



Original Research Article

Diarrhea, CD4 Cell Counts and Intestinal Parasitic Infection in HIV Seropositive Patients in a Tertiary Care Hospital

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ABSTRACT

Background: Diarrhea in HIV patients is a common complication seen in about 90% of patients in developing countries. It may be caused by parasites, bacteria, fungi, enteric viruses or by other non infectious etiology, but diarrhea due to intestinal parasites is more common in these patients and is usually associated with low CD4 count.

Aims and Objectives: To study the occurrence of diarrhea and intestinal parasites in HIV seropositive patients and its co-relation with CD4 cell counts.

Setting: Tertiary care hospital

Design: Cross-sectional study

Material and Methods: Hundred HIV seropositive patients were recruited in the study after informed consent. Patients were asked for history of diarrhea and its characteristics. Single stool and blood samples were collected from all of them. Stool samples were examined for presence of parasites. CD4 cell counts were estimated for all patients. 50 HIV seronegative clinically healthy individuals were used as controls.

Statistical analysis used: Percentages and chi square test.

Results: Diarrhea was seen in 66% of individuals in study group. Diarrhea was more common in patients with CD4 cell count of less than 200 cells/ μ l ($p < 0.01$). *Cryptosporidium* spp (30.7%) was the commonest parasite isolated in this group.

Conclusion: Diarrhea was common and was strongly associated in patients with low CD4 cell counts. Opportunistic protozoa were largely responsible for majority of diarrheal cases in the study. These parasites cannot be identified until routine examination of stool samples is done.

Keywords: CD4 counts, *Cryptosporidium*, Diarrhea, HIV

INTRODUCTION

HIV infection is a global pandemic and an extremely serious problem worldwide, causing dysfunction of both limbs of immune system resulting in overwhelming and fatal opportunistic infections. These infections are higher in developing countries due to higher prevalence of infections in general population. [1] Gastrointestinal involvement, primarily in the form of diarrhea affects 90% of HIV patients in developing countries. [2, 3] Diarrhea and intestinal parasitic infections are strongly associated with low CD4 cell count. [1]

Intestinal infections should be suspected in any HIV patient with advanced disease who presents with diarrhea. [4] Several species of protozoa have been associated with diarrhea in HIV patients. These include *Cryptosporidium parvum*, *Isospora belli*, *Cyclospora* spp, *Microsporidia* spp, *Giardia lamblia*, *Entamoeba histolytica*, *Blastocystis hominis*, *Dientamoeba fragilis* etc. [5] Helminthes such as hook worm, *Ascaris lumbricoides* etc are also seen in stool of HIV patients which may cause increase in plasma HIV viral load leading to acceleration towards AIDS. [6] Infections caused by these parasites cannot be differentiated clinically unless specific fecal examination is carried out. Thus, the study was aimed to determine the association of diarrhea, CD4+cell counts and to find out the frequent causes of intestinal parasites responsible for diarrhea in HIV patients in our area.

MATERIAL AND METHODS

Type and Place of study: An outcome study was conducted at Department of Microbiology, in a tertiary care hospital which caters for the health care of people in and around it.

Duration of study: The study was conducted between February 2007 and March 2008.

Study population: After obtaining an informed consent and approval from the ethical committee stool samples from hundred HIV seropositive patients with and without diarrhea were recruited randomly for the study. Control group comprised of 50 HIV healthy seronegatives.

Inclusion Criteria: In the study diarrhea was defined as having three or more loose bowel movements daily for at least one week prior to visit to hospital.

Exclusion Criteria: Patients who had received anti-parasitic treatment for diarrhea in past 3 weeks were excluded.

Methodology: HIV status was confirmed in all the participants according to NACO guidelines using Comb, Capillus and Tridot kits as per manufacturer's instructions (Span Diagnostics Ltd. (Surat) India, Trinity Biotech Plc, Ireland; J Mitra and Co Pvt Ltd., (Delhi).

A single stool sample was collected in a leak proof, labelled plastic container with 10% buffered formalin as a preservative.

The stool samples were concentrated by the formalin-ether sedimentation technique and were examined using saline and iodine preparation for the presence of trophozoites, oocysts, larvae, and ova of intestinal parasites [7] (both direct and concentrated specimens). Air dried smears of stool samples were stained by modified Ziehl-Neelsen (ZN) staining technique, [8] to identify oocysts of *Cryptosporidium* spp, *Cyclospora* spp, *Isospora belli*. No attempt was made to isolate bacteria and viruses.

Also 3 ml of venous blood was collected from HIV seropositives in a vacutainer containing K3 EDTA for estimation of CD₄ count by automated flow cytometry analyzer FACS calibur (Beckton Dickinson).

Statistical software: MS Excel and Epi-info was used.

RESULTS

A total of 100 HIV seropositives (with and without diarrhea) and 50 HIV seronegative healthy individuals were enrolled in this study [Table 1].

Table 1: Occurrence of infection in study and control groups.

Cases-Controls		No Infection	Infection present	TOTAL
AIDS Patients	Without diarrhea	29	05	34
	With diarrhea	42	24	66
	TOTAL	71	29	100
CONTROLS		43	07	50

$$\chi^2 = 4.11; df=1; P<0.042.$$

The study group consisted of 51 patients with CD4 <200 cells / μ l, 33 with 200-500 cells / μ l, and 16 with CD4 >500cells/ μ l. Diarrhea was highest in the group with CD4 <200cells/ μ l (76.4%) (p <0.01) [Table: 2].

Table - 2: Distribution of CD4 T cells and diarrhea in HIV seropositives.

CD4 cells/ μ l	Diarrhea Status			
	Diarrhea Positive		Diarrhea Negative	
	No	Percentage	No	Percentage
<200 cells / μ l	39	76.4%	12	23.5%
200-500cells/ μ l	21	63.6%	12	36.3%
>500 cells/ μ l	06	37.5%	10	62.5%

$$\chi^2 = 8.37; df = 2; p < 0.01$$

A total of 35 intestinal parasites were detected from HIV seropositive stool samples. Of which, 28 were coccidian parasites which were detected alone or in combination with others. The species-wise prevalence of various intestinal parasites in study group with reference to CD4 count and diarrhea is depicted [Table 3].

Table : 3 : Intestinal parasites in study group in relation to CD4 count and diarrhea.

Parasites	CD4<200cells/ μ l		CD4 200-500/ μ l		CD4>500cells/ μ l		TOTAL
	Diarrhea		Diarrhea		Diarrhea		
	present	absent	present	absent	present	absent	
<i>Cryptosporidium</i>	12	-	02	02	02	01	19
<i>Isospora belli</i>	04	-	02	-	-	-	06
<i>Cyclospora</i>	03	-	-	-	-	-	03
Hookworm	03	-	-	01	-	01	05
<i>Trichuris trichiura</i>	02	-	-	-	-	-	02

Also, enteric parasites were detected more in diarrheal cases 36.3% (24/66) compared to 14.7% (5/34) in non diarrheal cases.

No coccidian parasites were detected in controls, while, hookworm, *Trichuris trichiura*, *Ascaris lumbricoides* were seen in 4% each.

Dual infection was seen in 6% of HIV seropositives and in 2% of controls.

DISCUSSION

Diarrhea is the third common clinical presentation in HIV patients and is strongly associated with low CD4⁺ cell counts. Various studies have shown a rise in diarrhea caused by parasites which include both opportunistic agents causing severe form and non-opportunistic agents causing treatable diarrheal illness.^[9]

In the present study, diarrhea in the group with CD4<200cells/ μ l was 76.4%, which was statistically significant compared to other two groups ($p < 0.01$). Similar results have been given by Sadraei et al^[10] and Zali MR et al.^[11] Infection rate was 29% in HIV patients and 14% in controls. Of the 29%, diarrheal and non-diarrheal accounted for 24 and 5 cases respectively. Studies by other workers have reported rates of 39% - 72.6%^[14, 1, 3] in diarrheal and 12.9% - 23.4%^[1, 14, 3] in non-diarrheal cases.

Cryptosporidium spp was the commonest parasite found in 54.2% (19/35) of the cases. Of which, 12 (30.7%) were seen in group with CD4 < 200cells/ μ l and diarrhea itself, whereas it was found in 28.7% of cases at Madurai^[5], 17% in Chennai^[12], 17.8% in Andhra Pradesh^[15], and 10.8% - 46.3% in Delhi^[3,9,10,13]. Sadraei et al have reported a rate of 56.5% in CD4< 200 cells/ μ l with diarrhea.^[10] The parasite has been found by some in HIV seronegatives also as has been reported in South India, in 2.99% and 0.12% of diarrheic children and adults respectively.^[8] While in Delhi, it was reported in 34% in diarrhea and 6% in non diarrheal people.^[10] However, in our study no coccidian parasites were detected in HIV seronegatives. *Isospora belli*, was the next common parasite found in 17.1%, which is in agreement with studies made by Ramana

et al (17.1%)^[15] and Satheesh et al (18.6%)^[14], while studies by Prasad KN et al^[11] and Gupta et al at AIIMS^[13] have reported higher prevalence of *Isospora belli* than *Cryptosporidium* spp.^[4] Other parasites detected in the study were *Cyclospora* spp 8.5% , helminthes namely hookworm 14.2% and *Trichuris trichiura* 5.7% which correlated with other studies.^[13, 12]

It was seen that CD4 count was less in diarrheal cases with helminth infestation as compared to non-diarrheal cases. Co-infection with helminthes in HIV leads to chronic immune activation leading to hypo responsiveness and anergy resulting in increase in plasma HIV viral load thus accelerating progression to AIDS^[6]. Hence, screening of helminthes must be included in routine parasitic examination.

Various studies have reported multiple infections at rates of 17.5%^[5] and 36.2%^[3] in HIV respectively. Such infections are common in areas where multiple parasites prevail and also due to poor hygienic practices. In our study, dual infections were seen in 6% of the cases with CD4 <200 cells/ μ l with diarrhea and in 2% of controls. The higher rate of dual infection in HIV patients may be due to higher prevalence of certain parasites in the risk group, which facilitates their mixing up.^[17]

No cases of *Strongyloides stercoralis*, *Microsporidia* were detected in our study as like in other studies^[3, 18], while some have reported *Strongyloides stercoralis* in 0.9 – 3.33%^[18, 8] and 27.3%^[3] and *Microsporidia* in 1.67% of the cases.^[4]

These differences in the pattern of prevalence of parasites seen in the present study and others may be attributed to the regional variability (demographic and ecological factors) of the pathogen, behavioral activities, the diagnostic methods used, asymptomatic shedding of oocysts and the use of prophylactic drugs etc. Such differences can be seen between different

geographical areas both within the country and between the countries.

The immune status of study patients was assessed by measuring CD4 count. It was observed that diarrhea, *Cryptosporidium* spp and dual infections were significant in those with CD4 < 200 cells/μl. These findings almost co-relate with the findings made by Dwivedi K et al. [3]

Various studies have shown that, CD4 cells, IL-2, IFN-γ are needed and act independently to clear the parasite. Hence, any selective depletion of CD4 cells or MHC-II leads to chronic infection with *Cryptosporidium* spp, while, deficiency of CD8 cells or MHC-I showed no susceptibility to cryptosporidial infection. This clearly indicates that immunity is dependent on the number of CD4 cells. [3]

Also, in this study *Cryptosporidium* spp was seen in 3 patients in non diarrheal group, but these patients had higher CD4 count and there was no co-infection with other parasites.

No intestinal parasites were detected in 29 cases without diarrhea and in 42 cases with diarrhea which may be attributed to other diarrheagenic agents or mechanisms as discussed earlier, indicating a need for better studies to identify these causes which was not done in our study. [17]

Although, our study was limited by small sample size, inability to follow up of diarrheal episodes, lack of PCR tests etc, the study co-related positively with others as discussed above and shows a close relationship between diarrhea, CD4 count and intestinal parasites in HIV seropositives.

CONCLUSION

The present study highlights the importance of stool examination for enteric parasites in HIV patients with and without diarrhea and also helps us to know the

geographical variation seen in distribution of parasites. *Cryptosporidium* spp and dual infections were commonly seen in HIV patients with CD4 < 200 cells/μl with diarrhea.

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