ORIGINAL RESEARCH PAPER

Microbiology

THE IMPACT OF CD4 IN EVOLUTION OF PARASITIC DIARRHOEA IN SEROPOSITIVE PATIENTS

KEY WORDS: HIV, CD4, AIDS.

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ABSTRACT

Human immunodeficiency virus (HIV) infection has altered both the epidemiology and outcome of enteric opportunistic parasitic infections. This study was done to determine the prevalence and species/genotypes of intestinal coccidian and microsporidial infections among HIV/AIDS patients with diarrhea and/or a history of diarrhea alternately with an asymptomatic interval, and their association with CD4 T cell count. This crosssectional study was done from May 2016 to March 2017 in Sampurnanand Medical College and Hospital, Jodhpur. A blood sample was obtained from HIV-positive patients for a CD4 T cell count upon enrollment. Sociodemographic data and a history of diarrhea were collected by interviewing 195 consecutive participants (116 males and 79 females). Using routine microscopy ,trichrome staining,modified ZN staining incidence of coccidian parasites were found to be as Cryptosporidium 21.8% ,followed by Strongyloides 17.6% ,microsporidia, Isospors, Giardi, Trichuris,and taenea . A CD4 count < 200 cells/ll was significantly associated with the presence of opportunistic parasites and diarrhea ($p < 0.05$). Opportunistic intestinal parasites should be suspected in any HIV/AIDS patient with chronic diarrhea.

INTRODUCTION

Enteric parasitic infections are important and common features of Human Immunodeficiency Virus infection (HIV), causing significant morbidity and responsible for about 80% of Acquired Immune Deficiency Syndrome (AIDS)-related deaths [1,2]. Co-infections of HIV and opportunistic parasites, including intestinal protozoa and helminths, are of concern in resource-poor settings where the health status of the population is generally poor and these opportunistic parasites very common [3]. The resultant effect of such parasitic infections include chronic diarrhea, weight loss, and malnutrition, which has been associated with death among AIDS patients [4]. HIV has the capacity to circumvent and weaken human immune system providing the impetus for increased infection with parasites such as Cryptosporidium spp., Microsporidium spp, Giardia intestinalis, and Strongyloides (S) stercoralis [4]. Cryptosporidium spp, Microsporidia and other coccidian parasites have emerged as significant causes of persistent diarrhea in People living with HIV/AIDS (PLWHA) [5]. These pathogens have been recognized as worldwide causes of diarrhea in all age groups, yet their most significant impact have been felt among individuals with weakened immune systems, especially PLWHA and organ transplant recipients [6]. In immunocompromised individuals, diarrheal infections goes beyond the inconvenience of frequent watery stool but may result in severe and potentially life-threatening dehydration, electrolyte loss and malnutrition, and eventually death [7]. Transmission of Cryptosporidium is mainly through the fecal-oral route in contaminated water and food, as well as through person-to-person spread and contact with infected animals [8]. Microsporidiosis, caused by Microsporidia, another important opportunistic pathogen causing significant morbidity in PLWHA [9], The route of transmission is usually by ingestion of the spores, including evidence of spore inhalation or rectal transmission [12]. Antiretroviral therapy increases the length and quality of life and productivity of patients by improving survival and decreasing the incidence of opportunistic infections in PLWHA through the reduction of circulating viremia and increasing the level of CD4+ cells [13]. Previous studies in India have investigated intestinal parasitic infections in relation to ART and CD4+ count [14,15].

MATERIAL AND METHODS

Study patients were interviewed using the structured questionnaire and information was obtained on demographic characteristics, present and past history of diarrhoea and antibiotic treatment. Diarrhoea was defined as two or more liquid or three or more soft stools per day. Patients already on antibiotic treatment were excluded from the study. A total of 165patients were enrolled in the study. Blood samples (plain & EDTA) 5 ml each were obtained from enrolled patients. Serum samples were used for HIV testing. HIV serostatus of the patients was determined by using commercially available ELISA antibody tests (Genetic system, Biorad Labs, USA and Tridot, J Mitra & Co., New Delhi) using National AIDS Control Organisation (NACO) recommended algorithm(6). CD4 cell counts were measured by using a FACS count system (Becton Dickinson, Singapore BD).

Patients were categorized by their immune status according to the 1993 – revised classification system for the HIV infection by CD4 T-cell categories(16,17). Stool specimens were collected according to the WHO standard procedure and examined microscopically following direct and formalin-ether concentration methods(18). Stool samples were collected at home in labelled, leak proof, clean sterile plastic containers and were transported to the laboratory within three hours of collection. The stool samples were fixed in 10 per cent formalin saline, concentrated using formyl/ ethyl acetate and examined through direct observation in saline (0.85% NaCl solution). Lugol's iodine was used for the detection of ova, larvae, trophozoites and cysts of intestinal parasites. Smears of direct and concentrated specimens were examined by modified acid fast staining for *C. parvum, I. belli* and *Cyclospora*(18,19). Modified trichrome stain (Hi-media laboratories, India, Qualigens Fine Chemicals, India) was used for detecting *Microsporidia*(20).

***Statistical analysis*:** Data were analysed using SPSS software version 20.0 (SPSS Inc, USA). The proportion of opportunistic pathogens were compared between the CD4 groups by using Z test.

RESULTS AND DISCUSSION

Among the total of 195 HIV study subjects ,116(58.2%) males and 79(41.8%) females were enrolled in the study .Diarrhoea was seen in 142 (67.8%)while 53 (38.2%(TABLE 1))were HIV non-diarrhoea as a control group. Overall, *Cryptosporidium* (21.8%%) was the most frequently encountered pathogen in the study population followed by *S.stercoralis*(17.6%) ,*Ascaris ova*(11%),hookworm(6.6%) ,trichuris ova (4.8%) (TABLE 2). HIV infected patients, due to downregulation of the immune system. Gastrointestinal parasitic infection is a universally recognized problem in these patients. These infections largely present with diarrhoea leading to life threatening complications. In the present study, enteric parasites were detected in 45 per cent of HIV infected patients with

diarrhoea. Various studies from India and other countries have reported a high prevalence of intestinal parasite, ranging from 30 to 60 per cent(10,14,21-23). Almost half of the patients with CD4 count less than 200 cells/ l were found to have gastrointestinal parasitic infections and a majority of which were opportunistic parasites (43%). Among the 106 patients with CD4 count < 200 cells / l, parasites could be identified in 71(46%) patients and opportunistic parasites were detected in as many as 64 (41%)patients. Thus, like many other studies, we also found that infections with opportunistic pathogens were the leading cause of diarrhoea in HIV infected individuals,especially, in subjects with advanced disease. *C. parvum* and *I. belli*, were the most common pathogens. Among the non opportunistic pathogens *ascaris,hookworm and giardia* seemed to contribute significantly has shown earlier(23). Similar to other reports, *Microspordia* and *Isospora* were detected in a few patients only. There was some limitations in our study. The study was done on a small sample size as a majority of the patients who came to microbiology laboratory were referred from the general practioners or from primary or secondary care centers. Patients were also referred from Voluntary Counseling & HIV Testing centers. Majority of the patients seen at these centers had already received antibiotics prior to their visit and therefore the number of symptomatic patients was less. The result is almost similar to the findings from other countries but such differing prevalence rates might be due to differences in geographical location, sensitivity of diagnostic techniques, immune status of study participants, environmental hygiene and possible increased awareness, amongst others(24,25). Several species of intestinal opportunistic parasites have been reported among PLWHA [16], with Cryptosporidium spp, Microsporidium spp and S. stercoralis the most commonly encountered in other study. Cryptosporidium spp, which was found to be responsible for diarrhea in 10-20% of PLWHA worldwide [26], has been reported in our study, and more importantly in those with CD4+ T-cell counts of less than 200 cells/mm3 [18,25].

CONCLUSION

This study aims to highlight the parasitic causes of diarrhoea among HIV patients .Though it is already known about the causative organisms but it was not known in our area .The impact of CD4 count on the parasitic load is significant in cases of Cryptosporidia and S.stercoralis. Therefore, our study emphasizes the need for routine screening of enteric coccidiosis as well as education about practicing personal hygiene and taking timely and appropriate measures. In a developing country like India, the magnitude of intestinal parasitic infections in HIV patients further adds to the existing financial burden of the disease. Patients usually belong to poor socio-economic backgrounds and they can hardly afford treatment. Therefore, it is suggested that steps should be taken to prevent the occurrence of these diseases in AIDS patients, as often the disease may take a fulminant form. This can be done by drinking safe water and avoiding contact with contaminated soil.

TABLE 1

Microorganism	Cd4 COUNT		
	CD4<200	CD4>200	P-VALUE
Cryptosporium(n=36)	29(80.5%)	7(19.5%)	0.001
S.stercoralis(n=29)	23(79.3%)	6(20.7%)	0.006
Ascaris(n=18)	12(66.7%)	6(33.4%)	0.56
Isospora(n=13)	10(76.9%)	1(9.1%)	0.53
Giardia(n=14)	10(71.4%)	4(29.6%)	0.68
Microsporidia spp.(n=6)	5(83.3%)	1(17.7%)	0.042
Hookworm(n=11)	10(76.9%)	1(9.1)	0.67
Trichuris (n=8)	8	0	1

TABLE 2

Microorganism	Diarrhoeal stool	Non-diarrhoeal stool	p-value
Cryptosporium (n=36)	30(83.3%)	6(17.7%)	0.005
S.stercoralis(n=29)	22(75.8%)	7(24.2%)	0.679
Ascaris(n=18)	13(72.2%)	5(17.8%)	0.221
Isospora(n=13)	12(92.3%)	1(7.7%)	0.003
Giardia(n=14)	9(64.2%)	5(36.8%)	0.833
Microsporidia spp.(n=6)	6(100%)	0	1
Hookworm(n=11)	8(72.2%)	3(17.8%)	0.342
Trichuris (n=8)	7(87.5%)	1(13.5%)	0.27

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