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Original Article

Microbiology Section

Prevalence Of *Cyclospora Cayetanensis* In HIV Positive Individuals In A Tertiary Care Hospital

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ABSTRACT

BACKGROUND AND OBJECTIVES: The aim of this study is to determine the prevalence of *cyclospora cayetanensis* in HIV positive individuals in a tertiary care hospital and to correlate the findings with the CD4 cell count.

MATERIALS AND METHODS: Stool specimens (n = 50) from HIV Positive individuals were subjected to modified acid fast staining (Kinyoun's method) and safranin staining methods. CD4 cell count for all seropositive HIV patients was determined by Flowcytometry method.

RESULTS: Prevalence Of *Cyclospora cayetanensis* in HIV positive individuals is 24%.The occcurence is more common in men than in women.Patients with CD4 count of <200 cells/ microlitres revealed cyclosporiasis.

CONCLUSIONS: Hence, it is necessary to screen all the patients with or without diarrhea, so that we can diagnose and treat the patient which prolongs the life span of the patient.

Key Words: Intestinal coccidian parasite, *Cyclospora cayetanensis* , HIV infection, Diarrhoea, CD4 count.

INTRODUCTION

Coccidial infections of the gastrointestinal tract cause an acute, self-limited diarrhoeal illness in immunocompetent hosts [1]. *Cryptosporidium* and *Isospora belli* are the well recognized causes of chronic enteric infections in patients with the acquired immunodeficiency syndrome (AIDS) and other immunodeficiency states [2]. Recently, another *coccidial* parasite was identified in the faeces of immunocompetent and immunocompromised patients with diarrhoea [3]. This new pathogen was found to belong to the genus *Cyclospora*, based on results of electron microscopy, in vitro sporulation and excystation studies [4].

The coccidian, *Cyclospora* species (previously called the Cyanobacterium-like organism) is a newly recognized enteric pathogen [5]. *Cyclospora* cayetanensis is a coccidian pathogen which has been found in humans. Cyclosporiosis is characterized by mild to severe nausea, anorexia, abdominal cramping and watery diarrhoea. *Cyclospora* has now been reported from patients with protracted diarrhoeal illness in north, central and south America, The Caribbean, Africa, Bangladesh, south east Asia, Australia, England, and eastern Europe, and has been found to be characterized by its marked seasonality. Its routes of transmission are still unknown, although the faecal-oral route, either directly or via water, is probably the major one. A recent outbreak of cyclosporal infections in USA suggested that the transmission of *Cyclospora* was by the ingestion of contaminated berries.

The oocysts of *Cyclospora* can be detected by phase contrast microscopy, modified acid-fast staining, autofluorescence, and by amplification by the polymerase chain reaction. The oocysts are unsporulated when they are excreted in the faeces, and sporulated oocysts are needed for infection.

Each sporulated oocyst contains two sporocysts and each sporocyst contains two sporozoites. Humans seem to be the only hosts for this parasite. The histopathological examination of jejunal biopsies from infected individuals showed mild to moderate acute inflammation of the lamina propria and surface epithelial disarray. Parasitophorous vacuoles containing sexual and asexual forms of *C. cayetanensis* were found to be located in the cytoplasm of the epithelial cells. Cyclospora infections can be treated successfully with trimethoprim-sulfamethoxazole [6].

MATERIALS AND METHODS

This prospective study was conducted on HIV positive individuals who were admitted to the Antiretroviral Therapy Ward and the Integrated Counselling and Treatment Centre at Tirunelveli Medical College Hospital, Tirunelveli, Tamil Nadu, over a 6 month period (April' 2010 to September' 2010). The study was approved by the Institutional Scientific and Ethics Committee, and written informed consents were obtained from the patients.

HIV TESTING

The sera of the patients were separated on the same day and they were tested for HIV antibodies on the same/following day of their collection, by using WHO approved ELISA/Rapid kits which were supplied by MicroElisa, New Delhi, by following its testing guidelines.

The blood samples were processed immediately within 2 hours of their collection, for determining the absolute counts of the CD4+ and CD8+ cells and their ratios by two colour immunophenotyping on a single platform fluorescence activated cell sorting (FACS) count

system (Becton Dickinson Pvt. Ltd., Mountain View, CA.), by using fluorochrome labeled monoclonal antibodies to the CD4+/CD8+ T-cells. FACS count protocol software versions 1.0 (2005) (Becton Dickinson) were used for the data acquisition and analysis.

SAMPLE COLLECTION

Fifty seropositive HIV patients were included in the study and their stool samples were processed. The samples were collected in wide mouthed, plastic sample containers and they were transported to the laboratory for processing and staining.

STAINING METHODS

The stool specimens were subjected to the two staining methods, namely the Modified acid fast staining (Kinyoun's method) and the Safranin staining methods. The criteria which was used for the identification of the *Cyclospora spp*, was the presentation of rounded acid fast oocysts that were of intermediate size (8-10µm) between the *Cryptosporidium* (4-5 µm) and the *Isospora* (20-30 µm) sp.

RESULTS

The mean age of the patients was 40 years (range, 25 to 55 years); there were 35 men and 15 women. Among the HIV-infected persons, 22.9% (8/35) males and 26.7 % (4/15) females showed a prevalence of and the *Cyclospora* oocysts respectively [Table/Fig 1]. Chronic diarrhoea was the presenting complaint in 16 patients, while 34 patients were asymptomatic. Only 8 (16%) out of 34 patients without diarrhoea who were seropositive for HIV had Cyclospora oocysts in their faecal specimens and among the remaining 16 patients with diarrhoea, only 4 (8%) showed the presence of the Cyclospora species [Table/Fig 2].

The infection with Cyclospora was identified in 24% (12 of 50) of the subjects. The oocysts appeared as spherical structures which were 8–10 µm in diameter by modified acid-fast staining [Table/Fig 1]. Some stained dark red and had a variable number of dark inclusion bodies; others stained pink or remained unstained. Of the 12 *Cyclospora* positive individuals, 6 (12%) were co-infected with *Cryptosporidium parvum* (dual infection). Safranin staining gave an orange to red colour and showed a more clearly outlined membrane, but internal structure was not clear.

The diagnostic methods gave sensitivities of 24 % for modified acid fast staining and 12 % for safranin staining. The sensitivity of the safranin staining method was evaluated with that of the modified acid fast staining [Table/Fig 3] .

Patients with CD4 counts of <200 cells/ µl revealed *cyclosporiosis* while those with CD4 counts of 200-500 cells/ µl showed *Cryptosporidium* in their faecal samples [Table/Fig 4].

Cyclospora +ve	Male (n=35)	Female (n=15)
	8 (22.9%)	4 (26.7%)

[Table/Fig-1]: Prevalence of Cyclospora cayetanensis among Male and Female

CYCLOSPORA +VE	With diarrhoea (N=16)	Without diarrhoea (N=34)
	4 (8%)	8 (16%)

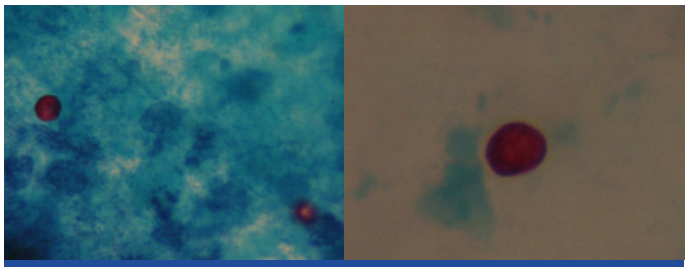
[Table/Fig-2]: Prevalence of Cyclospora cayetanensis among HIV patients with diarrhoeal and non-diarrhoeal complaints

Staining methods	Isolated Cyclospora	Associated coccidian infections (Cryptosporidium parvum)
Modified AFS	6	6
Modified Safranin	2	4

[Table/Fig-3]: The association between isolated Cyclospora oocysts and Staining methods

CD4 cell count	Total HIV Positive Patients	Cyclospora occurrence
< 200 cells/ µl	22	7
200-500 cells/ µl	23	5
> 500 cells/ µl	5	-

[Table/Fig-4]: The association between isolated Cyclospora oocysts and CD4 counts of AIDS patients.



[Table/Fig-5]: Oocyst of Cyclospora cayetanensis in modified Safranin stain (x 1,000)

DISCUSSION

The enteric coccidia are important causes of chronic and intermittent diarrhoea in infants, travellers, and immunocompromised patients, including those with AIDS. Previous studies have documented the occurrence of chronic diarrhoea in most of the patients with AIDS, which is usually associated with either Cryptosporidium or Isospora belli, and now the *Cyclospora* species [7,8] .The morphology of *Cyclospora* is similar to that of *Cryptosporidium* and it is called as the "Big Crypto".

The morphological characteristics of this organism were identical to those which were observed in patients with AIDS. Ortega et al reported that this organism belonged to the genus *Cyclospora*, as was determined by sporulation and excystation studies and by its appearance which was seen in transmission electron microscope studies [7] .

The clinical syndrome which was observed in immunocompetent persons and in patients with AIDS was virtually indistinguishable from that which was produced by Isospora belli and *Cryptosporidium*. In immunocompetent patients, the illness which was caused by *cyclosporiosis* was associated with prolonged but self-limiting diarrhoea which lasted for a period of 43 days. The organism was found to be waterborne and only limited epidemiological and environmental data were available. There have been few anecdotal reports on an association between *Cyclospora* species and prolonged diarrhoea in patients with AIDS.

Banu Sancak reported that males and females were equally susceptible to the *Cyclospora* infections. However, they proposed that the cause of the illness usually varied at all ages and in both immunocompetent and immunocomprised hosts [9,10]. In the present study, the incidence of the *Cyclospora* infections was

found to be more common in males (16%) than in females (8%).

Jean William et al. found that HIV seropositive patients who had chronic diarrhoea for more than 3 weeks had the Cyclospora spp in their faecal specimens. Other protozoa which were identified included *Cryptosporidium* in 135 patients (30%) among 1150 patients [11]. In the present study, 4 out of 16 HIV seropositive patients who had chronic diarrhoea for more than 3 weeks had the *Cyclospora* spp in their faecal specimens and 8 patients (30%) out of 34 patients without diarrhoea also showed the presence of this organism in their faecal specimens. Hence, the incidence of *Cyclospora* was suggested to be higher in non-diarrhoeal patients.

Jean William et al., reported that the sensitivities of other diagnostic methods for *Cyclospora* were evaluated by using faecal specimens that gave positive results with the modified acid-fast stain. The diagnostic methods gave sensitivities of 75% for modified AFS and of 30% for the safranin stain [11]. In this study, the diagnostic methods gave sensitivities of 24 % for modified AFS, and of 12% for the safranin stain. Hence, the diagnosis of *Cyclospora* was found to be better with modified AFS.

Jean Williams et al., reported that the CD4 counts of <200 cells/ microlitres revealed cyclosporiosis. The CD4 counts of 200-500 cells/microlitres showed *Cryptosporidium* in their faecal samples [11]. Similarly, the present study also revealedthat patients with a CD4 count of <200 cells/microlitres revealed cyclosporiosis and that CD4counts of 200-500cells/microlitres showed along with *Cryptosporidium* in their faecal samples.

The prevalence of *Cyclospora* cayetanensis in the HIV positive individuals was 24%.The occurrence was more common in men than in females. *Cyclospora* can be detected by using Modified Acid Fast Staining in patients without diarrhoea, who have a CD4 cell count of < 200 cells/ micro litres. Hence, it is necessary to screen all the patients with or without diarrhoea, so that we can diagnose and treat the patients, which prolongs the life span of the patients.

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