Jpn. J. Infect. Dis., 57, 268-270, 2004

Short Communication

Prevalence of Intestinal Parasitic Pathogens among HIV-Positive Individuals in Iran

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(Received January 28, 2004. Accepted July 5, 2004)

SUMMARY: Parasites are important enteric pathogens among patients with human immunodeficiency virus (HIV) infection. There have been very few reports on the prevalence of intestinal parasites among such patients in Iran. To determine the prevalence of intestinal parasites among HIV-positive individuals, we collected single stool samples and analyzed them for detection of various intestinal parasites from 206 HIV-positive individuals with different immune status visited in different medical centers in Iran. The data were tested for statistical significance with χ^2 and Mann-Whitney U tests. The overall prevalence of intestinal parasites was 18.4% (95%CI: 13.7, 24.3). More specifically, the following parasites were identified: *Giardia lamblia* (7.3%), *Blastocystis hominis* (4.4%), *Entamoeba coli* (3.9%), and *Cryptosporidium parvum* (1.5%). Other parasites observed included *Strongyloides stercoralis* and *Hymenolepis nana* in two cases and *Dicrocoelium dendriticum* in one. Of the 38 patients who tested positive for intestinal parasites, 15 (39.2%) had diarrhea. Intestinal parasites were significantly more common among patients with diarrhea than those without ($P < 0.001$). Further, CD4 counts were significantly lower among individuals with diarrhea than those without ($P < 0.001$). This study highlights the importance of testing for intestinal parasites among Iranian HIV-positive patients, especially those with low immunity presenting with diarrhea.

Intestinal parasitic infections cause morbidity and mortality in human immunodeficiency virus (HIV)-positive individuals worldwide (1). These infections would be expected to be appreciably higher in developing countries due to higher prevalence of infections in the general population. Although HIV infection is not highly prevalent in the Middle East (2), it is a rapidly spreading infection in this region (3). The prevalence of HIV infection has doubled in Iran in the past 3 years. As of December 2003, 5,780 HIV-positive cases had been detected; several experts said in the same report that the projected real figure was 30,000 - 40,000 cases (4).

The epidemiology of intestinal parasites in HIV-positive patients in Iran has not been properly studied. To investigate the prevalence of these pathogens in this population, a cross-sectional study was carried out in Tehran (the biggest metropolis in Iran; population: 6,758,845) and Kermanshah (western Iran; population: 678,245) (5) between September 2002 and December 2003. Tehran and Kermanshah have the greatest numbers of registered cases of HIV infection in Iran. The study population consisted of 206 HIV-positive patients visited in different inpatient and outpatient medical centers in these cities. These centers included the Department of Infectious Diseases, Imam Khomeini Hospital, Tehran; Health Center Clinic, Tehran; Behavioral Disease Clinic, Kermanshah; and several private offices in Tehran. To address ethical issues, the study design and performance were approved by the Medical Ethics Committee of the Research Center for Gastroenterology and Liver Diseases (RCGLD), Shaheed Beheshti University of Medical Sciences.

HIV-positive patients were defined as those who had tested positive for HIV infection by two sequential ELISA tests (HIV-EIA, Boi-Rad Laboratories, Milan, Italy). A single fecal sample was collected from each patient. Samples were collected in sterile plastic containers and transported to the laboratory of RCGLD (Taleghani Hospital, Tehran, Iran).

Specimens were fixed with 10% formalin for 30 min and then concentrated by a formalin-ether sedimentation technique (6). Samples were examined as wet saline mounts and in iodine preparation for detection of protozoan oocysts, cysts, helminthic eggs, and larvae. Permanent stained smears were performed for intestinal coccidia and microsporidia by the Ziehl-Neelsen technique modified according to Garcia (6), and a modified trichrome stain according to Ryan et al. (7), respectively. The Mann-Whitney U test and chi-square test were used, where appropriate, to determine the significance of differences.

Almost all of the 206 patients enrolled in the study (203, 98.5%) were adults, a preponderance of whom were male (86.9%), and most of them (173, 83.9%) were intravenous drug abusers. Twenty-eight patients (13.6%) had diarrhea. The mean CD4+ cell count was 496.47 ± 240.58 S.D./mm^3 in all examined subjects, 327.17 ± 174.44 S.D./mm^3 in diarrheic patients, and 523.14 ± 239.12 S.D./mm^3 in patients without diarrhea.

The overall prevalence of intestinal parasites among the study population was 18.4% (95% CI: 13.7, 24.3). There was

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Table 1. Intestinal parasites detected in 206 HIV-positive individuals and the association of these parasites with diarrhea

Parasite detected	Number (%)	Diarrhea (%)
Giardia lamblia	15 (7.3)	5 (33.3)
Blastocystis hominis	9 (4.4)	5 (55.5)
Entamoeba coli	8 (3.9)	2 (25.0)
Cryptosporidium parvum	3 (1.5)	3 (100)
Strongyloides stercoralis	2 (0.9)	2 (100)
Hymenolepis nana	2 (0.9)	0
Dicrocoelium dendriticum	1 (0.4)	0

no significant difference between males and females (18.5% versus 18.1%, respectively; $P > 0.05$, χ^2 test). Intestinal parasites were significantly more common among patients with diarrhea than those without (53.6% versus 12.9%, respectively; $P < 0.001$, χ^2 test). Further, CD4 counts were significantly lower among individuals with diarrhea than those without ($P < 0.001$, Mann-Whitney U test). More specifically, the following parasites were identified: *Giardia lamblia* in 15 patients, *Blastocystis hominis* in 9, *Entamoeba coli* in 8, *Cryptosporidium parvum* in 3, *Strongyloides stercoralis* and *Hymenolepis nana* in 2, and *Dicrocoelium dendriticum* in one. One patient was co-infected with *E. coli* and *H. nana*, and another patient with *G. lamblia* and *B. hominis* (Table 1).

Intestinal parasitic infection did not appear to be highly prevalent in our population; an intermediate to low level of prevalence, in comparison with data obtained from prevalence studies carried out in other regions, was found (8-11). This could be correlated to the relatively high level of immune status among the study population, and the fact that diarrhea and intestinal parasitic infections are strongly associated with lower CD4 counts (9). Moreover, only one sample was taken from each patient, and it has been shown that second specimens yield more pathogen detections (11). In addition, incorporation of more sophisticated methods of diagnosis including jejunal fluid examination and biopsy increase the total yield of pathogens (12).

Few studies on the prevalence of intestinal parasites among immunocompetent Iranian patients have been conducted, and isolation rates of 1-8% for *G. lamblia* and 0.4-5.1% for *B. hominis* from stool samples of these patients have been reported (13,14). Isolation rates of these pathogens in this study were within the ranges reported from other Iranian populations. Reports from other countries demonstrated that the prevalence of these pathogens was not influenced by moderate reduction of cell-mediated immunity (11,15). The results of our study are in accord with these findings. *C. parvum* infection is prevalent in communities with overcrowding and low level sanitation (9), and its prevalence reaches up to 36% in certain developing countries (15). Previous reports in Iran demonstrated that 0.3-3% of immunocompromised patients were infected with this pathogen (13,16). Its isolation rate was low in our subjects (1.5%). This could be attributed to the relatively conserved immune status of our study subjects. Notably, incorporation of newer methods in the study such as the capture ELISA technique may yield better detection of this pathogen (17). Although microsporidiosis in patients with relatively high CD4 count has been described (18), no cases were found in our study population. This may be attributed to the difficulty detecting these organisms in fecal specimens by conventional staining techniques due to their small size (1-3 μm) and close resemblance to bacteria (10), and partly due to different risk factors for HIV infection among this Iranian population, which included more intraveous drug abusers than homosexuals (11). Newer more sensitive molecular methods such as polymerase chain reaction with specific primers, according to da Silva et al. (19), could be useful in detecting microsporidia in our patients.

In our study, as in other reports, intestinal parasites were significantly more common among patients with diarrhea than those without (8-12). It is not clear that diarrhea in HIV-positive patients can be ascribed to these pathogens. Enterocytic or neural dysfunction related to HIV infection may be responsible for diarrhea in some patients (12). It would be interesting to examine this possibility and to compare it with pathogens isolated from HIV-negative patients with diarrhea. Moreover, knowledge of the pattern of infection may be useful in empiric therapy when lack of resources impedes accurate diagnosis of the etiological agents in HIV-associated diarrhea.

It should be also stressed that although our study was limited by sampling method and the number of stool samples analyzed per person, this baseline study provides an impetus for future in-depth studies of possible epidemiologic associations between HIV infection and intestinal parasites in this region of Iran.

ACKNOWLEDGMENTS

This study with the license number of CSE/02/197 was completely supported by grants from the RCGLD.

The authors acknowledge Dr. S. M. Sadat Hashemi for statistical assistance. Authors also appreciate technical help of A. Moghadam Golmohammadi, Drs. M. Taremi, K. MoezArdalan, and S. Ansari.

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