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Correlation between stool microscopy and level of immunosuppression in HIV/AIDS patients with diarrhea in East Godavari District, Andhra Pradesh

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Abstract

Introduction: Gastrointestinal infections are very common in HIV patients, and diarrhea is a common clinical condition seen in them. With this observation, a study was conducted to find the parasites that cause opportunistic infections (OIs) and its association with immune status in the HIV-infected patients.

Materials and Method: This study was conducted in the Department of Microbiology, Rangaraya Medical College, Kakinada, Andhra Pradesh, India, for 3 months, that is, from January to March 2013. Stool samples were collected and observed under microscope to detect diarrhea-causing parasites. Direct saline mount, iodine mount, and modified acid fast, modified trichrome stain were carried out. CD4 counts were measured by using FACS counting system.

Results: Among the 178 study participants who had diarrhea and who were on antiretroviral treatment, maximum parasites were observed in patients with CD4 counts <200 cells/µL (65%), followed by 200–499 cells/µL (35%).

Conclusion: OIs were identified in 54.5% of the HIV patients and the infections were more in patients with CD4 count <200 cells/µL. This would help the clinician to plan a proper treatment schedule.

Keywords: CD4; HIV patients; Opportunistic infection; Stool

Introduction

Because of progressive decrease in immunity, HIV-infected individuals are highly susceptible to opportunistic infections (OIs). In HIV patients, gastrointestinal (GI) infections are very common, and diarrhea is a common clinical condition seen in them.[1,2] As per the available literature, the incidence of GI infections in HIV patients ranged between 30% and 90%.[3,4] Various enteric pathogens caused GI infections in HIV patients.[4] Among these, parasites were very important, and they caused acute chronic diarrhea and weight loss.[5] Common parasites that caused GI infections in HIV patients were *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Isospora belli*, Microsporidia, *Strongyloides stercoralis*, and *Giardia lamblia*.[6,7]

Because of the changes in the lifestyle and the measures like improved personal hygiene, the incidence of parasitic infections was comparatively low in developed countries. Although in HIV patients, the incidence of parasitic infections depended on the endemicity of the parasites in the community[8] and the spectrum of OIs varied with respect to region.[9] CD4 cell population was the main target of the HIV pathogen. In HIV infection, there was a marked reduction in the number and the function of CD4 cells, which finally affected the immunity of the host. Hence, CD4 count was an important investigation in clinical evaluation as well as in deciding the stage of HIV infection.

This study was undertaken to find the prevalence of diarrhea-causing enteric parasites and their association with immune status among HIV patients in East Godavari District, Andhra Pradesh, India.

Materials and Method

Study was conducted in the Department of Microbiology, Rangaraya Medical College, Kakinada, Andhra Pradesh, India, for 3 months, that is, from January to March 2013. Study group consisted of HIV patients who were suffering from diarrhea. All the patients were registered in the Antiretroviral Treatment Center, Government General Hospital, Kakinada. Informed consent was taken from all the participants. For the participants below 18 years of age, consent was taken in the presence of a witness, that is, their parent/guardian. The consent was to collect history, CD4 estimation, and stool microscopy. Study was approved by the Institutional Ethics and Research Committee.

CD4 counts were measured by using FACS counting system, BD. All the volunteers were asked to submit three consecutive stool samples. Stool sample was collected in a sterile, wide mouth, leak-proof container. Direct microscopy of stool samples, that is, saline mount and iodine mount was performed. Lugol's iodine was used for iodine mount to detect ova/trophozoites/cysts in the intestinal parasites. Samples were also examined using formol ether concentration technique.[10]

Smears of direct and concentrated specimen were air-dried, fixed with methanol, and stained by modified acid fast staining for *C. parvum*, *I. belli*, and *C. cayetanensis*,[11,12] and by modified trichrome staining to detect Microsporidia.[2]

Results

During the study period, 178 HIV patients were included, their male: female ratio was 1.41, and their mean age was 52 years. The study population consisted of 2 (1.1%) patients with CD4 counts >500 cells/μL, 70 (39.3%) patients between 200 – 499 cells/μL, and 106 (59.6%) patients with <200 cells/μL. The mean CD4 count was 241 cells/μL and intestinal parasites were identified in 97 (54.5%) patients (Table 1).

Discussion

Our study also revealed that OIs caused by parasites were the leading cause of diarrhea in HIV patients. In this study, it was found to be 54.5%. Among these, *C. parvum* (39/97, 40%) was found to be the leading cause of diarrhea, followed by *I. belli* (34/97, 35%) (Table 1). De et al[13] reported that the prevalence of enteric pathogens among HIV patients was 39%. *Cryptosporidium* infection in HIV patients from different parts of India ranged from 0.7% to 87% as reported by Ajjampur et al[14] and it was reported to be 39% by Samantaray and Panda[15] and just 9% by Ballal.[16]

Table 1: Distribution of enteric parasites and CD4 counts

Parasites	CD4 count (cells/□L); n (%)			
	<200	**200–499**	**>500**	**Total**
Cryptosporidium parvum	28 (29)	11 (11.3)	**0**	39 (40)
Isospora belli	21 (22)	13 (13.4)	0	34 (35)
Entamoeba histolytica	7 (7.2)	3 (3.1)	0	10 (10)
Giardia lamblia	3 (3.1)	4 (4.1)	0	7 (7.2)
Strongyloides stercoralis	1 (1)	2 (2)	0	3 (3.1)
Ascaris lumbricoides	1 (1)	1 (1)	0	2 (2)
Ancylostoma duodenale	2 (2)	0	0	2 (2)
Total parasites	63 (65)	34 (35)	0	97 (100)
Total patients	106 (59.6)	70 (39.3)	2 (1.1)	178 (100)
Percentage of parasites identified	63/106 (59.4)	34/70(48.6)	0	97/178 (54.5)

In this study, majority of parasites were observed in the individuals with CD4 counts <200 cells/μL. Because of lowered CD4 counts, there was a reduction in immunity, which further increased the OIs. The lymphopoiesis diminished with age. In this study, HIV infection and fall in CD4 counts were the reasons for getting more number of OIs-causing pathogens.

Effective treatment with high-active antiretroviral therapy was accompanied with an increase in CD4 levels. This accompanying influx of CD4 cells into the lamina propria could help in eradicating OIs.[17] Enteric parasitic infections were generally less common in patients with high CD4 counts because of several factors.[10] Hence, even in this study too, parasites were not identified in the study participants with CD4 counts >500 cells/μL.

Endemicity of parasitic infections was not same in all parts of the world; this automatically influenced the OIs-causing parasites among HIV patients. A study from Nigeria[10] reported *Schistosoma mansoni*, *Dipylidium caninum*, *Enterobius vermicularis*, and *Fasciola* species as the common cause of OIs; study conducted in Varanasi reported Microsporidia species, *Cyclospora* species, *Trichuris trichiura*, and *Hymenolepis nana;*[18] another study from India reported that *Ancylostoma duodenale* was a diarrhea-causing agent in HIV-positive patients;[19] and Dalvi et al[20] reported Microsporidia (17.1%) species as the cause for diarrhea in HIV patients. In East Godavari District, no reports on Microsporidia species, *Cyclospora* species, *Fasciola* species, and *D. caninum* were found; however, *E. vermicularis* and *A. duodenale* were found to be the most common parasites that caused infections especially in the school-going children. The mean age of our study group was 52 years; this could be the reason for no identification of *E. vermicularis*, and identification of *A. duodenale* in just 2% of the patients in our study (Table 1).

Conclusion

Enteric parasitic infections affected both the quality of life and life span of HIV-infected patients. In this study, OIs were identified in 54.5% of the HIV patients. Most of the infections were in the patients with CD4 counts <200 cells/μL. This would help the clinician to plan proper treatment schedule. Other bacterial, fungal, and viral diagnostic methods were not attempted to save the grace. This was the limitation of our study because in the remaining 45.5% of the HIV-infected patients, diarrhea-causing agent was not identified.

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