Persistent diarrhoea in Zairian AIDS patients: an endoscopic and histological study

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SUMMARY To determine the aetiology of persistent diarrhoea in African patients with acquired immunodeficiency syndrome (AIDS), 42 patients with human immunodeficiency virus (HIV) and persistent diarrhoea were enrolled in a microbiological, endoscopic, and histological study. Cryptosporidium was the intestinal parasite most often identified (30%); Isospora belli was found in 12% of the patients. Histological examination of the duodenal mucosa showed a non-specific inflammatory reaction in a significantly higher number of HIV-seropositive patients (82%) than HIV-seronegative controls without diarrhoea (52%) (p=0·02). Lymphocytes were more likely to be found in inflammatory reactions in HIV-seropositive patients than in controls (p<0·0001). Pathogens were observed in histological sections of the duodenum of HIV-seropositive patients only (p=0·002) and included cryptosporidia (four patients) Isospora belli (one), Strongyloides stercoralis (one), and Cryptococcus neoformans (one). On histological examination the rectal mucosa of HIV-seropositive patients and controls was similar, except eosinphils were more likely to be present in inflammatory reaction in HIV-seropositive patients (p=0·05) and enteric pathogens were observed only in HIV-seropositive patients (cytomegalovirus inclusion bodies (one) and Schistosoma mansoni (two). The aetiology of persistent diarrhoea in most African AIDS patients remains unclear.

A persistent diarrhoeal wasting syndrome is frequently observed in African AIDS patients.1–2 In a study recently carried out in Kinshasa, Zaire, persistent diarrhoea was seen in 98 (40%) of 243 AIDS patients.3 Stool cultures for Salmonella, Shigella, Campylobacter, and Yersinia and parasitologic examinations were conducted on 104 HIV infected patients with persistent diarrhoea. No pathogens were identified in 38% of cases.3 Similarly, in Uganda, in 30% of 23 patients with enteropathic AIDS, the aetiology of the diarrhoea was not identified.4

We present endoscopic, histologic, and microbiologic findings in HIV seropositive patients with persistent diarrhoea.

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Methods

Patients
We carried out the study at Mama Yemo Hospital (MYH), a 2000 bed facility in Kinshasa in which an AIDS clinic has been functioning in the Department of Internal Medicine since February 1985.

During February–July 1986, all patients referred to the AIDS clinic for persistent diarrhoea were offered upper and lower gastrointestinal endoscopy. All HIV-seropositive patients who underwent an upper and/or lower gastrointestinal endoscopy were enrolled in the study, completed a questionnaire, and underwent thorough physical examination. Persistent diarrhoea was defined as a history of two or more loose stools per day for at least 30 days during the last two months.

Consecutive HIV-seronegative patients without
diarrhoea who underwent endoscopy for other reasons (abdominal pain/discomfort (36), gastrointestinal bleeding (four), other condition (seven), during the same period served as controls.

LABORATORY PROCEDURES
Fresh stool specimens were processed within two hours. They were inoculated onto MacConkey agar (Difco, Detroit, Michigan, USA), Salmonella-Shigella agar (Baltimore Biological Laboratories, Maryland, USA), Yersinia agar (Oxoid, Basingstoke, Hants, UK), selenite broth (Difco) and Columbia agar (Bio Merieux, Charboniere-les-bains, France) with a Campylobacter growth supplement (Oxoid) and selective antibiotic supplement (Institute Virion, Ruchlicon, Switzerland). Isolation and identification were done according to standard techniques.5

A saline wet mount preparation was examined by light microscopy for presence of ova, parasites, and leucocytes. In addition, a formol ether concentration method and a modified Sheather's sugar flotation method were used.6

ENDOSCOPY
Gastroscopy was carried out using an Olympus GIF Q10 gastroscope after topical anaesthesia of the oropharynx. Premedication (diazepam) was administered only to patients displaying anxiety.

Rectosigmoidoscopy was carried out using an Olympus CF type 10 M sigmoidoscope. No premedication was given for this procedure. Rectosigmoidoscopy was done after defecation but without special preparation in 70% of the patients. In the remaining 30%, a small enema was given before the procedure.

The totally immersible endoscopes and biopsy forceps were completely cleaned, then were disinfected with gluteraldehyde between procedures. Some patients were lost to follow up after the first endoscopic procedures; the conditions of others made a second endoscopic examination difficult to carry out, and finally, instruments were not always available. Therefore, only 60% of the patients underwent both upper and lower endoscopic examination.

HISTOLOGY
At least two duodenal biopsies were taken at the second or third part of the duodenum when this was technically possible. A minimum of two rectal and/or sigmoidal biopsies were undertaken in all patients who underwent a rectosigmoidal endoscopy.

One duodenal and one rectosigmoidal biopsy specimen were placed in 10% buffered formalin and processed using standard procedures. Sections were stained with haematoxylin eosin and periodic acid Schiff (PAS). All biopsies were read by at least two pathologists after a blinded protocol.

One duodenal and one rectosigmoidal biopsy specimen were placed in OCT-compound (Miles Scientific, Naperville, Illinois, USA), snap frozen in liquid nitrogen, and processed for immunofluorescent staining with cytomegalovirus (CMV) monoclonal antibodies (IGM mouse monoclonal antibodies recognising a 66000-K antigen of CMV, Biosoft, Paris, France) and HIV monoclonal antibodies against HIV core proteins (55-18) kindly provided by D Klatzmann, Hôpital de la Pitié Salpetrière, Paris.7

Intestinal biopsies of 13 patients were fixed in gluteraldehyde, postfixed in osmium tetroxide, and processed for electron microscopic (EM) examination.

SEROLOGY
Serum was tested for antibody to HIV by an enzyme linked immunosorbent assay (ELISA) (Organon Teknika, Oklahoma City, Oklahoma, USA). A Western blot assay (Du Pont de Nemours, Wilmington, Delaware, USA) was performed on all serum samples positive by ELISA and was considered positive in the presence of protein bands p24 and gp41. A patient was considered to be HIV-seropositive and included in the series only if the Western blot was positive.

STATISTICAL ANALYSES
Chi-square and Fisher's exact tests were used for statistical analysis.

Results
Forty two (93%) (25 men and 17 women) of 45 HIV-seropositive patients with persistent diarrhoea participated in the study. The diarrhoea was liquid without the presence of mucus or blood in 26 (62%). Other symptoms and signs of these patients included >10% body weight loss in 39 (93%), anorexia in 34 (81%), abdominal cramps in 27 (64%), nausea in 26 (62%) fever in 11 (26%), cough in 10 (24%), oral candidiasis in 10 (24%), dysphagia in eight (19%), persistent genital ulceration in six (14%), history of herpes zoster in three (7%), and Kaposi's sarcoma in one (2%).

ENTERIC PATHOGENS
An enteric bacterial pathogen (Salmonella group C) was found in the stools of only one (2%) of the 42 HIV-seropositive patients.

Pathogenic parasites were observed in the stools of 25 (60%) of the 42 HIV-seropositive patients and included cryptosporidia in 13 cases (31%), Trichuris trichiuris in nine (21%), Isospora belli in five (12%),
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Blastocystis hominis and Ascaris lumbricoides each in three patients (7%), Giardia lamblia two (5%), Ancylostoma duodenale or Strongyloides stercoralis each in one patient (2%).

In only 21 (50%) of the 42 patients with HIV infection and persistent diarrhoea an enteric pathogen (bacteria or parasite known to be able to cause diarrhoea) was identified.

**Endoscopic Findings**

Gastroduodenal endoscopy was carried out on 39 HIV-seropositive patients with persistent diarrhoea and on 35 controls. The duodenum could not be explored in one HIV-seropositive patient because of a technical problem.

Endoscopic findings were normal in 17 (44%) of the HIV-seropositive patients. White mucosal patches characteristic of candidal oesophagitis were noted in 10 (26%) (all these patients had oral candidiasis), minor gastric erythema or erosions in 12 (31%), gastric ulcerations in two (5%), and multiple tumoral Kaposi lesions in the stomach in one (3%). In 12 controls, endoscopic findings were normal. Endoscopic abnormalities observed in the other controls included bulbitis or bulbular ulcer (14), gastritis or gastric erosion (five), hiatal hernia (three), gastric ulcer (two), and a gastric polyp (one).

The second and third part of duodenal mucosa appeared endoscopically normal in all patients.

Rectosigmoidoscopy was undertaken on 31 HIV-seropositive patients with persistent diarrhoea and on 12 controls. In six of these patients, only the rectum was examined because of the presence of faeces. Among the HIV-seropositive patients, endoscopic findings were normal in 21 (68%), a minor erythema of the mucosa was noted in 14 (45%), and small mucosal ulcerations was seen in two (6%). Only one control was an endoscopically abnormality (sigmoidal cancer).

**Histologic Findings**

Adequately processed duodenal biopsies were obtained in 28 HIV-seropositive patients (20 men and eight women) and 34 controls (21 men and 13 women) (Table 1). An inflammatory reaction of the duodenal mucosa was observed more often in the HIV-seropositive patients 23 (82%) (Figure) than controls 17 (50%) (p<0.01). Inflammatory infiltrates consisted more frequently of lymphocytes in HIV-seropositive patients (22 [96%]) than in controls (six [35%]) (p<0.0001). Cryptosporidia were observed in four (14%) of the HIV-seropositive patients and in none of the controls (p=0.03).

Rectal and sigmoidal biopsies were obtained on 25
HIV-seropositive patients with persistent diarrhea (16 men and nine women) and in 12 controls (eight men and four women) (Table 2). In 18 (72%) of the HIV-seropositive patients and five (42%) of the controls, a non-specific inflammatory reaction was found. Inflammatory reactions more often consisted of increased numbers of eosinophils in HIV-seropositive patients (14 [78%]) than in controls (one [20%]) (p=0.03), and this remained so if the two HIV-seropositive patients with Schistosoma in the rectal biopsies were excluded from the analysis.

Histological findings of the intestinal mucosa were not significantly different between patients in whom enteric pathogens were and were not identified.

In four (40%) of the 10 HIV-seropositive patients with cryptosporidia and in one (20%) of the five HIV-seropositive patients with I belli in their stools, in whom duodenal biopsies were obtained, cryptosporidial I belli were identified in the duodenal mucosa. No cases of cryptosporidiosis/I belli were identified on biopsy that had not been seen on stool examination.

None of the 28 duodenal and 25 rectosigmoidal biopsies of HIV-seropositive patients examined by immunofluorescent staining with CMV and HIV monoclonal antibodies showed viral antigens.

The duodenal mucosa of 13 HIV-seropositive patients was examined by electron microscopy (EM). Isospora were observed in one patient and cryptosporidia in another. No tubuloreticular structures were noted.

In none of the patients were endoscopic and histologic findings helpful for patient management purposes.

Discussion

An aetiologic agent could not be identified in 50% of our patients with HIV infection and persistent diarrhea. Similar results have been reported for Haitian AIDS patients with diarrhea.4 In series of American homosexual AIDS patients with diarrhea in 68% and 85% of these patients at least one enteric pathogen was identified.

In a study done on enteropathic AIDS in Uganda, duodenal biopsies showed villi that were inflamed by European standards and mildly blunted with increased lamina propria plasma cells and increased intra-epithelial lymphocytes. Colonorectal biopsies were essentially normal. No controls were included in that study, however.4 In the present study, HIV-seropositive patients had an inflammatory reaction of the duodenal mucosa (82%) more often than controls (52%). The rectal mucosa was histologically similar in both patient groups.

Some differences in the histologic findings between American and African AIDS patients with persistent...
diarrhoea are apparent. In one series of American homosexual AIDS patients, all jejunal and rectal biopsies were histologically abnormal.11 Jejunal abnormalities in these patients included partial villous atrophy with crypt hyperplasia and increased numbers of intraepithelial lymphocytes. Rectal abnormalities included intranuclear viral inclusions, mast cell infiltration in the lamina propria, and focal cell degeneration near the crypt bases (apoptosis).11,12

In the present study, duodenal and rectal biopsies were normal in 18% and 28% of the cases, apoptosis was never observed, and rectal inclusion bodies were seen in only one patient. None of our patients presented a cytomegalovirus enteritis, a condition frequently observed in American homosexual AIDS patients.16 These histologic differences might be because of differences in the microbiologic environments of Africa and North America and to the absence of documented genitouretal intercourse in our series of heterosexual patients. In contrast with what has been reported in American AIDS patients with persistent diarrhoea,13 tubuloreticular stuctures were not observed by EM examination of the intestinal mucosa of study participants. As others have previously reported, intestinal biopsies were less sensitive in diagnosing cryptosporidiosis infection than were parasitologic stool examinations.

Our study suggests that for patients management purposes systematic endoscopy in an HIV seropositive patient with persistent diarrhoea is not indicated.

The persistent diarrhoea of HIV infected patients could be a direct or indirect effect of HIV itself or an as yet unidentified pathogens. The report that HIV RNA was detected in the epithelium of two of four bowel biopsy specimens from AIDS patients with chronic diarrhoea of unknown origin suggests that HIV may directly cause some of the gastrointestinal disorders of the AIDS patients.10 Normal enteric flora may also play a pathogenic role in HIV infected patients. Persistent diarrhoea, malabsorption, and abnormalities of the small intestinal mucosa have been described in patients with primary immunodeficiency.15 Perhaps in AIDS similar mechanisms are causing the diarrhoea.

Further investigations, including malabsorption and bacterial overgrowth studies, intestinal biopsy and stool cultures for viruses known to cause diarrhoea, and studies to show HIV (viral RNA or HIV antigens) in the intestinal mucosa, are essential to clarify the pathogenesis of persistent diarrhoea in HIV infected patients so that an effective treatment for this condition can be developed.

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