

Study of Opportunistic Intestinal Parasitic Infections in Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome Patients

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ABSTRACT

Introduction: Intestinal parasites predominantly coccidian parasites are a common cause for diarrhea in human immunodeficiency virus (HIV)-positive patients. **Materials and Methods:** The study was conducted during January 2009-December 2010. A total of 1,088 stool samples from 544 seropositive HIV positive cases were examined microscopically for ova and cyst using wet mount preparations and stained smears. Out of 544 patients, 343 had prolonged diarrhea for more than 4 weeks, 57 had acute diarrhea of lesser than 7 days and 144 were asymptomatic cases who attended out-patient department; included in this study after taking consent from patients. Enteric pathogens were detected in 274 (50.36%) of the 544 patients. **Results and Conclusions:** The parasites identified were *Cryptosporidium* (135), *Isospora belli* (42), *Cyclospora* (12), *Microsporidia* (02), *Entamoeba histolytica* (49), *Hookworm* (34). Intestinal parasites in chronic diarrhea were significantly higher than the acute diarrhea (63.05% vs. 7.35%; $P < 0.05$). Parasitic pathogens were frequently associated with HIV-positive patients with diarrhea in Western India. Stools of all HIV-positive patients with diarrhea should thoroughly be investigated to identify etiologic agents for proper management.

Key words: Chronic diarrhea, Coccidian parasites, *Entamoeba histolytica*

INTRODUCTION

Infectious diseases appeared to be receding from certain parts of the world in the mid-1950s, but due to the emergence of certain diseases today, microbial health threats are once more a source of concern. These diseases are in some cases resurgent, like tuberculosis and some completely new to humans, such as cryptosporidiosis, isosporiasis, microsporidiosis and cyclosporiasis. The reasons for emergence differ by disease. The emergence of diseases affecting humans is due to many different factors, depending upon the particular disease.^[1] A new niche for infection may also be created by environmentally acquired or genetic immune deficiencies on the part of the host. The epidemic of acquired immune deficiency syndrome (AIDS) is one such example, which has left us vulnerable to many opportunistic infections.^[2]

Several enteric infections occur with increased frequency in persons infected with human immunodeficiency virus (HIV)/AIDS and some of these are more likely to be severe, recurrent and persistent and associated with extra intestinal disease. Intestinal parasitic infections of the gastrointestinal tract are a major cause of morbidity in developing countries. Though in most of these cases, pathogenic organism could not be identified, but there are many possible causes of diarrhea, including common bacterial and parasitic infections. Common parasites associated with HIV infected persons are either well-established enteric pathogens, e.g., *Entamoeba histolytica*, *Giardia lamblia* and *Strongyloides stercoralis* or an opportunistic pathogen, e.g., *Cryptosporidium*, *Microsporidia*, *Isospora* and *Cyclospora*. Only a small percentage of individuals harboring the intestinal pathogenic parasites suffer from symptomatic disease in an immunocompetent host, but with the advent of HIV/AIDS the scenario has changed. The rate of infection with a particular enteric parasite in HIV/AIDS patient depends upon the endemicity of a particular parasite in the community.^[3,4]

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Since the diarrheal diseases due to parasitic infections in HIV/AIDS patients is on the rise during recent times, the present study was conducted on seropositive HIV/AIDS patients with or without history of diarrhea over a period of 2 years in the Department of Microbiology of M.P. Shah Medical College, Jamnagar. The aim of this study was to determine the intestinal parasites in HIV/AIDS patients with and without diarrhea.

MATERIALS AND METHODS

Retrospective data of Guru Gobind Singh Hospital, Jamnagar has been reviewed to find out the intestinal parasitic load in HIV patients. A total of 1,188 stool samples from 544 patients were examined for coccidian parasites during January 2009-December 2010. In the laboratory, macroscopic and microscopic examination was carried out from all the freshly passed stool samples of patients. The sample was concentrated by formal ether concentration technique and subjected to normal saline and iodine wet mount preparation from the concentration material. Smears were prepared from the concentration material and stained by modified Ziehl Neelsen stain for detection of intestinal coccidian parasites.^[5] Prepare a smear from the sediment obtained by the formal ether oocyst concentration technique. Air-dry the smear fix the smear with methanol for 2-3 min. In modified Ziehl Neelsen stain, slides were made from concentrated smear and heat fixed slides were stain with unheated carbolfuchsin for 15 min followed by decolorization with 1% acid alcohol for 10-15 s.

Smears were washed off with water and counterstain with methylene blue (0.5%) for 30 s. Wash off with water and stand the slide in a draining rack for the smear to dry. Smears were examined using oil immersion objective.

RESULTS

In this study, a total of 1,088 stool samples from 544 seropositive HIV patients were examined for parasitic infections. Out of 544 seropositive cases, 400 were symptomatic cases and 144 were asymptomatic patients. The symptomatic patients were further divided into two groups according to the duration of diarrhea as acute (57) and chronic diarrhea (343). Among the total seropositive patients, 274 (50.36%) showed parasitic infections, which include 42.27% from chronic diarrhea stool samples, 7.35% from acute diarrhea samples and 0.73% from the stool of asymptomatic cases. Among the total seropositive patients, 98.54% of the parasites were identified from symptomatic patient samples and 1.45% parasites in asymptomatic cases [Table 1].

In this study, five different types of intestinal parasitic cysts and one helminthic ova were observed and the most common among them was *Cryptosporidium parvum* (135), followed by *E. histolytica* (49), *Isospora belli* (42), *Hookworm* (34), *Cyclospora* (12) and *Microsporidium* (02). Age distribution in this study group showed that the lowest age of the case whose stool examined was 20 years and highest age was 65. The age distribution results showed maximum number of intestinal parasites were identified from the age group 20 years to 30 years followed by 31-40, 41-50 and >51 year age groups. The study showed that more than 95% of parasites were identified in young and middle age group of patients, i.e., between 20 years and 50 years. In the age group, greater than 50 year, which was 20.65% of total study population, the intestinal parasites were observed in only 2.55% subjects [Table 2].

This study showed that approximately 69.70% of the identified intestinal parasites were coccidian parasites, whereas 30.29% were the *protozoans* and helminthes. The

Table 1: Distribution of intestinal parasitic cyst in HIV seropositive cases

Total number of HIV seropositive patients	Number of patients	Positive for parasitic infection (%)	P value
Symptomatic patient (400)			
Acute diarrhea	57	40 (7.35)	<0.05
Chronic diarrhea	343	230 (63.05)	
Asymptomatic patient (144)	144	4 (0.7)	
Total	544	274 (50.36)	

HIV: Human immunodeficiency virus

Table 2: Intestinal parasites in different age groups

Age	Number of cases	Distribution of parasites						Total number of parasites
		<i>C. parvum</i>	<i>Isospora belli</i>	<i>Microsporidia</i>	<i>Cyclospora</i>	<i>E. histo</i>	<i>Hookworm</i>	
20-30	154	43	26	2	4	24	17	116
31-40	263	57	14	1	3	17	7	99
41-50	105	32	2	0	4	6	8	52
>51	22	3	0	0	1	1	2	7
Total	544	135	42	2	12	49	34	274

C. parvum: *Cryptosporidium parvum*; *E. histo*: *Entamoebahistolytica*

most common coccidian parasite identified was *C. parvum* 135 (49.27%). In this study, only two cysts were identified as *Microsporidial* cyst — a least commonly identified parasitic cysts. *E. histolytica* was 49 (9%) and *Hookworm* ova were 34 (6.25%) [Table 3].

DISCUSSION

The emergence and pandemic spread of the AIDS is the greatest challenge to public health in modern times. HIV infection, a world-wide infection, is a serious problem in the present day. A high rate of infection is found in many regions of the world, including the Southeast Asia. In India, HIV infection is a major problem. Continued progression of AIDS pandemic and its association with intestinal parasitic infections is now a serious concern.^[6,7]

In the present study, 50.36% of intestinal parasites were identified from the stool samples of seropositive HIV patients. It is in contrast to studies by Pape *et al.* from Peru where 57.33% of parasites were identified. In the year 1993, Cotte *et al.* in France revealed the prevalence of intestinal parasitic infestations to be 70.6%, which was very high as compared with our studies. In another study, Brandonisio *et al.* in Italy revealed the prevalence of intestinal parasitic infestation in HIV/AIDS patients to be 27.92%.^[8-10]

These parasitic infections have been commonly reported from different centers of India. Prasad *et al.* from Lucknow had reported that intestinal parasitic infestation is seen in >50% seropositive HIV patients.^[11] In the study, conducted by Mohandas *et al.* from Chandigarh observation was on 120 HIV seropositive patients and reported the prevalence of intestinal parasitic infestations to be 30%.^[12] Kumar *et al.* during 2002 in his study on 150 HIV/AIDS patient found the prevalence of intestinal parasitic infestations to be 30% in Chennai.^[13] The difference in prevalence of intestinal parasitic infestations can be due to the difference in geographical distribution of parasites and personal hygienic and sanitary habits. The difference in prevalence can also be

due to different selection of cases with different immune status and difference in method of stool examination. The patients might acquire multiple parasitic infections because of poor sanitary conditions.

In the present study, the most predominant parasite was *Cryptosporidium* and was reported in 24.80% seropositive HIV patients. In symptomatic patients, i.e., patients having diarrhea, the *Cryptosporidium* cyst was found to be 24.26%. Other studies have revealed similar findings. In Brazil, Moura *et al.* in the year 1989, found the prevalence of *Cryptosporidium* to be 18.2% whereas in another study conducted in the Brazil by Cimerman *et al.* revealed the prevalence to be 7%. Chacin *et al.* in Venezuela, studied the prevalence of *Cryptosporidium* in HIV/AIDS patients found *Cryptosporidium* in 41.3% of the patients. Anand *et al.* from Canchipur (Manipur) in the year 1996 reported the prevalence of *Cryptosporidium* as 46.6%.^[14-17] In Northern part of India, the prevalence of *Cryptosporidium* was found to be 11%, by Prasad *et al.* in the year 1995-98.^[11] In another study, conducted in Chandigarh by Mohandas *et al.* 2002 found *Cryptosporidium* in 13% of HIV/AIDS seropositive patients.^[12] In Chennai, Kumar *et al.* found that 12%, of the stool samples were positive for *Cryptosporidium* and was opposite to studies of Northern part of India.^[13] *Cryptosporidium* was found to etiological agent for diarrhea in 10-20% of patients with AIDS worldwide.^[16]

Isospora is infrequently associated with diarrhea due to AIDS in the USA and Europe (about 2%) and is in contrast to our study where 7.70% of the total stool samples were found to be positive for *I. belli*. In some of the countries i.e., Brazil (9.9%), Zaire (12%), Zambia (16%) and Haiti (12%) *I. belli* is a commonly isolated in patients with AIDS and chronic diarrhea.^[15] *Cyclospora* coccidian parasite is a common finding in Haiti (11%), but it was not a common isolate in this study (<1%). This study similar to the results of US and Tanzanian where patients with AIDS and chronic diarrhea (<1%) were positive for *Cyclospora*.^[15,17] Studies from various parts of the world show contrasting prevalence rates with

Table 3: Type of intestinal parasites in HIV seropositive patients

Parasites	Symptomatic cases		Asymptomatic cases (%)	Total parasites (%)
	Acute diarrhea (%)	Chronic diarrhea (%)		
<i>Cryptosporidium parvum</i>	6	126	3	135 (24.81)
<i>Isospora belli</i>	3	39	0	42 (7.72)
<i>Cyclospora</i>	1	11	0	12 (2.20)
<i>Microspora</i>	0	2	0	2 (2.20)
<i>Entameobahistolytica</i>	17	31	1	49 (9.0)
<i>Hookworm</i>	13	21	0	34 (6.25)
Total	40 (7.35)	230 (42.27)	4 (0.73)	274 (50.36)

HIV: Human immunodeficiency virus

Table 4: Comparative study of intestinal parasites in HIV/AIDS patients

Author	Place	Year	Isolation rate (%)
Pape et al. ^[8]	Peru	1990-1993	57.33
Punpoowong et al. ^[58]	Thailand	1994-1995	50
Abaza et al. ^[19]	Egypt	1995	23
Prasad et al. ^[12]	Lucknow, India	1995-1998	50
Brandonisio et al. ^[10]	Italy	1998	27.92
Escobedo and Núñez ^[2]	Cuba	1999	51
Cimerman et al. ^[15]	Brazil	1999	40
Mohandas et al. ^[12]	Chandigarh, India	2002	30
Kumar et al. ^[13]	Chennai	2002	30.67
Present study	Jamnagar	2009-2010	50.36

HIV: Human immunodeficiency virus; AIDS: Acquired immunodeficiency syndrome

marked geographical variations. This emphasizes the need for thorough investigations of these patients to identify pathogens for proper management [Table 4].

CONCLUSION

Parasitic infection is a common finding in HIV associated chronic diarrhea cases. The present study has shown that *Cryptosporidium* was the most important emerging pathogen in HIV-positive patients with diarrhea in Western India. Simple direct, concentrated and stained smear of stool examination can help in identification of enteric parasites in the majority of patients. Detection of etiologic agents will help clinicians to decide appropriate management strategies.

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