Original Article

Prevalence of isosporiasis in relation to CD4 cell counts among HIV-infected patients with diarrhea in Odisha, India

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Abstract

Background: To determine the prevalence of *Isospora belli* and its correlation with CD4+ cell counts in HIV-positive patients with diarrhea in this region.

Materials and Methods: Stool samples from 250 HIV-positive patients, including 200 with diarrhea and 50 without diarrhea included in the study were examined for the presence of enteric parasites under microscopy. Prevalence of the enteric parasites with special reference to *I. belli* in HIV-positive patients with and without diarrhea were calculated and correlated with their CD4+ cell counts.

Results: Enteric parasites were detected in 39% of the HIV patients with diarrhea compared to 30% without diarrhea. *I. belli* was detected in 22% of the patients with diarrhea and in 4% without diarrhea ($P = 0.0019$). *I. belli* was the most common parasite, followed by *Entamoeba histolytica/dispar* (8%) and *Cryptosporidium parvum* (5%) in HIV-positive patients with diarrhea. In HIV-positive patients without diarrhea, the most common parasite detected was *E. histolytica/dispar* (12%) followed by *C. parvum* (6%) and *I. belli* (4%). The mean CD4 cell count of HIV-positive patients with diarrhea suffering from isosporiasis was 138.35 ± 70.71. In patients with CD4 cell counts <200/µl, *I. belli* was seen in 36/123 stool samples and 2/27 stool samples which was statistically significant ($P = 0.0157$).

Conclusion: *I. belli* was the predominant parasite with a prevalence of 22% among HIV-positive patients with diarrhea, majority having CD4 cell count <200/µl. This study highlights the importance of routine screening for coccidian parasites in HIV-positive patients with and without diarrhea especially in those with low CD4 cell counts.

Key Words: CD4 cell count, diarrhea, HIV, *Isospora belli*, isosporiasis

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INTRODUCTION

Isosporiasis is an intestinal disease of humans caused by the coccidian parasite *Isospora belli*. Isosporiasis mainly affects children and causes self-limiting diarrheal illness in healthy individuals. It is also recognized as a cause of persistent diarrhea in children and severe, prolonged diarrhea in persons with acquired

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immunodeficiency syndrome (AIDS). Progressive decline in immunologic response makes HIV-infected patients vulnerable to opportunistic infections (OI). Diarrhea is one of the major complications, occurring in 90% of patients living with HIV/AIDS (PLHA) in developing countries.[1] Many studies have outlined the emergence of opportunistic intestinal coccidian parasites notably *Cryptosporidium* species and *I. belli* in HIV-infected individuals.[2] As isosporiasis can be effectively treated with trimethoprim-sulfamethoxazole combination, investigation of the parasitic etiology of diarrhea can help in decreasing the morbidity in such cases.[3] Keeping the above facts in view, this study was conducted to know the prevalence of isosporiasis and its correlation with CD4 count.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology, M.K.C.G. Medical College, Berhampur University, from April 2010 to March 2012. A total of 250 stool samples from HIV sero-positive individuals attending the antiretroviral treatment (ART) center were included in the study, comprising 200 HIV patients with diarrhea and 50 HIV patients without diarrhea. The age group of the patients ranged from 21 to 55 years. The HIV sero-positive patients were defined as those who had been tested positive for HIV antibodies by the three Rapid tests as per recommendations given by the WHO. A verbal informed consent was obtained from all the patients prior to specimen collection. Fresh stool samples were collected in a clean, dry, and wide-mouthed universal container. All the patients were advised to give three consecutive stool samples. Diarrhea was defined as the passage of two or more liquid or three or more soft or unformed stools per day. The stool samples were observed macroscopically for the presence of blood, mucus, and adult helminthes worms. The samples were first subjected to wet-mount examination (saline and iodine) under low power (×10) and high power (×40) magnification for the presence of ova, cysts, and trophozoites of intestinal parasites. After formal-ether concentration, a smear was made in a clean and grease-free slide, fixed with methanol and stained using the modified Ziehl-Nelseen technique for the detection of coccidian parasites. The CD4 cell counts of the patients were analyzed using flow cytometry (Becton and Dickinson, USA, FACS caliber). Three antibody panels were used, i.e., BD Tri TEST™ CD3 fluorescein isothiocyanate (FITC)/CD4 phycoerythrin (PE)/CD45 peridinin-chlorophyll-protein (PerCP), a three-color direct immunofluorescence reagent to identify and determine the percentages and absolute counts of mature T-lymphocytes (CD3+) and helper T-lymphocyte ($CD3^+CD4^+$) subsets in erythrocyte-lysed whole-blood, by using Tru Count™ tubes. The findings were noted and tabulated.

Statistical analysis

Graph Pad software was used for calculation of mean, median, standard deviation, range, *P* value using Fisher's extract test.

RESULTS

A total of 250 stool samples from HIV-infected patients were studied which included a study group of 200 patients with diarrhea and a control group of 50 patients without diarrhea. The mean age of the patients with diarrhea was 34.93 (SD 6.89; median 35) and those without diarrhea was 32.50 (SD 7.55; median 32). The ages ranged from 21 to 55 years. Out of the 200 HIV-positive patients with diarrhea, 118 (59%) were male and 82 (41%) were female. Even in the control group, there was male (66%) preponderance. Enteric parasites were seen in 78 (39%) stool samples in the study group and in 15 (30%) stool samples in the control group. In the study group, the most common parasite seen was *I. belli* (22%), [Figure 1], followed by *Entamoeba histolytica / dispar* (8%), *Cryptosporidium* spp. (5%), *Microsporidium* spp. (2%), *Giardia lamblia* (1%), and *Ascaris lumbricoides* and *Strongyloides stercoralis* (0.5% each). In the control group (HIV-positive without diarrhea), the most common entero-parasite detected was *E. histolytica / dispar* (12%), followed by *Cryptosporidium* spp. (6%), *I. belli* (4%) and *A. lumbricoides, A. duodenale, Cyclospora cayetenensis* and *Microsporidium* spp. (2% each) [Table 1]. The association of *I. belli* infection among HIV-positive individuals with diarrhea was significant ($P = 0.0019$, Fisher's extract test).

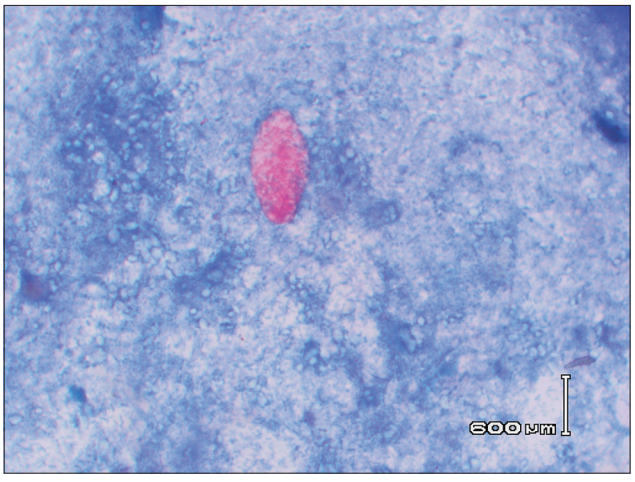

Figure 1: Microphotograph showing acid fast *Isospora belli* in stool sample

The study and the control groups were categorized into three groups based on their CD4 cell counts, i.e., <200, 200-499 and >500 cells/µl. In HIV-positive patients with CD4+ cell counts <200 cells/µl, *I. belli* was seen in 36/123 stool samples with diarrhea and 2/27 stool samples without diarrhea, which was statistically significant (P = 0.0157, Fisher's extract test). Eight cases of *I. belli* were seen in HIV-positive patients with diarrhea having CD4+ cell counts in the range of 200-499 cell/µl. No cases of *I. belli* were seen in the CD4 cell count range >500 cells/µl [Table 2]. The mean CD4+ count of the patients who harbored *I. belli* in was 138.35 (SD 70.71; minimum 55; median 106; maximum 316).

DISCUSSION

Intestinal parasitic infections are the commonest among the OI's and are the major cause of morbidity and mortality in HIV-positive patients.[4] The coccidian parasites like *Cryptosporidium* spp., *Isospora* spp., *Cyclospora* spp. and *Microsporidium* spp. are the foremost among the enteric parasites in HIV-positive patients with diarrhea. These organisms cause self-limiting illness in immunocompetent individuals, but in the case of immunocompromised patients, they can cause life threatening, profuse, watery diarrhea.[5] The line of treatment being different for the diverse parasites, necessitates a definitive diagnosis of the etiological agents causing diarrhea, especially when the outcome can be fatal in this group of individuals.

In the present study, enteric parasites were detected in 39% of the HIV-positive patients with diarrhea. Various studies from India and other countries have reported a high prevalence of intestinal parasites ranging from 18.4% to 70%.[4,6-11] Like many studies we found that coccidian parasites were the leading cause of diarrhea in HIV-positive patients. Among the non-opportunistic pathogens, *E. histolytica/dispar* was the commonest in HIV-positive patients with and without diarrhea.

I. belli (22%) was identified as the most prevalent coccidian parasite among the HIV-positive patients with diarrhea in the present study. Vignesh *et al*. showed similar detection rates of *Isospora* spp. in HIV-positive patients with diarrhea but it was slightly higher than our study.[2] Similarly, higher prevalence of isosporiasis has also been reported from southern India ranging from 18% to 26.1%[1,2,12-14] [Table 3]. The prevalence of isosporiasis in the western part of India revealed a lower prevalence of *I. belli* (4.7-8%) in comparison to our study.[9,10,15] But in the northern part of India, studies have shown a wide variation in the prevalence of isosporiasis ranging from 2.5% to 50%.[4,11,16,17] Studies from countries like Nigeria, France, and Spain have shown the prevalence of isosporiasis as 9.8%, 0.44%, and 5.0%, respectively[7,8,18-21] [Table 3]. The frequency of isosporiasis is often underestimated due to asymptomatic shedding of oocysts and treatment of

Table 1: Parasites detected from the stool samples of HIV-positive individuals with and without diarrhea

Parasites	HIV patients		Total
	With diarrhea (*n*=200) (%)	Without diarrhea (*n*=50) (%)	
Ascaris lumbricoides	1 (0.5)	1 (2)	2
Ankylostoma duodenale	0 (0)	1 (2)	1
Strongyloides stercoralis	1 (0.5)	0 (0)	1
Entamoeba histolytica/dispar	16 (8)	6 (12)	14
Giardia lamblia	2 (1)	0 (0)	2
Isospora belli	44 (22)	2 (4)	46
Cyclospora cayetenensis	0 (0)	1 (2)	1
Cryptosporidium parvum	10 (5)	3 (6)	13
Microsporidium spp.	4 (2)	1 (2)	5
Total	78 (39)	15 (30)	85

Table 2: Correlation of CD4+ cell counts with isosporiasis

CD4 cell counts	HIV-positive with diarrhea (*n*=200)		HIV-positive without diarrhea (*n*=50)	
	No. of cases	*Isospora* positive	No. of cases	*Isospora* positive
<200/µl	123	36*	27	2
200-499/µl	49	8	14	0
<500/µl	28	0	9	0

*P=0.0157, Fisher's extract test. Mean CD4 count in HIV-positive with isosporiasis 138.35±70.71

Table 3: Prevalence of isosporiasis in HIV-positive patients with diarrhea in different studies within India and abroad

Place	Prevalence of isosporiasis (%)	Reference number
Studies outside India		
Nigeria, 2010, 2012	7.8; 9.8	[8,7]
Ethiopia, 2009	5; 12	[19,20]
Spain, 1987	5	[21]
France, 2008	0.44	[22]
Studies within India		
Vellore, 1999, 2007	18; 20	[1,13]
Lucknow, 2000	31	[11]
Chennai, 2002, 2007	18; 26.1	[14,2]
Chandigarh, 2002	2.5	[17]
Madurai, 2007	1.2	[12]
New Delhi, 2008	50	[4]
Pune, 2009	8	[9]
Wardha, 2010	4.7	[10]
Ahmednagar, 2011	5.72	[15]
Jaipur, 2012	10.9	[16]
Our study	22	

other OI's with trimethoprim-sulfamethoxazole which may confer some protection against this parasite. In most studies, *Cryptosporidium* spp. was the most common coccidian parasite responsible for diarrhea in HIV-positive patients.[9,10,15] There has been no documented report on the prevalence of coccidian parasite from this region prior to this study. We report a high detection rate of *I. belli* in comparison to other coccidian parasites among HIV-positive patients with diarrhea. *E. histolytica*/*dispar* (8%) was the next common parasite followed by *Cryptosporidium* spp. (5%) in the present study. A comparison listed in Table 3, on the studies conducted throughout the world showed a marked geographical variation in the prevalence of isosporiasis.

In the present study, *I. belli* was the predominant pathogen among patients having CD4+ cell counts <200/µl which was similar to the study done by Gupta *et al*.[4] There are very few studies on the correlation of isosporiasis with CD4+ cell counts. Opportunistic parasitic infections may be acquired any time during the course of HIV infection and cause self-limiting illness in individuals with normal CD4+ cell counts, but as the immune status decreases, life-threatening diarrhea results. Spontaneous clearing of these opportunistic parasites generally occurs as the CD4+ counts increases. All the patients included in this study were attending ART center and were on Highly active anti retroviral therapy (HAART) and other antibiotics for OI's including trimethoprim-sulfamethoxazole prophylactically for these infections. In spite of the prophylactic treatment with trimethoprim–sulfamethoxazole, the patients were suffering from isosporiasis. This may be due to under dosage, non-compliance, relapses after discontinuation, or emergence of drug resistance.

CONCLUSION

Screening of HIV-patients with diarrhea is not done routinely in most of the cases even though coccidian parasites are considered as AIDS-defining opportunistic pathogens according to CDC guidelines. The incidence and prevalence of infection with a particular enteric parasite in HIV/AIDS patients is likely to depend upon the endemicity of that particular parasite in the community.[22] Intestinal parasitic infections in HIV-positive individuals would be expected to be higher in developing countries due to the higher prevalence of infection in the general population. As most protozoan infections are treatable, an early and accurate diagnosis can decrease the morbidity and mortality in most cases. As the techniques used in the diagnosis of coccidian parasites are simple, rapid, and do not require sophisticated or costly equipments, they can be used as screening tests in all diarrheal cases in HIV-positive patients. In resource-poor settings like ours, the patients usually present late in the course of illness with low CD4+ counts due to delay in diagnosis.

It should be stressed that routine screening of stool samples of HIV-positive patients with diarrhea should be done to prevent morbidity and mortality. As most of the enteric pathogens gain entry through the feco-oral route, improvement of sanitation and proper drinking water and health education can help in decreasing the prevalence. The health practitioners should also be made aware about the routine screening methods for coccidian parasites.

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