

Study of Opportunistic Intestinal Parasitic Infections in Stool of HIV Seropositive Patients of A Tertiary Care Hospital in India



Microbiology

KEYWORDS : opportunistic infections, HIV, parasites, diarrhea

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ABSTRACT

Introduction: This study was meant to determine the prevalence of intestinal opportunistic parasites with or without diarrhea in stool of HIV sero-positive patients and to study age and sex in relation with the parasite isolated. **Material & Methods:** Stool samples from 90 HIV positive patients were collected at Microbiology Laboratory, Sir Takhtasinhji Hospital, Bhavnagar and examined for enteric parasites by wet mount (saline wet mount, iodine wet mount, LPCB wet mount) and stained smear preparation by modified ZN stain. **Results:** Out of 90 seropositive patients of HIV, 60 patients were found to be positive for opportunistic parasitic infections. The higher percentage of parasitic infection was found in adults. The maximum numbers found were of *Cryptosporidium* followed by *Cyclospora*, *Microspora*, *Isospora* and *Giardia lamblia*. **Conclusion:** A regular screening program is mandatory for HIV positive patients for opportunistic parasitic infection in form of routine stool examination.

Introduction:

People with advanced HIV infections are vulnerable to multiple infections and malignancies that are called opportunistic infections because they take advantage of the opportunity offered by a weakened host immune system. Most of the patients die of infections other than HIV. ⁽¹⁾ HIV causes progressive decline in the immune system of the patients which makes them susceptible to opportunistic parasitic infections. Diarrhoea is one of the major complications of HIV positive patients causing cachexia and weight loss which is seen in 90% of the total HIV patients in developing countries like India. Following are some data reflecting the current status of opportunistic infections in HIV positive cases in India. ⁽²⁾

Though the highly active antiretroviral therapy (HAART) has reduced the incidence of opportunistic infection by 50 – 80%, in recent years, multiple surveillance programmes have revealed the emergence of important GI protozoan species like *Microsporidia*, *Cryptosporidia*, *Isospora*, *Giardia* and moreover, newly emerging coccidian parasites *Cyclospora cayetanensis* from HIV seropositive patients with severe diarrhea in India, Peru, Latin America, United States and Papua new Guinea. ^(3,4,5,6,7) Non opportunistic parasites such as *Trichuris trichiura*, *Ascaris lumbricoides*, *Strongyloides stercoralis* and *Ancylostoma duodenale* are frequently found in developing countries but are not currently considered opportunistic in AIDS patients. ^(8,9) In immunocompromised patients, the intestinal opportunistic parasites probably play a major role in causing chronic diarrhea accompanied by weight loss. The incidence and prevalence of infection with a particular enteric parasite in HIV/AIDS patients is likely to depend upon the endemicity of that particular parasite in the community. ⁽⁶⁾ *Cryptosporidium parvum*, *Isosporidium belli* and *Entamoeba histolytica* have been reported as the most frequently identified organisms in HIV infected individuals with diarrhea from India and other parts of the world. ^(7,10,11,12)

Cryptosporidia are coccidian protozoan parasites and strongly associated with diarrhoea in HIV positive patients in developing countries. In HIV infected individuals, cryptosporidial infection may present as a variety of ways, ranging from a self – limited to an intermittent diarrhoeal illness in the early stages of HIV disease to a severe life threatening diarrhoea in severely immunodeficient individuals. *Microsporidia* is obligate intracellular parasites and different studies have revealed prevalence of 6-60% among all the AIDS patients. *Isospora belli*, *Giardia lamblia*, *Microsporidia*, *Entamoeba histolytica* and *Strongyloides stercoralis* are other enteric parasites that can infect the individual with HIV/AIDS. ⁽¹³⁾

Materials and Methods:

Our aim was to study and determine the prevalence of intestinal opportunistic parasites with or without diarrhoea in stool of HIV positive patients and to study age and sex relationship with the parasites isolated. The study was carried out during October 2008 to January 2009 at Microbiology Laboratory, Sir Takhtasinhji General Hospital, Bhavnagar with multiple stool samples of total 90 HIV seropositive patients with or without diarrhoea. Before collecting the samples, patient information such as name, age, sex, occupation, clinical history as well as history of diarrhea, antibiotic and anti helminthic treatment history was obtained. Patients already on anti helminthic and antibiotic treatment were excluded from the study.

Stool samples were collected in clean wide mouthed, leak proof plastic containers from each patient. Stool specimens were examined microscopically for ova, cysts, oocysts, or parasites, using normal saline, iodine mounts and LPCB wet mounts on grease-free slides. Following this, each fresh stool samples were preserved in 10% formal saline. The preserved samples were concentrated using formal-ether concentration methods and examined for oocyst of *Cryptosporidium* spp., *Isospora belli*, and *Cyclospora cayetanensis* were identified using modified Ziehl-Neelsen staining technique. ⁽¹⁴⁾

Cryptosporidium oocyst were identified as pinkish or orange spherical bodies measuring 4-6 µm in ZN stained smear and *Cyclospora*, which appeared as pinkish spherical body measuring 8-10 µm. *Microsporidial* spores were appeared ovoid, refractile and was stained bright pinkish red approximately 1.5 µm size and *Isosporal* oocyst were 20 – 30 x 10 – 19 µm size, elongated and narrow at one pole with neck like restricted region. Trophozoites of *Giardia lamblia* were identified from normal saline and iodine wet mount as pear shaped 10 – 12 x 5 – 6 µm with falling leaf motility. ⁽¹⁵⁾

Results:

Out of 90 seropositive patients of HIV, 60 patients were found to be positive for intestinal opportunistic parasitic infections, while 30 patients were negative for enteric parasites. *Cryptosporidium* oocysts were found in highest proportion followed by *Cyclospora*, which appeared as pinkish spherical body followed by *Microspora*, which spores were appeared ovoid, refractile and was stained bright pinkish red followed by *Isospora*. Trophozoites of *Giardia lamblia* were identified from normal saline and iodine wet mount.

Out of 60 positive patients for enteric parasites, 40 patients were male, 16 were female and 4 were children. The most infected age group was 31 – 45 years (31 cases), followed by 16 – 30 years (17

cases), 46 – 60 years (06 cases), 1 – 15 years i.e. children (04 cases) and least infected age group was 61 – 75 (02 cases).

Cryptosporidium parvum (36 isolates) was found to be the most prevalent parasite causing opportunistic enteric infection in our study, followed by *Cyclospora* (14), *Microsporidia* (07), *Isospora* (02) and *Giardia lamblia* (01).

Table 1: Gender wise distribution of enteric parasitic infection in AIDS patients

Gender wise distribution of enteric parasitic infection in AIDS patients	
Male	40 (70 %)
Female	16 (30 %)

Table 2: Age group wise distribution of enteric parasitic infection in AIDS patients

Age group wise distribution of enteric parasitic infection in AIDS patients	
Age group(years)	Distribution of parasites (%)
1 -15	4 (4.44 %)
16 – 30	17 (18.89 %)
31 – 45	31 (34.44 %)
46 – 60	06 (6.67 %)

Table 3: Distribution of enteric parasitic infection in AIDS patients

Distribution of enteric parasites in AIDS patients	
Parasite	Frequency of infection (%)
<i>Cryptosporidium parvum</i>	36 (60 %)
<i>Cyclosporidium</i>	14 (23.33 %)
<i>Microsporidium</i>	07 (11.66 %)
<i>Isospora belli</i>	02 (3.33 %)
<i>Giardia lamblia</i>	01(1.66 %)

Discussion:

Enteric parasitic infections still remains an important cause of morbidity and mortality in developing countries especially among HIV-infected persons with and without diarrhea. The World Health Organization 2006 defines diarrhea wasting syndrome along with HIV-seropositive patients, the etiology of such diarrhea could either be parasites, bacteria, fungal, enteric virus or HIV itself.^(16,17)

In the present study the enteric parasites were detected in 66.66% from the samples with and without diarrhea.

One or more enteric parasites were detected in 60 patients and frequency of protozoa was more than helminths. Most of the total parasitic infections were caused by *Cryptosporidium* (60%) followed by *Cyclospora* (23.33%), *Microsporidia* (11.66%), *Isospora* (3.33%) and *Giardia lamblia* (1.66%).

Parasitic infection among people of Gujarat region is documented previously.⁽¹⁸⁾ Opportunistic parasitic infection should be suspected in any HIV seropositive patient with diarrhea. Kumar et al documented *C. parvum* as the predominant pathogen (11.5%) in HIV/AIDS patients with diarrhea.⁽¹⁹⁾ In the present study *C. parvum* was isolated in 60 % of proportions whereas it was isolated from 28.7% cases at Chennai, 11 % cases at Mumbai and 2.3 % cases at Kolkata.^(20,21,22) *E. histolytica* was next common parasite in previous mentioned studies, unlike our results. Some other studies have also highlighted *Cryptosporidium* as a major pathogen associated with diarrhoea in HIV positive patients^(19, 23). While some studies have concluded *Isospora* as major pathogen associated with diarrhoea in HIV positive patients^(24,25) Because of the fact that the frequency of *Isosporiasis* in AIDS is likely to be underestimated due to asymptomatic shedding of oocyst and treatment with TMP-SMX for other infections which may confer protection against this protozoan.⁽²⁶⁾ The variation of these parasite isolates is the difference in the prevalence rate of the particular parasite in particular region and difference in advancement of the disease.

The research study from Singh et al reveals that the prevalence rate was higher in male (70%) than female (30 %) in their study, which is similar to the study of ours.⁽²³⁾ Prevalence of enteric parasitic infections was seen more commonly in adult patients in age group 31-45 years followed by 16-30 years and prevalence was the least in 61-75 years age group due to the fact that 31-45 years is being the common age group infected by HIV while enteric parasitic infection rate was more common in males as compare to females because the prevalence rate of HIV is more common in males than in females.

Difference in the incident rate of intestinal parasitic infection reported by different researcher can be attributed to the difference in geographical distribution of parasites, sanitary practices and different selection of cases. Though the reasons for the same were not ascertained, this could be attributed to the limited study sample.

The present study documents the higher prevalence of opportunistic enteric parasitic infections in HIV positive patients. A regular screening programme is mandatory for HIV positive patients for opportunistic parasitic infection in form of routine stool examination and steps should be taken to prevent the occurrence of these diseases by advising the patients to drink safe water and avoiding contact with contaminated soil.

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