction related to HIV infection[13,14] may be responsible for diarrhoea in some. Alternatively, there may be quantitative differences in parasite burden between patients with and without diarrhoea. Pathogens infecting HIV patients with diarrhoea may differ in genotype or phenotype from those in HIV patients without diarrhoea. It would be interesting to examine this possibility and

to compare the above with pathogens isolated from HIV-negative patients with diarrhoea.

Figures from various studies demonstrate striking geographic variations in the prevalence of individual pathogens in HIV infected patients[6,7,15-18]. These variations may relate both to the prevalence of pathogens within the community, and to drugs used prophylactically in patients with HIV infection and AIDS. Isolation rates, in this study, of *Cryptosporidium* (10%, reported rates 6-37%), *Giardia* (15%, reported rates 1-11%) and *Microsporidia* (6%, reported 2-39%) were within the ranges reported from elsewhere[6,7,15-18]. The isolation rate of *I. belli* was high in our subjects (18%) compared to most studies (0-3%), although this protozoan was isolated from 28 per cent of Zambian patients with AIDS and diarrhoea[18]. The present study, appears to be the first report of the detection of *Cyclospora cayatenensis* from India. This parasite was detected both in non-diarrhoeal and diarrhoeal patients. Enteric polymicrobial infection was noted in 8 per cent of non-diarrhoeal HIV patients and in 21 per cent of diarrhoeal patients. This is comparable to a 11 per cent poly-infection rate reported in HIV patients with diarrhoea[7].

Previous studies in HIV patients have not differentiated between acute and chronic diarrhoea. Such a differentiation may be useful in practice, since the majority of acute episodes of diarrhoea were self-limited. The limited follow up in this study did not permit significant inferences regarding the course or recurrence of diarrhoea in HIV patients.

Lack of resources often limits investigation in developing countries. Knowledge of the pattern of infection in HIV patients with diarrhoea may be used in empiric treatment strategies. In Zambia, albendazole was tried as monotherapy since it acted against *Microsporidia, Strongyloides* and *Giardia*[18]. Empiric therapy with albendazole resulted in sustained remission of diarrhoea in 26 per cent of patients[18]. Based on our experience, we propose that HIV patients with diarrhoea of less than two weeks be treated symptomatically. Diarrhoea will cease in up to two-thirds. If diarrhoea persists, and in those with longer duration diarrhoea, empiric therapy aimed at bacterial enteropathogens and *I. belli* may be commenced. Typically, this would include a quinolone (for bacterial enteropathogens) and co-trimoxazole (for *Isospora*). The value of adding metronidazole (to cover *Giardia*) is questionable, but may need to be tested in practice. Patients who do not respond to these measures may be investigated, first with three stool examinations and culture, followed, if negative, by jejunal biopsy and fluid examination. Such a management strategy will need to be carefully examined in practice.

Acknowledgment

The authors thank Sh. A.H. Moody (Hospital for Tropical Diseases, London) for kindly confirming the identification of *Cyclospora cayatenensis* from patients in this study. Smt. Sheela Roy provided excellent technical help. The Wellcome Trust Research Laboratory received support from the Wellcome Trust, London, UK and from the Indian Council of Medical Research, New Delhi.

References

1. Lew EA, Poles MA, Dieterich DT. Diarrheal diseases associated with HIV infection. *Gastroenterol Clin North Am* 1997; *26*: 259-90.
2. Chacko S, John TJ, Babu PG, Jacob M, Kaur A, Mathai D. Clinical profile of AIDS in India. A review of 61 cases. *J Assoc Physicians India* 1995; *43*: 535-8.
3. Lubeck DP, Bennett CL, Mazonsen PD, Fifer SK, Fries JF. Quality of life and health service use among HIV-infected patients with chronic diarrhoea. *J Acquir Immune Defic Syndr* 1993; *6*: 478-84.
4. Justice AC, Feinstein AR, Wells CK. A new prognostic staging system for the acquired immunodeficiency syndrome. *N Engl J Med* 1989; *320*: 1388-93.
5. Lindan CP, Allen S, Serufilira A, Lifson AR, Van de Perre P, Chen-Rundle A, *et al*. Predictors of mortality among HIV-infected women in Kigali, Rwanda. *Ann Intern Med* 1992; *116*: 320-8.
6. Sharpstone D, Gazzard B. Gastrointestinal manifestations of HIV infection. *Lancet* 1996; *348*: 379-83.
7. Ullrich R, Heise W, Bergs C, L'age M, Riecken EO, Zeitz M. Gastrointestinal symptoms in patients infected with human immunodeficiency virus: relevance of infective agents isolated from gastrointestinal tract. *Gut* 1992; *33*: 1080-4.
8. Wilcox CM, Schwarts DA, Cotsonic G, Thompson SE 3rd. Chronic unexplained diarrhea in human immunodeficiency virus infection: determination of the best diagnostic approach. *Gastroenterology* 1996; *110*: 30-7.
9. Colebunders R, Lusakumuni K, Nelson AM, Gigase P, Lebughe I, Van Marck E, *et al*. Persistent diarrhoea in Zairian AIDS patients: an endoscopic and histologic study. *Gut* 1988; *29*: 1687-91.
10. World Health Organization. Acquired immunodeficiency syndrome (AIDS). WHO/CDC case definition of AIDS. *Wkly Epidemiol Rec* 1986; *61*: 69-70.

11. Mathan MM, Venkatesan S, George R, Mathew M, Mathan VI. Cryptosporidium and diarrhoea in southern Indian children. *Lancet* 1985; *2*: 1172-5.

12. Casemore DP, Roberts C. Guidelines for screening for Cryptosporidium in stools. Report of a joint working group. *J Clin Pathol* 1993; *46*: 2-4.

13. Bartlett JG, Belitsos PC, Sears CL. AIDS enteropathy. *Clin Infect Dis* 1992; *15*: 726-35.

14. Batman PA, Miller AR, Sedgwick PM, Griffin GE. Autonomic denervation in jejunal mucosa of homosexual men infected with HIV. *AIDS* 1991; *5*: 1247-52.

15. Antony MA, Brandt LJ, Klein RS, Bernstein LH. Infectious diarrhea in patients with AIDS. *Dig Dis Sci* 1988; *33*: 1141-6.

16. Laughon BE, Druckman DA, Vernon A, Quinn TC, Polk BF, Modlin JF, *et al*. Prevalence of enteric pathogens in homosexual men with and without acquired immunodeficiency syndrome. *Gastroenterology* 1988; *94*: 984-93.

17. Cotte L, Rabodonirina M, Piens MA, Perreard M, Mojon M, Trepo C. Prevalence of intestinal protozoans in French patients infected with HIV. *J Acquir Immune Defic Syndr* 1993; *6*: 1024-9.

18. Kelly P, Lungu F, Keane E, Baggaley R, Kazembe F, Pobee J, *et al*. Albendazole chemotherapy for treatment of diarrhoea in patients with AIDS in Zambia: a randomized double blind controlled trial. *Br Med J* 1996; *312*: 1187-91.

Reprint requests : Dr B.S. Ramakrishna, Professor, Department of Gastrointestinal Sciences
Christian Medical College & Hospital, Vellore 632004