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Research Paper

Study of Cryptosporidium, Cyclospora, Isospora Species Among Human Immunodeficiency Virus (HIV) Positive Patients and its Correlation with CD4 Count.

Medical Science

KEYWORDS : Cryptosporidium parvum, Cyclospora caytenesis, Isospora belli, HIV, CD4 count.

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ABSTRACT

Intestinal parasitic infection is a common entity in patients infected with human immunodeficiency virus (HIV). Diarrhoea is a common complication of infection with HIV leading to weight loss and cachexia. *Cryptosporidium parvum* is a major cause of diarrhoea in developing countries, mainly affecting children and HIV infected individuals with low CD4 counts. The infection is self limiting in immunocompetent hosts but can be severe and persist in the immunocompromised and malnourished individuals. **Objectives:** To identify oocysts of *Cryptosporidium parvum*, *Cyclospora caytenesis* and *Isospora belli* in stool sample of HIV positive patients and its correlation with CD4 count of patient. **Material and Methods:** Prospective study was conducted in which stool samples from 62 HIV positive patients were collected and routine microscopy was performed and for coccidian parasites modified acid fast staining (Kinyoun method) was used. The CD4 count of each patient was done by BD FACS machine with flowcytrometry technique. **Results:** Out of 62 stool sample, 36 patients were symptomatic while 26 were asymptomatic. Total 40 patients were positive for all enteric parasite out of these 35 patients were positive for *Cryptosporidium parvum*, 8 for *Cyclospora caytenesis* and 3 for *Isospora belli*. Enteric parasites were detected in 67.5% HIV-infected patients with CD4 count <200 cells/µl in which *Cryptosporidium parvum* (56.45%) was the most common parasite with CD4 count <200 cells/µl, followed by *Cyclospora Species* (12.9%) and *Isospora belli* (4.8%). **Conclusion**: In a developing country like India it is very easy to prevent all of these parasitic diseases which were commonly seen in AIDS patient. This can be done by drinking pure water, avoiding contact with contaminated soil and also by giving education about practicing personal hygiene, proper food preparation, private good sanitation facility and taking timely and appropriate prophylactic measures.

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 cayetanensis, 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 with low CD4 counts[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 diarrhea 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 diarrhea 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 upto 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].

The present study was undertaken To identify oocysts of *Cryptosporidium parvum*, *Cyclospora caytenesis* and *Isospora belli* in stool sample of HIV positive patients and its correlation with CD4 count of patient.

Material and Methods:

Study population: This prospective study was conducted between the month May 2014 to July 2014 on HIV-infected patients with diarrhoea and without diarrhoea visiting at Anti-retroviral therapy (ART) centre in SCSM Solapur. 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 Con-

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trol Organisation (NACO) recommended algorithm16. HIV- infected patients were defined as those who had tested positive for HIV antibodies by two sequential ELISA/rapid tests as per the recommendations given by the WHO. Five ml of blood sample was collected in an EDTA bulb from each enrolled patients. Serum samples were used for HIV CD4 cell count. CD4 cell counts were measured by using a FACS count system (Becton Dickinson, Singapore BD). Sixty two stool samples from HIV positive patients were included in our study. The patients were provided a wide mouthed, clean, dry, properly labeled 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

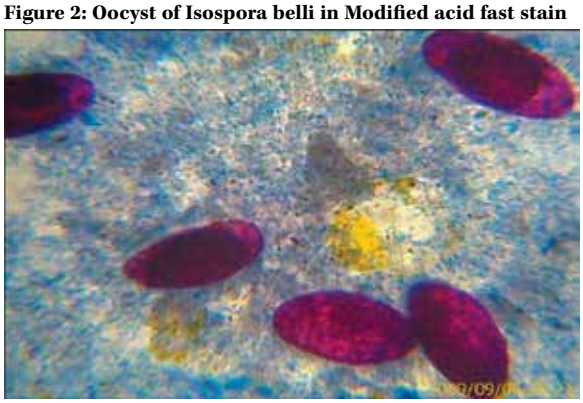


Figure 3: Oocyst of *Cryptosporidium* in Modified acid fast st ain

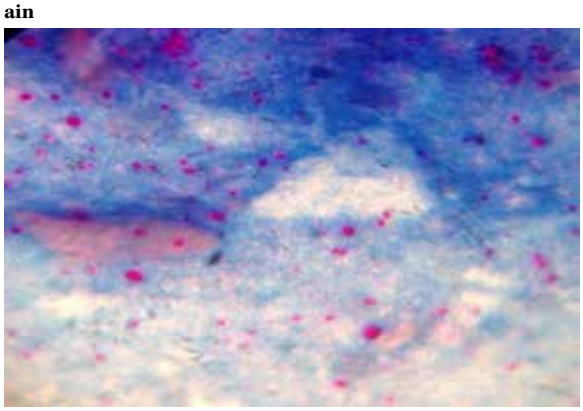
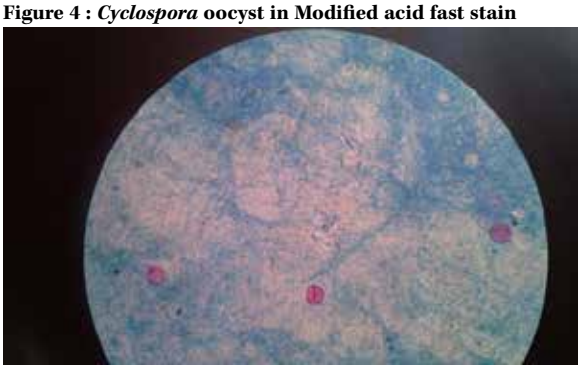


Figure 4 : *Cyclospora* oocyst in Modified acid fast stain



Results:
Out of total 62 stool samples collected from HIV positive patients, 36 patients presented with diarrhoea and 26 without diarrhea.

Total of 40 patients were positive for all enteric parasite out of these, 35 patients positive for *cryptosporidium parvum,* 8 were positive for *cyclospora caytenesis* and 3 were positive for *Isospora belli.* Among these forty patients, 8 patients had mix infection out of which 7 had *cryptosporidium & cyclospora* infection and 1 patient was *with isospora & cyclospora* infection**.**

Table 1: Percentage of Parasite in Stool Samples

Total stool samples collected	62	Percentage
Cryptosporidium parvum	35	56.45%
Cyclospora cytenesis	8	12.90%
Isospora belli	3	4.8%

Cryptosporidium parvum (56.45%) was the most common parasite with CD4 count <200 cells/µl, followed by *Cyclospora Species* (12.9%) and *Isospora belli* (4.8%).

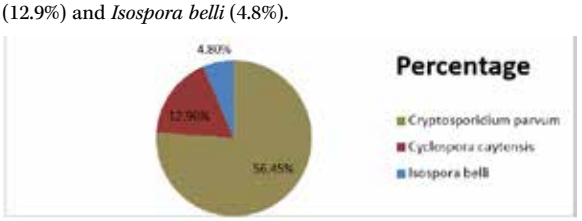


Figure 5: Diagram shows Percentage of parasite in stool samples

Enteric parasites were detected in 67.5% HIV-infected patients with CD4 count <200 cells/µl as compared with 32.5% in patients with CD4 count >200 cells/µl.

Table 2: Correlation of CD4 Count with number of patients

CD4 count	No patients	Percentage
CD4 count < 200 cells/µl	27	67.5 %
CD4 count > 200 cells/µl	13	32.5 %

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., Isospra belli, cyclospora spp. and Micro-*

sporidium 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 Haiti[10] but it was higher than the prevalence reported from Shrihari narayan et al[5], Rina das et al[6] and Kulkarni et al[2]. It has been observed that prevalence of cryptosporidiosis ranges from 8.5 to 81% in India[12]. The prevalence of *Cyclospora cyetensis* 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 al[10]. The prevalence of *Isospora belli* is 4.80% in present study which was similar to the study performed by Basak et al[9]. 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[8] 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.

In present study it was observed that coccidian parasitic infections were common with low CD4 count (<200cells/µl) and the similar finding were observed in Srihari narayan et al[5], Rina das et al[6], Kulkarni et al[2], S Gupta et al[15], Amatya et al[8].

Conclusion:

The prevalence of intestinal parasites was higher among those HIV infected individuals with diarrhea, low CD4 count. 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. 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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