Original Article

PARASITIC ENTEROPATHOGENS IN HIV SEROPOSITIVE PATIENTS IN RELATION TO CD4 COUNT AT A TERTIARY CARE HOSPITAL

Susmita Sahu, Indrani Mohanty, Narasimham MV, Banojini Parida

Department of Microbiology, M.K.C.G Medical College, Berhampur, Orissa, India

ABSTRACT

Background: Parasitic Entero pathogens causing diarrhoea is considered as one of the major health problems in HIV infected patients especially those with AIDS. The knowledge of prevalence of these pathogens in a particular area can guide clinicians to provide early clinical management in HIV-associated diarrhea. **Objectives:** The primary objective of this study was to study the prevalence of parasitic enteropathogens and compare the intestinal parasitic pathogens in HIV infected diarrhoeal (acute and chronic) and in HIV infected non diarrheal patients and to elucidate the relation between CD4 counts and intestinal parasitic infections **Materials and Methods:** This study was conducted between Nov 2016 and November 2017 in the Department of Microbiology. Stool samples from 105 HIV seropositive cases with diarrhea and without diarrhea were examined by routine parasitological laboratory procedures. CD4 testing was done to know the CD4 cell count. **Results:** Out of 105 cases of HIV seropositive cases 15 cases had acute diarrhea, 66 cases had chronic diarrhea and 24 HIV seropositive cases were without diarrhea. Enteric pathogens were detected in 48 (45.7%) number of patients. *Isospora* sp. was the most common parasite. Other parasites included *Ancylostoma duodenale* and *Entamoeba histolytica*. The diarrheal HIV-positive patients had lower mean CD4 counts as compared to those without diarrhea. **Conclusions:** Identification of the etiological agent of diarrhea in patients with HIV/ AIDS is very important as it can help in the initiation of appropriate therapy which helps in reduction of morbidity and mortality in these patients.

KEYWORDS: Prevalence; Enteric parasites acute; Chronic Diarrhea; HIV; CD_4 count.

INTRODUCTION

Patients with HIV infection are prone to develop different types of opportunistic infections during their lifetime due to Immunosuppression caused by it. Among them, diarrhoea is a significant cause of morbidity and mortality observed in a majority of reports [1,2]. Diarrhoea has significant impact in reducing the quality of life and causes weight loss dehydration and nutritional deficiency. The etiology of diarrhea in HIV infection could be parasitic, bacterial, fungal, enteric viruses, or HIV itself may contribute to diarrhea. In addition to microbes, other factors such as medication, immune deregulation, autonomic dysfunction, and nutritional supplementation play a substantial role in diarrhea in patients with HIV/AIDS. The CD4 cell count has been significantly lowered in these individuals with diarrhoea

The most common opportunistic parasites encountered in HIV patients with diarrhoea include *Cryptosporidium parvum*, *Cyclosporacayatenensis*, *Isospora belli* and *Microsporidia spp*. Other parasites which are non opportunistic commonly reported are *Entamoeba histolytica*, *Giardia lamblia, Ancylostoma duodenale, Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercoralis*. Risk factors include homosexuality, oro-anal sex and poor hygiene can increase the incidence of diarrhoea due to parasites. Studies indicate that diarrhoea occurs in 30-60% of patients with AIDS in developed countries and in about 90% of such patients in developing countries [3].

In immunocompetant individuals the clinical manifestations of diarrhea do not prolong for more than 14 days but in immunocompromised patients like HIV these symptoms continue for long time. It interferes with the nutrient absorption which lead to weight loss [4]. The world health organization defines (WHO) chronic unexplained diarrhoea for more than one month as clinical stage III and chronic cryptosporidiasis or chronic isosporiasis with diarrhoea as clinical stage -IV in adults and adolescents with confirmed HIV infection [5]. In tropical countries, chronic diarrhoea associated with weight loss is the frequently associated presenting illness in HIV infected individuals [6].

Knowledge of the prevalence of the etiological agents in a particular geographycal area can often guide em-

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Correspondence: Dr. Susmita Sahu, Department of Microbiology, M.K.C.G Medical College Berhampur, Orissa, India.
Email: susmitakumarisahu@gmail.com

pirical treatment when the exact diagnosis of the etiological agent in HIV-associated diarrhea is delayed. The primary objectives of this study were to study the parasitic causes of diarrhea in HIV infected individuals in our area, compare them with HIV sero positive patients without diarrhea in relation to CD4 count.

MATERIALS AND METHODS

Study design: Prospective observational study.

Ethics approval: institutional ethical committee permission was taken before commencing the present study.

Study location: The study was conducted in the department of microbiology in collaboration with Department of Medicine, ART Centre

Study duration: From Nov2016– Nov2017

Sample size: Total of 210 stool samples from 105 HIV seropositive cases were examined.

Exclusion criteria: Persons who had received antibiotics/antiparasitic treatment for diarrhea within the past 14 days were excluded from our study.

Methodology: Stool samples of HIV/AIDS cases attending the ART centre were collected under aseptic conditions. CD4 cell count was done by FACS counter BD [7] biosciences, San Jose CA, USA. After receipt of informed consent from 105 HIV seropositive patients with and without diarrhea two consecutive stool samples were collected, information on duration of diarrhea and treatment history were noted. Out of 105 seropositive cases Eighty one were HIV seropositive cases with diarrhea. These patients were further categorized into two groups according to duration as acute (15 cases, having less than 7 days duration) and chronic diarrhea (66 cases, diarrhea lasted for more than 4 weeks duration) and 24 were HIV seropositive cases without diarrhea.

Laboratory examination; Microbiological examination of stool samples were done according to standard protocol. Freshly collected Stool specimens were processed within 2 hours of collection. Parasite detection was done by direct examination after formalin-ether-concentration of stool specimens and wet saline and iodine mount techniques done for the detection of protozoan trophozoites, cysts, helminthic eggs and larva. Additionally, all samples were subjected to modified acid-fast stain and modified trichome staining for oocysts of coccidian parasites [8].

Statistical Analysis: Data analysis was done using chi-square test and the values were considered to be statistically significant.

RESULTS

From the total no of 105 HIV seropositive cases forty eight number of parasites (45.7%) were identified. Out of which 33.3% from chronic diarrhoea samples and 6.6% from acute diarrhoea cases and 5.7% from asymptomatic cases.

In this study Males outnumber females in all age groups both in symptomatic and asymptomatic groups. Maximum number of patients were in the age group of 31-40 yr. Patients having CD4 count less than 200 cells/µL have more number of parasites detected when compared to higher CD4 counts.

In our study most commonly identified parasitic enteropathogens are oocysts of *Isospora* spp (21) followed by *Ancylostoma duodenale* (10), *Entmoeba histolytica* (7), *Giardia lamblia* (3),*Cryptosporidium parvum* (2), *Microsporidia* (2) *Ascaris lumbricoides* (1) and *Strongyloides stercoralis* (1). Around 52% of intestinal parasites were Coccidian parasites,and 50% were other helminths and protozoan parasites.

DISCUSSION

HIV/AIDS, is the leading causes of morbidity and mortality in developing countries like in India. Diarrhoea is a very common clinical condition in HIV patients. Enteric parasitic infections are major cause of morbidity causing acute, chronic and persistent diarrhea. As the prevalence of these enteropathogens show wide geographic variations identification of the etiological agents of diarrhea in a patient with AIDS is very important especially in advanced level of immunosuppression [9]. As Coccidian group of parasites detected more frequently than other parasites, they cause severe, prolonged chronic and recurrent diarrhoea in the presence of HIV infection. Many studies have reported the emergence of important protozoan parasites and helminths as a major cause of morbidity and mortality in patients with AIDS [10,11]. There are many studies regarding the frequency of various enteropathogens causing diarrhea from different parts of India[12] Some studies reported regional variability and endemicity of pathogens [13], as well as changing trends of etiology in the same population.

In our study 31-40 years old was the most predominant age group which constitutes 50.47% of the total patients. Previous studies have also indicated HIV prevalence to be most common in the 21-30 year old age group [14,,15].

We found an overall prevalence of parasites in this study was 45.7%. A previous study reported that enteric pathogens was identified from 49% of the HIV-positive individuals [14]. Another study documented a prevalence of enteric parasites is of 36% and 14% in patients with HIV with and without diarrhea, respectively15 whereas some other studies in India have shown a higher percentage of enteric parasitic pathogens in patients with HIV with diarrhea [16].

Table 1. Age and sex distribution in cases and controls.

Age group in years	HIV seropositive with diarrhea cases (n=81)			HIV seropositive without diarrhea control (n=24)		
	Male	Female	Total	Male	Female	Total
21-30 yrs	15	10	25	05	02	07
31-40yrs	24	17	**41**	08	04	**12**
41-50yrs	06	05	11	03	01	04
>50 yrs	03	01	04	01	00	01
TOTAL	**48**	**33**	81	**17**	7	24

Table 2. Distribution of parasitic enteropathogens

Diarrhoeal status of HIV sero positives	No of patients	Positive for parasites	p-value
Acute diarrhoea	15	07	<0.05
Chronic diarrhoea	66	35	
HIV Seropositive without diarrhoea	24	06	
Total	105	48	

Table 3. Parasites detected and their correlation with CD4 count

CD4 count (cells/µL)	No of HIV patients with diarrhoea	No of HIV patients with parasitic infections
<200	**55**	36
200-350	12	08
350-500	14	04

Table 4. List of parasites identified in HIV seropositive patients

Parasites	HIV +ve patients with diarrhoea Acute (n=15)	chronic (n=66)	HIV+ve patients without diarrhoea (n=24)
Protozoa			
Giardia lamblia		02	01
Entamoebahistolytica	01	**05**	01
Helminths			
Ascarislumbricoids		01	00
Strongyloidesstercoralis		01	00
Ancylostomaduodenale	02	**05**	03
Coccidian parasites			
Isosporaspp	02	**19**	00
Cryptosporidium spp	0	01	01
Cyclosporaspp	01	0	00
Microsporidia	01	01	00
Total	**07**	**35**	**06**

Our study showed the higher proportion of protozoan parasites in the cases previous authors have also reported a higher prevalence of protozoa as compared with helminthes in enteric parasitosis in HIV/AIDS [17,18]. These findings also suggest that patients with HIV/AIDS having diarrhoeal episodes had more intestinal parasite infections than the asymptomatic group and the parasites more prevalent in this area were Isospora *belli,* followed by *Ancylostoma duodenale*. A recent study from North India reported the emerging parasites, *Isospora belli* was most frequently detected followed by *Cryptosporidium, E. histolytica and Giardia lamblia respectively* [19]. The high rate of infection with *Isospora belli* poses a threat to HIV-positive patient. HIV opportunistic infections tend to vary from one locality to another and from one country to another depending on the level of contamination in the water, food, and contact with animals, which are important factors in the dissemination of the parasite. Our findings tend to support the view that the more common parasites *Ancylostoma duodenale*, *Giardia lamblia*, and *Entamoeba histolytica* are not opportunistic in patients with AIDS and identification of these common parasites in HIV sero-positive patients without diarrhoea is a reflection of poor environmental hygiene. More number of parasitic infections were reported in the range of CD4 count <200 /µl. Studies from various parts of the world show different prevalence rates with marked geographical

variations [20, 21]. This emphasizes the need for thorough investigations of these patients to identify pathogens for proper management.

CONCLUSION

Better understanding of HIV induced mucosal immunosuppression, effective clinical management, careful diagnostic evaluation, the development of newer antimicrobial agents, and judicious patient management should help to meet this challenge and may help to reduce morbidity and mortality for patients with HIV/AIDS in India.

Conflict of interest: None

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