

STUDY OF CRYPTOSPORIDIUM, CYCLOSPORA, ISOSPORA SPECIES AMONG HIV POSITIVE PATIENTS IN A TERTIARY CARE HOSPITAL, BIHAR, INDIA

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Abstract

Background: In HIV infected patients, progressive decline in their immunological responses makes them extremely susceptible to a variety of common and opportunistic infections. Gastrointestinal infections are very common in patients with HIV infection or AIDS. It leads to fatal complications in the immune-suppressed individuals. Diarrhoea is a common clinical presentation of these infections. Reports indicate that diarrhoea occurs in 30-60 per cent of AIDS patients in developed countries and in about 90 per cent of AIDS patients in developing countries. **Material & Methods:** This prospective study was conducted between the month March 2023 to August 2023 on HIV-infected patients with diarrhoea and without diarrhoea visiting at Anti-retroviral therapy (ART) centre in JLNMC, Bhagalpur. All the tests were done after due patients consent and in accordance with institutional ethical guidelines. Five ml of blood sample was collected in an EDTA bulb from each enrolled patients. Serum samples were used for HIV. Sixty stool samples from HIV positive patients were included in our study. The patients were provided a wide mouthed, clean, dry, properly labelled plastic and advised to collect freshly passed stool in the container. **Results:** Out of total 60 stool samples collected from HIV positive patients, 35 patients presented with diarrhoea and 25 without diarrhoea. Total of 40 patients were positive for all enteric parasite out of these, 20 patients positive for cryptosporidium parvum, 7 were positive for cyclospora caytanensis and 3 were positive for Isospora belli and 7 patients had mix infection out of which 6 had cryptosporidium & cyclospora infection and 1 patient was with isospora & cyclospora infection. **Conclusion:** The prevalence of intestinal parasites was higher among those HIV infected individuals with diarrhoea. The coccidian parasites are significantly more frequently seen in the stool of HIV positive patients. Among these coccidian parasites Cryptosporidium parvum is a most common parasite which can cause life threatening diarrhea.

INTRODUCTION

In HIV infected patients, progressive decline in their immunological responses makes them extremely susceptible to a variety of common and opportunistic infections.^[1] Gastrointestinal infections are very common in patients with HIV infection or AIDS. It leads to fatal complications in the immune-suppressed individuals. Diarrhoea is a common clinical presentation of these infections. Reports indicate that diarrhoea occurs in 30-60 per cent of AIDS patients in developed countries and in about 90 per cent of AIDS patients in developing countries.^[2]

The presence of opportunistic parasites Cryptosporidium parvum, Cyclospora caytanensis, Isospora belli and Microsporidia are documented in patients with AIDS.^[3] Non opportunistic parasites such as Entamoeba histolytica, Giardia lamblia, Trichuris trichiura, Ascaris lumbricoides, Strongyloides stercoralis and Ancylostoma duodenale are frequently encountered in developing countries but are not currently considered opportunistic in AIDS patients.^[4] Cryptosporidium parvum is a major cause of diarrhoea in developing countries, mainly affecting children and HIV infected individuals.^[5,6]

Cryptosporidium parvum is an intestinal coccidian parasite that causes infection of the small intestine. There are currently 11 valid species of *Cryptosporidium*, of which *C. parvum* and *C. hominis* are the main cause of disease in humans. The infection has been frequently diagnosed in patients with AIDS. Humans acquire infection by the ingestion of food or drink that is contaminated with faeces, which contain the sporulated thick walled oocyst. The cholera like voluminous watery diarrhoea is the key feature of cryptosporidiosis in the patients with AIDS. It causes self-limited diarrhoeal illness in immunocompetent healthy persons while severe prolonged life threatening diarrhoea in patients with AIDS. Oocysts are excreted variably in stool samples therefore multiple stool samples have to be examined. *Cryptosporidium* infection can be treated with nitazoxanide 500mg twice daily for 3-5 days. Hand washing, improved personal hygiene can help to minimise the risk of acquiring the infection.^[7] *Cyclospora cayentanensis* is the only species that is known to cause infection in humans. It inhabits the small intestine of humans. Humans acquired infection by ingestion of water or vegetables that are contaminated with the sporulating oocyst. Diarrhoea is the most common clinical feature seen in patients with Cyclosporiasis. It lasts for up to 2 weeks in immunocompetent hosts while it is watery and profuse and lasts prolonged in immunocompromised host. Detection of oocyst in stool is the method of diagnosis of cyclosporiasis. Co-trimaxazole (trimethoprim 160 mg and sulfamethaxazole 600 mg) twice daily for seven days is the treatment of choice in these patients. Boiling of drinking water and washing of fruits and vegetables before eating helps in prevention of infection.^[7]

Isospora belli is the coccidian parasite which also inhabits the small intestine of humans and cause self-limited mild diarrhoea in immunocompetent hosts and life threatening diarrhoea and dehydration in immunocompromised hosts, patients with AIDS. Humans acquire infection by drinking of water and eating food that is contaminated with mature oocyst. For the diagnosis of infection oocyst can be demonstrated in stool of patient. Co-trimoxazole is the drug of choice in these patients.^[7]

Aim & Objective

The present study was undertaken to identify oocysts of *Cryptosporidium parvum*, *Cyclospora cayentensis* and *Isospora belli* in stool sample of HIV positive patients.

MATERIALS AND METHODS

Study population: This prospective study was conducted between the month March 2023 to August 2023 on HIV-infected patients with diarrhoea and without diarrhoea visiting at Anti-retroviral therapy (ART) centre in JLNMCH, Bhagalpur. All the tests were done after due patients consent and in accordance with institutional ethical guidelines. HIV

serostatus of the patients was determined by using commercially available ELISA antibody tests using National AIDS Control Organisation (NACO) recommended algorithm. Five ml of blood sample was collected in an EDTA bulb from each enrolled patients. Serum samples were used for HIV. Sixty stool samples from HIV positive patients were included in our study. The patients were provided a wide mouthed, clean, dry, properly labelled plastic and advised to collect freshly passed stool in the container. These samples were transported to laboratory within one hour of collection for identification of parasites.

Stool Examination

Iodine and saline wet mount preparation of stool samples were made and examined under microscope. Samples were screened for oocysts of coccidian parasites by wet mount and Iodine mount preparations. Smears were stained by Modified acid fast method and examined under 1000x for red coloured acid fast oocysts of *Cryptosporidium*, *Isospora* and *Cyclospora* with their typical morphological features.

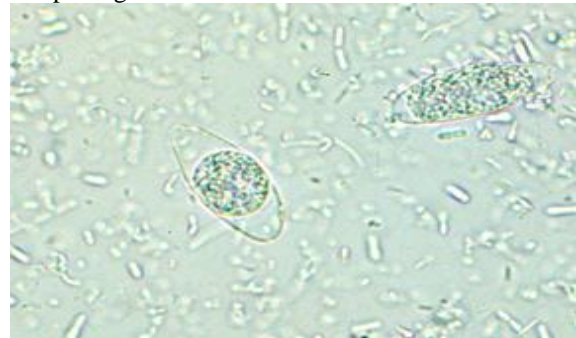


Figure 1: Oocyst of *Isospora belli* in stool wet mount

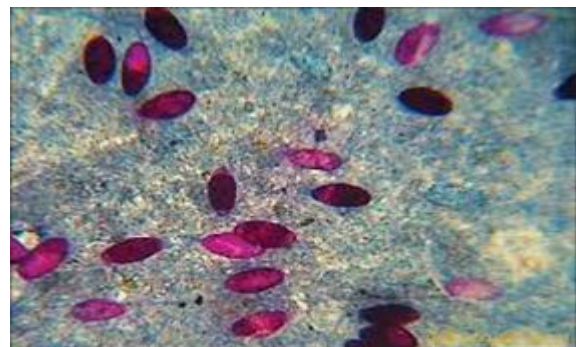


Figure 2: Oocyst of *Isospora belli* in Modified acid fast stain

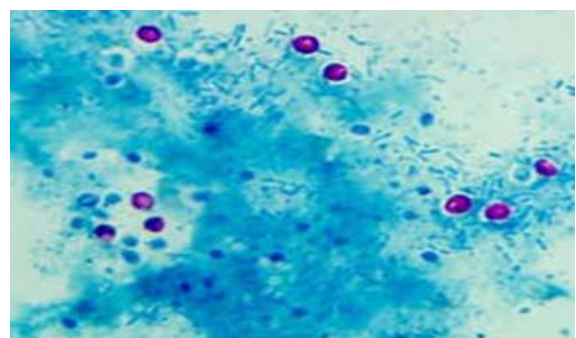


Figure 3: Oocyst of *Cryptosporidium* in Modified acid fast stain

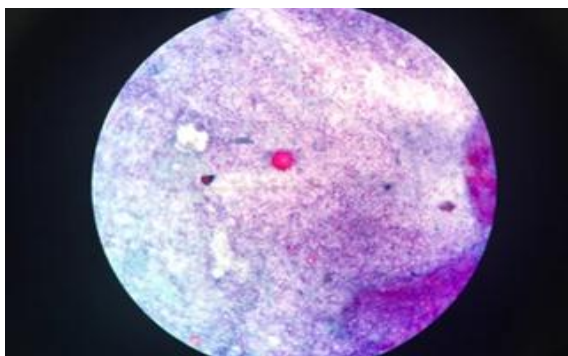


Figure 4: Cyclospora oocyst in Modified acid fast stain

RESULTS

Out of total 60 stool samples collected from HIV positive patients, 35 patients presented with diarrhoea and 25 without diarrhoea.

Total of 40 patients were positive for all enteric parasite out of these, 20 patients positive for cryptosporidium parvum, 7 were positive for cyclospora caytenesis and 3 were positive for Isospora belli and 7 patients had mix infection out of which 6 had cryptosporidium & cyclospora infection and 1 patient was with isospora & cyclospora infection.

Table 1: Percentage of Parasite in Stool Samples

Infection	No. of positive samples	Percentage
cryptosporidium parvum	23	57.5%
cyclospora caytenesis	7	17.5%
Isospora belli	3	7.5%
cryptosporidium & cyclospora	6	15%
isospora & cyclospora	1	2.5%
Total	40	100%

DISCUSSION

In the HIV/AIDS era, the infections by opportunistic agents are on the rise. Opportunistic infections of the gastrointestinal tract are one of the major causes of morbidity and mortality in HIV positive individuals worldwide.^[8] The coccidian parasites (Cryptosporidium spp., Isospora belli, cyclospora spp. and Microsporidium spp.) are foremost among the enteric parasites in these patients.^[6] The organisms usually cause a self-limiting illness in immunocompetent individuals but as the immune status of the patient falls they are known to cause life threatening profuse watery diarrhea.^[6] The present study documents that infections with intestinal protozoan parasites are common in HIV seropositive individuals. In present study, Cryptosporidium parvum infection is predominant and causes diarrhoea, this finding correlates with Rina das et al study. Studies conducted by Basak et al.^[9] Tuli et al,^[10] and Sadraei et al,^[11] have also reported the similar observation. Prevalence of Cryptosporidium is 56.49% in this study which is similar to the prevalence observed by Rina das et al.^[6] Prevalence of cryptosporidium observed in present study was similar to the prevalence reported in Africa and Haiti¹⁰ but it was higher than the prevalence reported from Shrihari narayan et al,^[15] Kulkarni et al,^[12] It has been observed that prevalence of cryptosporidiosis ranges from 8.5 to 81% in India¹². The prevalence of Cyclospora caytenesis was observed as 12.90% in present study which was higher than the prevalence observed in other studies 6,2,8 but lower than a study which is performed by Tuli et al¹⁰. The prevalence of Isospora belli is 4.80% in present study which was similar to the study performed by Basak et al. The reported prevalence rates of Isospora belli from various studies in India are 2.5%, 13.7%, 16%, 17%, 18%, and 31%.^[13] The

lower prevalence of both parasites in this study might be due to that our study participants are in the ART care who were taking ART and/or treatment for opportunistic infection. The other reason might be due to difference in immunity, diarrheic status, environmental and personal hygiene of the study participants and also geographical distribution.^[14] In present study, eight patients showed mix infection amongst which cryptosporidium and cyclospora mix infection was observed in seven patients. Amatya et al also found the mix infection with more than two parasites in which cryptosporidium and cyclospora infection was second most common cause of mixed infection. Whereas in a study conducted by Gupta et al,^[15] found that the mix infection caused by Isospora and cryptosporidium were more common.

CONCLUSION

The prevalence of intestinal parasites was higher among those HIV infected individuals with diarrhea. The coccidian parasites are significantly more frequently seen in the stool of HIV positive patients. Among these coccidian parasites Cryptosporidium parvum is a most common parasite which can cause life threatening diarrhoea. The routine screening of the stool samples of HIV seropositive patients with diarrhoea should be done for prompt patient care, to prevent the fulminant form of the disease. Timely detection and treatment would avoid the serious consequences of infection and also prevent the transmission. As most of the opportunistic parasitic infections occur through the faecal oral route, they can be prevented by using safe drinking water and food, also by giving education about practicing personal hygiene, proper food preparation, private good sanitation facility and taking timely and appropriate prophylactic measures.

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