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Prevalence of Intestinal Parasitic Infections among HIV Infected Individuals

C. Baragundi Mahesh*, K. Gokale Shilpa, S. Solabannavar Shivakumar and B. Kulkarni Ramakant

Department of Microbiology, S.N.M.C. Bagalkot - 587 102, India.

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Human immunodeficiency virus (HIV), the causative agent of AIDS is fast becoming a major threat in Indian subcontinent, with an estimated 5.2 million persons being infected with HIV with 1, 11,000 AIDS cases among them.[1] Enteric parasitic infections are one of leading causes of morbidity and mortality in HIV infected patients. Study was conducted to know the prevalence of different enteric parasites in HIV-seropositive patients. The study was conducted on 75 HIV seropositive patients. Fresh morning stool sample was collected from each patient and examined macroscopically and by saline wet mount, iodine wet mount and modified Ziehl-Neelsen's staining before and after formol ether concentration technique. Statistical analysis was done by Fisher's test and Odd's ratio with 95% confidence interval was calculated. Of the 75 patients 51(68%) patients presented with diarrhea. Maximum number of patients were in the 21-50 years age group with male preponderance. Parasitic infection was seen in 35(46.66%) patients and a total of 38 parasites were detected. Poly parasitism was seen in 3(4%) patients. *Cryptosporidium parvum* was the predominant (18.66%) parasite detected followed by *Isospora belli* (9.33%), *E.hystolytica*/*dispar* (9.33%) and others. *Cyclospora cayetanensis* was detected in only 2(2.6%) patients. A higher proportion of diarrhoeal patients were found to harbour enteric parasites that non-diarrhoeal cases (67.74% Vs 25%). Of the 38 parasites detected, 92.10% were protozoan parasites while only 7.89% were helminthic parasites. Opportunistic and non opportunistic parasites are highly prevalent in HIV seropositive patients with or without diarrhoea. The parasitic infections in HIV infected patients are more predominantly caused by protozoal and intracellular parasites than helminthic and extracellular parasites. Routine examination of stool samples and prompt treatment of parasitic infections can significantly benefit HIV-infected individuals.

Key words: *Cryptosporidium parvum, Isospora belli, Cyclospora,* Opportunistic infections, HIV.

Human immunodeficiency virus (HIV), the causative agent of AIDS is fast becoming a major threat in Indian subcontinent, with an estimated 5.2 million persons being infected with HIV with 1, 11,000 AIDS cases among them.[1] The progressive decline in mucosal immunologic defensive mechanisms predisposes HIV positive individuals to gastrointestinal infections.[2] In developing countries, acute gastroenteritis caused by intestinal parasites is complicated and it is major cause of illness and kills millions of AIDS patients annually.[3] Reports indicate that diarrhoea occurs in 30-60% of AIDS patients in developed countries and in about 90% of AIDS patients in developing countries.[4] The intracellular intestinal protozoans, *Cryptosporidium parvum, Cyclospora cayetanensis, Isospora belli and the Microsporidia* are opportunistic in patients with HIV infection and are often the major cause of uncontrollable debilitating diarrhea.[5,6,7,8.] Little however has been reported on the presence of

* To whom all correspondence should be addressed.
Mob.: +91-9945471374
E-mail : baragundimc@rediffmail.com

other protozoan and helminthic infections in HIV-positive individuals in developing countries. Based on high prevalence, non opportunistic parasites may also be major contributors to morbidity in patients with HIV infection.

Figures from various studies demonstrate the striking geographical variations with respect to the prevalence of enteric parasites in HIV infected patients.[9,10,11] Because many of these infections are treatable, an early and accurate diagnosis is important. At present there is no data available about the prevalence of intestinal parasitic infections in HIV positive individuals in our geographical area. Hence the present study was undertaken to investigate the frequency and distribution of intestinal parasitic infections in HIV infected individuals.

MATERIALS AND METHODS

A total of 75 patients diagnosed as HIV seropositive according to NACO guidelines[12] were included in the study. The standards of ethical committee on Human experimentation were followed during the study. Ethical clearance was obtained by institutional ethical committee. Consent was taken from all subjects of the study.

Fresh morning stool sample was collected in a wide mouthed leak proof clean container. About 5gms of solid stool or about 10 ml of liquid stool was collected. Data regarding age, sex, history of diarrhoea was also collected. In the study, diarrhoea was defined as having three or more loose bowel movements within 24 hrs period. Samples were processed without delay within half an hour. Macroscopic examination was done to look for structures like tape worm proglottids, scolices, adult enterobius, ascaris, trichuris or hook worm. Samples were concentrated by formol ether sedimentation technique. Both direct and concentrated samples were subjected to microscopic examination. Unstained saline wet mount preparation was done to detect protozoal trophozoites, helminthic eggs or larvae. Iodine wet mount was done to detect cysts.[13] Modified Ziehl-Neelsen's staining[14] was done to detect coccidian parasites. Statistical analysis was done using Fisher's test and Odd's ratio with 95% confidence interval (CI) was calculated.

RESULTS

Of the 75 enrolled cases, 51(68%) patients presented with diarrhoea, where as 24(32%) did not have diarrhoea. Maximum number of HIV positive patients and maximum number of patients with diarrhoea were in the age group 21-50 years. Among the 75 subjects, 50(66.66%) were males and 25(33.32%) were females. Diarrhoea was more common in male (53.33%) patients than female (14.66%) patients (Table -1&2).

Table 3 shows detection of 12(31.57%) parasites only after formol-ether concentration. Out of the 75 cases, 35(46.66%) patients were found to harbour the enteric parasites and a total of 38 parasites were detected. Poly parasitism was seen in 3(4%) patients.

Tables 4 & 5 show the parasites detected. *Cryptosporidium parvum* (18.66%) was the predominant parasite detected followed by *I.belli* (9.33%), *E.hystolytica/dispar* (9.33%) and others. Triple parasitic infection with *Cryptosporidium parvum, Hook worm* and *Entamoeba coli* was seen in one patient. Double parasitic infection was seen

Table 1. Age and diarrhoea wise distribution of HIV Positive patients

Age group (years)	Patients with diarrhoea (%)	Patients without diarrhoea (%)	Total (%)
0-10	3(4)	1(1.3)	4(5.3)
11-20	2(2.6)	2(2.6)	4(5.2)
21-30	10(13.33)	6(8)	16(21.33)
31-40	14(18.66)	6(8)	20(26.66)
41-50	15(20)	6(8)	21(28)
>50	7(9.33)	3(4)	10(13.33)
Total	51(68)	24(32)	75(100)

Table 2. Sex and diarrhoea wise distribution of HIV-Positive patients

Sex	Patients with diarrhoea (%)	Patients without diarrhoea (%)	Total (%)
Male	40(53.33)	10(13.33)	50(66.66)
Female	11(14.66)	14(18.66)	25(33.32)
Total	51(68)	24(32)	75(100)

Table 3. Comparison between direct microscopy and fomol-ether concentration technique

Total parasites detected	Positive by direct microscopy	Positive only after concentration
38	26(68.42%)	12(31.57%)

Table 4. Prevalence of parasitic infections in HIV –positive patients

Parasite detected	Total no (%)
Cryptosporidium parvum	14(18.66)
Isospora belli	7(9.33)
Entamoeba hystolytica/dispar	7(9.33)
Giardia lamblia	3(4)
Cyclospora cayetanensis	2(2.6)
Entamoeba coli	2(2.6)
Hook worm	2(2.6)
Hymenolepis nana	1(1.33)
Total	38(50.66)

in two subjects. One was positive for *Cryptosporidium parvum* and *E.coli* and the other for *E.hystolytica/dispar* and *Hook worm.*

Cryptosporidium was predominant parasite detected in patients with diarrhoea (23.52%) and without diarrhea (8.33%). A higher proportion of diarrhoeal cases were found to harbour enteric parasites than non-diarrhoeal cases (62.74% Vs 25%). This was analyzed by Fisher's test. *Cryptosporidium* was 3.38 times more common in diarrhoeal patients than non diarrhoeal patients. Similarly *Isospora* was 3.07 times, *E.hystolytica/dispar* was 3.07 times, *Giardia*

Table 5. Diarrhoea wise distribution of parasites in HIV positive patients

Parasite detected	No of Patients with Diarrhoea. n=51(%)	No of Patients without diarrhoea. n=24 (%)	Odd's ratio with 95% CI*
Cryptosporidium parvum	12(23.52)	2(8.33)	3.38(0.62-24.16)
Isospora belli	6(11.76)	1(4.16)	3.07(0.33-71.69)
E.hystolytica/dispar	6(11.76)	1(4.16)	3.07(0.33-71.69)
Giardia lamblia	2(3.92)	1(4.16)	0.94(0.06-27.65)
Cyclospora cayatensis	2(3.92)	0(0)	-
Entamoeba coli	1(1.96)	1(4.16)	0.46(0.01-17.76)
Hookworm	2(3.92)	0(0)	-
Hymenolepis nana	1(1.96)	0(0)	-
Total	32(62.74)	6(25)	-

*Fisher's test .

lamblia was 0.94 times and *E.coli* was 0.46 times more common in diarrhoeal patients. Out of the 38 parasites detected in the present study, 35(92.10%) were protozoan parasites and only 3(7.89%) were helminthic parasites.

DISCUSSION

Although HIV is the causative agent in AIDS, most of the morbidity and mortality seen in HIV infected patients results from opportunistic infections.[16] A wide variety of infections are encountered in HIV positive individuals at different stages including bacterial, fungal, viral and parasitic infections.[17] The present study focused on detecting various intestinal parasitic infections in HIV-seropositive individuals.

Out of total 75 HIV seropositive cases majority of patients presented with diarrhoea (68%), indicating that diarrhoea is a common presenting symptom. Diarrhoea was more common in males than females (53.33% Vs 14.66%). Similar findings are reported by Patel SD et al[18] and Ayyagiri et al.[19] Males are more commonly affected by enteric pathogens as they are more exposed to outside home environment and food than females. Maximum number of HIV seropositive cases were in the age group of 21-50 years, with a male preponderance. Similar results were seen in other studies also.[18,20,21.] This could be due to the high risk behavior of males than females in this age group.

The study results demonstrated that using concentration method (Formol-ether) improves the chances of finding the parasite in stool samples. This signifies the importance of technical skill and expertise in the parasitological laboratory.

The overall prevalence of parasitic infections in the present study was 46.66%. Various studies from India have reported a high prevalence parasitic infections ranging from 30-60%.[22-24.] Poly parasitism was seen in 3 patients. *Cryptosporidium* was the predominant (18.66%) parasite detected followed by *Isospora* (9.33%), *E.hystolytica/ dispar* (9.33%) and others. A high prevalence of *Cryptosporidium* (18.66%) was seen the present study as compared to other studies that have reported the prevalence ranging from 3.7-17.8% in India [25-28.] and 1.5-13.3% in other countries.[9,10,29.] In contrast to the present study, *Isospora* was reported as predominant parasite, from northern part of India by Ayyagiri etal.[19] The present study also reveals a low prevalence *Isospora* and a very low prevalence of *Cyclospora* as compared to Cryptosporidium. These observations may be justified by the explanation that, co-trimoxazole which is taken by HIV positive patients as prophylactic measure for *Pneumocystis carinii* pneumonia is also effective against *Isospora* and *Cyclospora.* It is possible that some parasites were not detected in the present study because not all techniques such as trichrome staining for *Microsporidia*, NIH swab for *Entrobius* were not used. Non opportunistic parasites like *E.hystolytica/dispar*, *Giardia lamblia, E.coli*, *Hookworm* and *H.nana* were also detected in few patients. As cysts of *E.hystolytica* and *E.dispar* are morphologically identical and therefore, they could not be differentiated morphologically.

Diarrhoeal patients were more commonly infected with the parasite than non-diarrhoeal patients for all parasites detected. Asymptomatic infection with these parasites requires only a time before symptomatic conditions develop, leading to chronic diarrhoea that may facilitate HIV infection and progression to clinical AIDS. Routine examination of stool samples for parasites will benefit HIV-infected non-diarrhoeal patients also.

Present study reveals predominance of protozoal and intracellular parasites in HIV infected patients in contrast to predominance of helminthic and extracellular parasites in HIV-negative individuals in a previous study done in the same institution.[30] These finding are in agreement with the reports of other studies.[31-33] This may be due to the fact that HIV induced enteropathy doesn't favor the establishment of extracellular/luminal parasites. Intracellular and mucosal dwelling organisms however may benefit from pathological changes and reduced local immune responses induced by the virus.[9]

In conclusion, Intestinal parasitic infection vary with geographical areas. Opportunistic and non opportunistic parasitic infections are highly prevalent in HIV seropositive patients, with or without diarrhoea. The study emphasizes the need for health education, on good sanitation, personal hygiene, proper cooking of food and safe drinking water. Routine examination

of stool samples for parasitic infections and prompt treatment of parasitic infections can significantly benefit HIV infected individuals by contributing to reduce morbidity and mortality.

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