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Original Article

Frequency of opportunistic and other intestinal parasitic infections in patients infected with human immunodeficiency virus in Bangladesh

Rashed Noor [a,*], Shekhar Ranjan Saha [a], Farjana Rahman [a], Saurab Kishore Munshi [a], Md. Aftab Uddin [a], M. Majibur Rahman [b]

[a] Department of Microbiology, Stamford University Bangladesh, Dhaka, Bangladesh
[b] Department of Microbiology, University of Dhaka, Dhaka, Bangladesh

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ABSTRACT

Objective: One of the major health problems in patients with human immunodeficiency virus (HIV) is superimposed infections due to reduced immunity. It has long been recognized that the interactions between HIV and other infective agents, including parasites, significantly influence the health status of people living with HIV/acquired immunodeficiency syndrome. This study attempted to detect enteric parasites in HIV-positive and HIV-negative patients with diarrhea.
Materials and Methods: Stool samples were collected and examined for enteric parasites by light microscopy, modified acid fast stain, and modified trichrome stain methods.
Results: Intestinal parasitic pathogens were detected in 77.14% of patients infected with HIV and they were found to be significantly associated with opportunistic infections. Major pathogenic populations identified are *Cryptosporidium* spp. (47.14%), *Blastocystis hominis* (44.28%), *Entamoeba histolytica* (37.14%), *Hymenolepis nana* (30%), *Isospora belli* (28.57%), *Giardia lamblia* (25.71%), *Cyclospora* species (24.29%), *Ascaris lumbricoides* (22.85%), and *Trichuris trichiura* (18.57%).
Conclusion: Cryptosporidium spp. prevailed in HIV-positive patients with diarrhea, and interestingly, polyparasitic infections were demonstrated in chronic cases with a low CD4 count. Therefore, regular antienteric parasitic prophylactic trials are recommended in for patients with low CD4 cell count.

1. Introduction

Parasitic infections are among the most widespread of all chronic human infections worldwide [1]. Individuals with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) are threatened by a great number of diseases including those caused by different kinds of biological agents. The progressive decline and ultimate collapse of immune system functions, which are characteristic of AIDS, usually result in morbidity leading to death due to opportunistic bacterial, viral, and parasitic infections, especially those that cause chronic diarrhea [2–14].

Infections caused by helminths and HIV have been reported to exhibit major adverse effects on the host immune response and such a co-infection is widespread [15]. With the progressive development of AIDS, especially once $CD4^+$ T-lymphocyte counts have fallen below 200 cells/L, patients often become co-infected with bacteria, parasites, or viruses [15,16]. Such co-infections are probable causes of death in patients with AIDS [17]. Several parasites have been implicated as major contributors of morbidity in individuals infected with HIV who are living in developing countries like Bangladesh. The parasites frequently encountered are *Cryptosporidium* spp., *Isospora belli*, *Microsporidia* spp., *Entamoeba histolytica*, *Giardia lamblia*, *Trichuris trichiura*, *Ascaris lumbricoides*, *Strongyloides stercoralis*, and hookworm [1,4,18,19]. All of these parasites have been reported to be associated with diarrhea and iron-deficiency anemia.

Parasitic infections in patients infected with HIV are common and are a lasting public health challenge. A number of studies have been carried out to determine the magnitude of opportunistic and nonopportunistic intestinal parasitic infections among patients with AIDS and HIV-positive carriers. All of these cases showed that diarrhea was a common symptom among patients infected with HIV [20–25]. Based on this background, our study focuses on intestinal parasitic infections in relation to clinical manifestations,

Conflict of interest: none.

* Corresponding author. Department of Microbiology, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka 1217, Bangladesh. Tel.: +880 2 8355626, +880 2 8355596x472; fax: +880 2 9143531.
E-mail address: noor.rashed@yahoo.com (R. Noor).

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Table 1
Intestinal parasites detected in HIV-positive (n = 70) and HIV-negative (n = 70) individuals and their correlation with diarrhea.

Parasite detected	Number (%)		Diarrhea (%)	
	HIV positive	HIV negative	HIV positive	HIV negative
Cryptosporidium spp.	33 (47.14)	04 (07.14)	27 (81.80)	01 (25.00)
Isospora belli	20 (28.57)	02 (02.86)	13 (65.00)	01 (50.00)
Blastocystis hominis	31 (44.28)	20 (28.57)	22 (70.97)	13 (59.09)
Cyclospora species	17 (24.29)	01 (01.42)	12 (70.59)	00 (00.00)
Giardia lamblia	18 (25.71)	32 (45.71)	15 (83.33)	25 (78.12)
Entamoeba histolytica	26 (37.14)	40 (57.14)	22 (84.60)	30 (75.00)
Ascaris lumbricoides	16 (22.85)	28 (40.00)	08 (50.00)	11 (39.28)
Hymenolepis nana	21 (30.00)	08 (11.42)	05 (38.09)	04 (50.00)
Trichuris trichiura	13 (18.57)	08 (11.42)	08 (61.53)	06 (75.00)

HIV = human immunodeficiency virus; n = total number.

patient characteristics, and immune status. Intestinal parasitic infection, which is a basic health problem in tropical regions, is common in patients infected with HIV. Thus, we performed a cross sectional study to document the frequency of intestinal parasitic infection in Bangladeshi patients infected with HIV with different immune statuses.

2. Materials and methods

2.1. Study design

This cross sectional study was carried out between April 2011 and September 2011 at different hospitals and HIV clinics in the city of Dhaka in Bangladesh. A total of 140 subjects consisting of 70 (46 men and 24 women) HIV-positive patients and 70 (36 men and 34 women) apparently healthy HIV-negative individuals were included in this study. The patients with HIV infection were between 20 and 60 years of age, while that of the HIV-negative individuals were between 6 months and 60 years.

2.2. Data collection

We used a predesigned structured questionnaire (see Supplement) to collect data from the patients.

2.3. Collection of stool samples

A 30-g sample of freshly passed feces was collected, without urine or toilet tissue contamination, into a sterile container. For adults, and older children, the sample was collected by passing feces into a plastic wrap that was stretched loosely over the toilet bowl. A portion of the sample was then transferred into a supplied container. With young children and infants wearing diapers, the diaper was lined with plastic wrap and a urine bag was attached to the child to ensure that the stool sample was not contaminated with urine. For bedridden patients, the sample was collected in a bedpan lined with plastic wrap and a portion of the feces was transferred into an appropriate container.

2.4. Identification of parasites

Stool samples were collected and microscopically examined using direct and formalin–ether concentration methods [26]. The samples were fixed in 10% formalin, concentrated using formyl/ethyl acetate and examined by direct observation in saline (0.85% NaCl solution). Lugol's iodine was used for the detection of ova, larvae, trophozoites, and cysts of intestinal parasites. *E histolytica* and *Entamoeba dispar* were studied by light microscopy (Carl Zeiss Inc., Oberkochen, Germany). Erythrophagocytosis (ingestion of red blood cells by the parasite) is the only morphologic characteristic used for differentiating between *E histolytica* and the nonpathogenic *E dispar* [27]. Smears of direct and concentrated samples were examined by modified acid fast staining for *Cryptosporidium parvum*, *I belli*, and *Cyclospora* species [28]. Modified trichrome stain

Table 2
Magnitude of single and multiple intestinal parasitic infections of HIV-positive and HIV-negative patients in relation to diarrhea.

Patients		Number of patients infected with parasites				
		No parasite	Single parasite	Two parasites	Three parasites	Four parasites
		N (%)	N (%)	N (%)	N (%)	N (%)
HIV positive	With diarrhea (n = 40)	2 (5.0)	22 (55.0)	8 (20.0)	5 (12.5)	3 (7.5)
	Without diarrhea (n = 30)	14 (46.7)	12 (40.0)	3 (10.0)	1 (3.3)	0 (0.0)
HIV negative	With diarrhea (n = 42)	13 (30.9)	22 (52.4)	7 (16.7)	0 (0.0)	0 (0.0)
	Without diarrhea (n = 28)	15 (53.6)	8 (28.6)	3 (10.7)	2 (7.1)	0 (0.0)

HIV = human immunodeficiency virus; n = total number; N = number.

Table 3
Prevalence of intestinal parasites among patients infected with HIV and HIV-negative persons in relation to gender.

Parasite identified	HIV infected			HIV negative		
	Male (n = 48)	Female (n = 22)	Total (n = 70)	Male (n = 38)	Female (n = 32)	Total (n = 70)
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Cryptosporidium spp.	21 (43.75)	12 (54.55)	33 (47.14)	2 (5.26)	2 (6.25)	4 (5.71)
Isospora belli	11 (22.92)	9 (40.90)	20 (28.57)	0 (0.00)	2 (6.25)	2 (02.86)
Blastocystis hominis	20 (41.66)	11 (50.00)	31 (44.28)	12 (31.58)	08 (25.00)	20 (28.57)
Cyclospora species	9 (18.75)	10 (45.45)	17 (24.29)	1 (2.63)	0 (0.00)	1 (01.42)
Giardia lamblia	10 (20.83)	8 (36.36)	18 (25.71)	16 (42.10)	16 (50.00)	32 (45.71)
Entamoeba histolytica	15 (31.25)	11 (50.00)	26 (37.14)	21 (55.26)	19 (59.37)	40 (57.14)
Ascaris lumbricoides	08 (16.67)	11 (50.00)	16 (22.85)	12 (31.57)	16 (50.00)	28 (40.00)
Hymenolepis nana	12 (25.00)	9 (40.90)	21 (30.00)	5 (13.15)	3 (9.37)	8 (11.42)
Trichuris trichiura	6 (12.5)	7 (31.81)	13 (18.57)	4 (10.52)	4 (12.5)	8 (11.42)

HIV = human immunodeficiency virus; n = total number; N = number.

(HiMedia Laboratories, Mumbai, India) was used to detect *Microsporidia* species [29].

2.5. Modified acid fast stain

A smear was prepared by adding one to two drops of the sample (direct and concentrated) on the slide and then drying it at 60°C. The smear was fixed with absolute methanol for 30 seconds. Staining was performed with Kinyoun carbol fuchsin for 1 minute and then rinsed with distilled water. The sample was decolorized with acid alcohol for 2 minutes, followed by counterstaining with malachite green for 2 minutes. The slide was then dried at 60°C for about 5 minutes and was examined under a microscope using 100× objective lenses.

2.6. Modified trichrome stain

Using a 10-μL aliquot of the concentrated specimen, the smear was prepared by spreading the material over a 45 × 25 mm^2 area. The smear was air dried and placed in absolute methanol for 5 or 10 minutes. Trichrome stain was then added. After 90 minutes the slide was placed in 95% alcohol for 5 minutes, then in 100% alcohol for 10 minutes, and finally in a xylene substitute for 10 minutes. A microscopic examination of the smear was performed under oil immersion (100×).

3. Results

3.1. Distribution of intestinal parasites

Cryptosporidium spp. and *Blastocystis hominis* were the main parasites causing diarrhea in the HIV-positive respondents (Table 1). *E histolytica* and *G lamblia* were the main parasites in the HIV-negative respondents (Table 1). The magnitude of multiple parasitic infections was very high in HIV-positive patients, but very low in HIV-negative patients (Table 2).

3.2. Prevalence of intestinal parasites by gender in patients with HIV

Interestingly, women with HIV were more susceptible to intestinal parasitic infection than men with HIV (Table 3). The reason might be that the female patients in Bangladesh still reside at home and do not go to HIV clinics for treatment, and therefore, their chance of infection is higher than that of males. Consequently, the intestinal parasitic infection rates among women were higher due to direct and indirect exposure of individuals when milking cattle and cleaning animal dung.

3.3. Profile of intestinal parasitic infections among HIV-positive and HIV-negative patients

The effects of patient characteristics which were assumed to be risk factors for these infections on the frequency of intestinal parasitic infections among HIV-positive patients were studied. The level of education among HIV-positive patients significantly affected the prevalence of intestinal parasitic infections, and patients with no institutional education had the highest prevalence of infection (93.18%) (Table 4). All parasitic species were detected in those with lower educational levels. The finding that parasitic infections declined with an increased educational level in the study participants indicated the overall improvement of hygienic conditions and sanitation with higher education. Patients infected with HIV who used water from a stream/river (100%) and the municipal water supply (75%) were significantly affected by parasitic infections (Table 4). The rate of infection among those who had contact with animals ranged from 100% (dogs) to 80% (cats) while the rate for those who had no contact with animals was 74% (Table 4). The occupation of the people infected with HIV also significantly affected the prevalence of intestinal parasites (Table 4).

Table 4
Effect of demographics on the prevalence of intestinal parasitic infections among HIV-positive ($n = 70$) and HIV-negative ($n = 70$) patients.

Demographics	No. of patients tested		No. of patients infected with parasites (%)	
	HIV positive	HIV negative	HIV positive	HIV negative
Educational status				
Literate	26	44	13 (50.00)	18 (40.90)
Illiterate	44	26	41 (93.18)	24 (92.30)
Occupation				
Service	12	36	8 (66.67)	21 (58.33)
Business	25	13	19 (76.00)	6 (46.15)
Student	7	10	4 (57.14)	6 (60.00)
Others	26	11	23 (88.46)	7 (63.64)
Source of water				
Municipal water	32	35	24 (75.00)	22 (62.85)
Tube well	34	28	26 (76.47)	14 (50.00)
River or pond	4	7	4 (100.00)	6 (85.71)
Type of toilet				
Sanitary	58	63	43 (74.13)	35 (55.55)
Non Sanitary	12	07	11 (91.67)	7 (100.00)
Animal contact				
Dog	2	6	2 (100.00)	4 (66.67)
Bird	1	4	0 (00.00)	2 (50.00)
Cat	5	2	4 (80.00)	2 (100.00)
Cattle	27	13	22 (81.48)	7 (53.84)
None	35	45	26 (74.28)	27 (60.00)
Living condition				
Poor	49	45	44 (89.80)	33 (73.33)
Good	21	25	10 (47.61)	9 (36.00)
Status of personal hygiene				
Poor	46	40	45 (97.82)	36 (90.00)
Average	14	22	6 (42.85)	5 (22.72)
Good	10	8	3 (30)	1 (12.50)

HIV = human immunodeficiency virus; n = total number; No. = number.

Supplement

Questionnaire

Patient Profile

Age:

Gender:

1. What is your educational status? Literate/Illiterate
2. What is your occupation? Service/Business/Student/Other
3. What is the source of your drinking water? Municipal water/Tube well/River or Pond
4. Which type of toilet you use? Sanitary/Nonsanitary
5. Are you in contact with animals? Dogs/Birds/Cats/Cattle/None
6. Your living conditions are …… Poor/Good
7. What is your personal hygiene status? Poor/Average/Good
8. Do you have diarrhea? Yes/No

4. Discussion

Numerous opportunistic infections have been reported in patients infected with HIV, mostly due to downregulation of the immune system. Gastrointestinal parasitic infections with diarrhea may lead to life-threatening complications in these patients. In our study, the overall frequency was found to be quite high (77.14%); diarrheal and nondiarrheal cases accounted for 95% and 53.33%, respectively. A high prevalence of *Cryptosporidium* spp. (47.14%) was noted, followed by *B hominis* (44.28%), compared with other studies which reported a prevalence range between 3.7% and 17.8% in India, and 1.5% and 19.2% in other countries for these parasites [4,30–35]. Such a high prevalence may be due to overcrowding, low levels of sanitation, and poor living conditions, as the majority of infected individuals lived in slums and practiced poor personal hygiene practices.

In this study, high prevalence of three helminths, *A lumbricoides* (22.85%), *Hymenolepis nana* (30%), and *T trichiura* (18.57%), were observed. Therefore, screening for helminths is proposed during routine parasitic examination, as our studies showed that helminthic infection in individuals infected with HIV accelerated the progression of HIV disease (Table 1). Seven risk factors were considered, of which five, namely, living conditions, exposure to pets and animals, source of water, type of toilet used, and personal hygiene practice, were found to be significant factors for acquiring parasitic infections. This emphasizes the need to educate people on safe sexual practices, good sanitation and personal hygiene, using boiled or properly filtered water, and avoiding contact with domestic pets.

Our study highlights the magnitude of enteric parasites in individuals infected with HIV, both with and without diarrhea. Our study was the first attempt in Bangladesh to determine exclusively the frequency of parasitic infections in HIV-positive patients and also to compare the results with that of non-HIV-infected individuals who were considered as controls. Nevertheless, one limitation of our study was the apparently small sample size, because most people infected with HIV refused to consent to collect stool samples, and most importantly because of the low prevalence of individuals infected with HIV in Bangladesh [36–38].

In our study, *Cryptosporidium* was found to be an important parasite in HIV-positive patients with diarrhea, which is of clinical significance. Polyparasitic infection was observed in patients with diarrhea (chronic), particularly in individuals infected with HIV, thereby reflecting the seriousness of parasitic infections among HIV-positive patients compared with healthy individuals (Table 2). Moreover, patients infected with HIV are very much neglected because of low educational levels and socioeconomic status in Bangladesh, which also accounts for the significantly high prevalence of parasitic infections in them (Table 4). Therefore, from the findings of our study, we recommend that the routine antienteric parasitic prophylactic measures should be provided in clinical settings on a regular basis to persons with a CD4 count <300 cells/L.

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