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## Study of Enteroparasitic Pathogens Among HIV Seropositive Patients at A Tertiary Hospital

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### ABSTRACT

Since the beginning of the HIV pandemic, opportunistic infections have been recognized as common complications of HIV infection. Among these opportunistic infections, gastrointestinal tract (GIT) infections are very common in patients with HIV infection or AIDS. Present study was aimed to study enteroparasitic pathogens among HIV seropositive patients at a tertiary hospital. Present study was single-center, prospective conducted in patients of age 19-60 years, either gender, HIV seropositive with and without diarrhea. In present study, 140 PLHIV subjects were studied. Majority were male (65.71%), from 40-49 years age group (32.86%). Mean age of study subjects was  $47.8 \pm 11.4$  years. Among PLHIV subjects, majority had CD4 T cell count  $\geq 500$  cells  $\mu\text{L}^{-1}$  (42.14%) followed by 200-500 cells  $\mu\text{L}^{-1}$  (32.14%) and  $<200$  cells  $\mu\text{L}^{-1}$  (25.71%). Among PLHIV subjects, 78 were symptomatic (55.71%) while remaining were asymptomatic (44.29%). Among symptomatic subjects, majority had chronic diarrhea (47.86 %) as compared to subjects with acute diarrhea (7.86 %). Parasitic infestation was noted common in CD4 T cell count  $<200$  cells  $\mu\text{L}^{-1}$  (80.56%), 200-499 cells  $\mu\text{L}^{-1}$  (40%) and  $\geq 500$  cells  $\mu\text{L}^{-1}$  (8.47%). Common protozoas noted were *Cryptosporidium* spp. (17.86%), *Isospora* spp. (5.71%), *Cyclospora* spp. (2.14%), *G. lamblia* 4 (2.86%) and *E. histolytica* (2.14%). While helminths noted were *S. stercoralis* (2.86%), *A. lumbricoides* (1.43%), *A. duodenale* (1.43%) and *Taenia* spp. (0.71%). Parasitic infections were detected in 37 % of the HIV infected patients and a low CD4 count was significantly associated with an parasitic infection.

## INTRODUCTION

Since the beginning of the HIV pandemic, opportunistic infections have been recognized as common complications of HIV infection. Among these opportunistic infections, gastrointestinal tract (GIT) infections are very common in patients with HIV infection or AID<sup>[1]</sup>. The inter-relationship of HIV and intestinal parasite infection is complex and yet to be fully understood. The higher prevalence of intestinal parasitic infection in HIV-infected individuals has been reported to be associated with many factors including lower CD4 T-cell count, diarrhoea, living in a rural area, and poor nutritio<sup>[2,3]</sup>.

Studies have shown that parasitic infections could disturb the balance of anti-HIV immune responses and contributed to HIV replication, which could accelerate progress to AIDS. The reduced immune response caused by an HIV infection might also lead to a higher susceptibility to parasitic infection<sup>[4-6]</sup>.

Diarrhea in HIV positive patients may occur due to various etiologies, among them, infection is been an important cause. The infectious etiological agents include both opportunistic agents and non-opportunistic infections. The opportunistic agents cause severe, chronic or frequent gastrointestinal diseases, while non-opportunistic agents usually cause acute, treatable diarrheal illness. The common organisms implicated are *Cryptosporidium parvum*, *Isospora belli*, *Microsporidia* species, *Entamoeba histolytica*, *Cyclospora cayetanensis*, *Giardia* species, *Strongyloides stercoralis*, *Ascaris* species and *Ancylostoma* species<sup>[7]</sup>. Present study was aimed to study enteroparasitic pathogens among HIV seropositive patients at a tertiary hospital.

## MATERIALS AND METHODS

Present study was single-center, prospective conducted in department of Microbiology, at a tertiary hospital India. Study duration was of 1 year (February 2022 to January 2023). Study approval was obtained from institutional ethical committee.

**Inclusion criteria:** Patients of age 19-60 years, either gender, HIV seropositive with and without diarrhea, willing to participate in present study.

**Exclusion criteria:** Patients who had received anti-parasitic treatment for diarrhea in past 3 weeks.

Study was explained to patients in local language and written consent was taken for participation and study. Patient related information was obtained such as name, age, sex, occupation, clinical history including history of diarrhea, antibiotic and antiparasitic

treatment history. Diarrhea was defined as having three or more loose bowel movements daily for at least one week prior to visit to hospital.

Stool samples were collected in sterile, screw capped, disposable plastic containers and were immediately transported to microbiology laboratory for parasitological study. All stool samples were examined for cyst, trophozoites and larvae of parasites using direct (saline and iodine wet mount) microscopy and after formalin ether concentration technique. Modified acid fast staining was done for detection of oocysts of *Cryptosporidium*, *Isospora* and *Cyclospora*.

Blood samples (2 mL) for CD4 T cell count was collected aseptically. The estimation of CD4 T cell count was done. HIV status was confirmed in all the participants according to NACO guidelines.

Data was collected and compiled using Microsoft Excel, analysed using SPSS 23.0 version. Statistical analysis was done using descriptive statistics.

## RESULTS

In present study, 140 PLHIV subjects were studied. Majority were male (65.71%), from 40-49 years age group (32.86%). Mean age of study subjects was 47.8±11.4 years. Among PLHIV subjects, majority had CD4 T cell count  $\geq 500$  cells  $\mu\text{L}^{-1}$  (42.14%) followed by 200-500 cells/ $\mu\text{L}$  (32.14%) and  $<200$  cells  $\mu\text{L}^{-1}$  (25.71%).

Among PLHIV subjects, 78 were symptomatic (55.71%) while remaining were asymptomatic (44.29%). Among symptomatic subjects majority had chronic diarrhea (47.86%) as compared to subjects with acute diarrhea (7.86%).

Parasitic infestation was noted common in CD4 T cell count  $<200$  cells  $\mu\text{L}^{-1}$  (80.56%), 200-499 cells  $\mu\text{L}^{-1}$  (40%) and  $\geq 500$  cells  $\mu\text{L}^{-1}$  (8.47%). Common protozoas noted were *Cryptosporidium* spp. (17.86%), *Isospora* spp. (5.71%), *Cyclospora* spp. (2.14%), *G. lamblia* 4 (2.86%), and *E. histolytica* (2.14%). While helminths noted were *S. stercoralis* (2.86%), *A. lumbricoides* (1.43%), *A. duodenale* (1.43%) and *Taenia* spp. (0.71%).

Table 1: General characteristics

Age groups (years)	No. of patients	Percentage
19-29	22	15.71
30-39	36	25.71
40-49	46	32.86
50-60	36	25.71
Mean age (mean±SD)	47.8±11.4	
<b>Gender</b>		
Male	92	65.71
Female	48	34.29
<b>CD4 T cell count</b>		
$\geq 500$ cells $\mu\text{L}^{-1}$	59	42.14
200-499 cells $\mu\text{L}^{-1}$	45	32.14
$<200$ cells $\mu\text{L}^{-1}$	36	25.71

Table 2: Distribution of intestinal parasitic infections in HIV seropositive cases

	No. of patients (%)		Positive for intestinal parasitic infection (%)	
	No.	Percentage	No.	Percentage
Symptomatic patients	78	55.71	47	33.57
• Acute diarrhea	11	7.86	6	4.29
• Chronic diarrhea	67	47.86	41	29.29
Asymptomatic patients	62	44.29	5	3.57
Total	140		52	37.14

Table 3: Association of parasites with CD4 T cell count

Parasites	CD4 T cell count			
	<200 cells $\mu\text{L}^{-1}$ (n = 36) (%)	200-499 cells $\mu\text{L}^{-1}$ (n = 45) (%)	>500 cells $\mu\text{L}^{-1}$ (n = 59)	Total (n = 140) (%)
<b>Protozoa</b>				
<i>Cryptosporidium</i> spp.	14 (38.89)	9 (20)	2 (3.39)	25 (17.86)
<i>Isospora</i> spp.	4 (11.11)	3 (6.67)	1 (1.69)	8 (5.71)
<i>Cyclospora</i> spp.	1 (2.78)	1 (2.22)	1 (1.69)	3 (2.14)
<i>G. lamblia</i>	3 (8.33)	1 (2.22)	0	4 (2.86)
<i>E. histolytica</i>	2 (5.56)	1 (2.22)	0	3 (2.14)
<b>Helminths</b>				
<i>S. stercoralis</i>	2 (5.56)	1 (2.22)	1 (1.69)	4 (2.86)
<i>A. lumbricoides</i>	1 (2.78)	1 (2.22)	0	2 (1.43)
<i>A. duodenale</i>	1 (2.78)	1 (2.22)	0	2 (1.43)
<i>Taenia</i> spp.	1 (2.78)	0	0	1 (0.71)
Total	29 (80.56)	18 (40)	5 (8.47)	52 (37.14)

## DISCUSSIONS

Diarrhoeal complications are often found in HIV infected individuals, and parasitic involvements in those cases are very significant. In some cases, diarrhoea becomes the starting point for the diagnosis of HIV. Diarrhoea is a life threatening complication often associated with HIV, causing severe weight loss; both conditions are independent predictors of mortality in HIV/AIDS.

Studies have been carried out from various parts of India to determine the prevalence of intestinal parasites which ranges from 20-50%<sup>[1-9]</sup>. In spite of the use of HAART in decreasing the incidence and severity of these parasitic infections, the problem of opportunistic infections especially diarrhoea, continues to plague developing countries like India<sup>[10]</sup>. The treatment varies with each type of the parasite infecting the HIV patient. Hence, it is absolutely essential to precisely identify the causative agent so that appropriately targeted therapy could be initiated, and complications can be prevented<sup>[10,11]</sup>. An association was noted between low socioeconomic status and intestinal parasitic infestations may be due to unhygienic source of water supply and poverty, which is closely related to malnutrition, crowded living conditions, lack of access to free or affordable health care services and dependence on traditional healers.

The progressive decline of the mucosal immunologic defense mechanisms in HIV/AIDS patients predisposes them to early, intermediary, or late gastrointestinal infections<sup>[12]</sup>. Progressive HIV infection have been characterized by an increase in Th2-like responses, which may either be a consequence or a cause of the immune deterioration<sup>[13]</sup>. This has been shown to be responsible for increasing host susceptibility to a

myriad of intestinal opportunistic agents, such as *Cryptosporidium parvum*, *Cystoisospora belli* and *Microsporidia* species<sup>[12,13]</sup>.

Tomar *et al.*<sup>[14]</sup> studied 139 HIV patients, mean age of patients was 37 years with male female ratio of 1.8:1. Intestinal parasites were detected in 64.02% HIV patients. Out of these, most common parasite was *Cryptosporidium* species (38.84%) followed by *Entamoeba histolytica/dispar* (26.61%), *Giardia lamblia* (7.19%), *Strongyloides stercoralis* (2.15%), *Isospora belli* (1.43%), *Microsporidia* (0.71%) and *Cyclospora* (0.71%). The prevalence of intestinal parasites was significantly higher in patients having CD4 count <200 cells<sup>-1</sup> mm<sup>3</sup>.

Sherchan *et al.*<sup>[15]</sup> studied 146 HIV patients the protozoan parasitic infection was found in 30.13% (44/146). Out of 146 patients, 78 had diarrhea in which parasitic infection was 39 (50%) and 7.35% (5/68) protozoal parasites positive cases did not have diarrhea. A significant difference ( $p < 0.05$ ) was observed in the level of infection of intestinal protozoan between the HIV seropositive with diarrhea and HIV-seropositive without diarrhea. Out of 43 patients whose CD4+ T cells were <200  $\mu\text{L}^{-1}$ , 29 (67.4%) had opportunistic parasitic infection whereas out of 103 patients whose CD4+T cells were >200  $\mu\text{L}^{-1}$ , only 15 (14.56%) had opportunistic parasitic infection ( $p < 0.05$ ).

In study by Swathirajan *et al.*<sup>[16]</sup> coccidian parasitic infection accounted for about 23.4% of parasitic infections and of these, *Cystoisospora belli* was observed to be the most common cause of diarrhoea (88.8%), followed by *Cryptosporidium* spp. (9.9%) and *Cyclospora* spp. (1.3%). Trend analysis of coccidian aetiology during the study period revealed a significant rise in the positivity of *C. belli* and *Cryptosporidium* spp.

( $p = 0.001$ ). Among the HIV patients with CD4+ T cell counts  $<200$  cells  $\mu\text{L}^{-1}$ , *Cryptosporidium* infection was most common (90%), followed by infection with *C. belli* (61.4%).

In study by Gupta *et al.*<sup>[17]</sup> enteric parasites were detected in 62% of patients with diarrhea in HIV seropositive individuals. Among them, *Cryptosporidium parvum* (48.3%) was the most common parasite followed by *Isospora belli* (16.1%). The prevalence of enteric parasites was significantly higher ( $p<0.01$ ) in patients with chronic diarrhea (88.2%) compared to acute diarrhea (48.4%). There was a significant difference ( $p<0.01$ ) in opportunistic enteric parasitic infection rate in patients with CD4 counts  $<200$  and  $>200$  cells  $\mu\text{L}^{-1}$  (81.48% vs. 39.1%).

In case of immunocompetent hosts, only some individuals harboring pathogenic intestinal parasites suffer from symptomatic disease but the scenario has changed with the advent of HIV/AIDS. In HIV/AIDS patient, the rate of infection with a particular intestinal parasite depends on the endemicity of a particular intestinal parasite in the community. The detection of intestinal parasites in HIV seropositive patients will help in proper management of these patients as drugs are available for the treatment of most of the infections<sup>[18,19]</sup>. Hence, routine screening of the stool samples of HIV seropositive patients with and without diarrhoea should be done for prompt patient care, to prevent the fulminant form of the disease.

There were some limitations in this study, which include a limited sample size; resource constraints to perform sensitive tests such as polymerase chain reaction and immunofluorescence for confirmation of parasites.

## CONCLUSION

Parasitic infections were detected in 37% of the HIV infected patients and a low CD4 count was significantly associated with a parasitic infection. Regular monitoring of CD4 counts and screening for these opportunistic agents in the HIV infected will help to reduce the mortality and morbidity associated with infections by these agents.

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