

## Original article

# Colonization of *Cryptosporidium spp* in asymptomatic HIV infected individuals and its correlation with CD4 T cell counts

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## Abstract

**Introduction:** Present study was undertaken to detect colonization of coccidian parasites in HIV infected patients without diarrhoea and its correlation with CD4 T cell counts.

**Materials and Methods:** 100 stool samples from HIV seropositive patients without diarrhoea attending ART OPD were included in present study. Stool samples were screened microscopically after staining with Modified Ziehl-Neelsen staining method to identify coccidian parasites. CD4 T cell counts of these patients were noted.

**Observations and Results:** Forty one out of hundred samples studied were positive for *Cryptosporidium spp*. Not a single sample was positive for *Cyclospora cayetanensis* or *Isospora belli*.

**Conclusion:** The screening of stool samples by Modified Ziehl-Neelsen staining method should be done routinely to look for coccidian parasites in all HIV positive individuals without diarrhoea especially with low CD4 T cell counts.

**Keywords:** HIV seropositive asymptomatic individuals, *Cryptosporidium spp*, CD4 T cell counts.

**Keynotes:** *Cryptosporidium spp* was the only enteric coccidian parasite found as colonizer in asymptomatic HIV positive patients. Colonization was more in patients with CD4 T cell counts less than 200 cells/ $\mu$ L.

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## Introduction

HIV infection being a global pandemic disease has affected almost each and every part of the world. Due to progressive immunosuppression, HIV infected persons are vulnerable to spectrum of overwhelming and fatal opportunistic infections. Gastrointestinal infections are a significant cause of morbidity, including wasting

and cachexia, in these persons.<sup>1</sup> Coccidian parasites, which include *Cryptosporidium spp.*, *Isospora belli* and *Cyclospora cayetanensis*, are well established as opportunistic parasites in AIDS patients.<sup>2</sup> With the increased burden of HIV infection, the prevalence rate of coccidian parasites detected as opportunistic parasites increased, leading to studies done addressing

different aspects of coccidian parasitic infection in different parts of world.<sup>2,3,4</sup> While most of the studies conducted underlined the importance of coccidian parasitic infection as causative factors in diarrhoea, few studies addressed its significance in asymptomatic HIV positive patients.<sup>5</sup> HIV seropositive patient carries a 10% lifetime risk of infection with *Cryptosporidium spp.*<sup>6</sup>

Patterns of enteric parasitic infections in developing countries, where hygiene is poor and intercurrent infection rates are high, may differ from patterns of developed countries.<sup>7</sup> Literature shows variations in types and prevalence rate of coccidian parasites in HIV infected people.<sup>5, 7-15</sup> Due to geographical disparities, regional and parameter specific studies on enteric parasites are required in areas with high rates of HIV infection. So this study was undertaken to determine the colonization of coccidian parasites in asymptomatic HIV positive patients. On the basis of immunological characteristics of HIV infection, the natural history of parasitic infection is likely to be altered in an unfavourable way.<sup>16</sup> Thus the present work also studied the correlation of coccidian parasites in asymptomatic HIV positive patients with CD4 T cell counts.

#### **Aim & Objectives**

1. To ascertain the common coccidian parasites as colonizers in HIV positive patients.
2. To compare the findings obtained with regard to the CD4 T cell counts and to assess the impact of CD4 T cell levels on probability of detecting coccidian parasites in HIV positive patients without diarrhoea.

#### **Materials & Methods**

A cross-sectional type of study was carried out in patients attending the ART centre. This study was initiated after the approval from the institutional ethical committee. The study was carried out over a period of two months and in line with ethical norms and guidelines issued by the ethical committee.

100 HIV positive asymptomatic patients were included in the study.

#### **INCLUSION CRITERIA-**

HIV positive patients defined as those who tested positive for HIV antibodies by three sequential ELISA / rapid test as per the guidelines recommended by NACO.

#### **EXCLUSION CRITERIA-**

1. Patients who received any anti-parasitic treatment in the past 3 weeks.
2. Patients of paediatric age group.

Consent of the patients through informed consent form was requested and confidentiality about their identity and HIV status was maintained.

HIV serostatus of the patients was determined by three sequential rapid methods as adopted by NACO.<sup>17</sup>

The first rapid test performed was SD BIOLINE HIV-1/2 3.0 (Standard Diagnostics, INC., Korea).

The second was Pareekshak HIV 1/2 Rapid Spot {Bhat Bio-Tech India (P) Ltd., Bangalore, Karnataka}.

The third test was The HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device {ACON Biotech (Hangzhou) Co. Ltd., China}

The CD4 T cell counts of HIV positive patients were estimated by FACS **Calibur** (Becton

Dickinson, Singapore BD). The most recent CD4 T cell counts of the patients were used for analysis.

The following technique of investigation was followed in detail.

1) Collection of Sample: -

Fresh stool sample was collected from patients in wide-mouth sterile container. Patients were advised to take care so as to avoid any kind of contamination with urine or water. The samples obtained were processed immediately within an hour.

2) Microscopic Examination: - The microscopic examination of all the samples was carried out with stained preparation. All samples were stained by using modified acid fast staining for detection of any coccidian parasites.

**Modified Ziehl-Neelsen staining:** - Sample were fixed on slide by heat and then flooded with strong carbol fuchsin. Heat was applied until steam rises from the stain. After cooling the slide, the material was decolorized using 3% HCL in 97% of ethanol. The material was counter-stained with methylene blue and after two minutes the slide was washed with distilled water. After drying the slide, it was observed under oil-immersion lens of light microscope.<sup>18</sup>

Statistical analysis: -

Data obtained from the investigation regarding the relationship between the CD4 counts of the patients who showed coccidian parasites in stained preparation was assessed using chi-square test

**Observations & Results**

Table 1: Age wise distribution of HIV positive asymptomatic patients enrolled in study

Age group (years)	Study group (n=100) (%)
11-20	2 (2%)
21-30	26 (26%)
31-40	46 (46%)
41-50	19 (19%)
>50	7 (7%)

Maximum number of asymptomatic HIV positive cases were from the age group of 31 to 40 years accounting for 46% (Table 1).

Table 2: Gender wise distribution of study subjects

Gender	study subjects n=100 (%)
Males	57 (57%)
Females	43 (43%)

Males were more as compared to females in the study group (57%).

Table 3: Prevalence of specific coccidian parasites

Parasites	HIV positive patients n=100 (%)
Coccidian parasites	
<i>Cryptosporidium spp.</i>	41 (41%)
<i>Isospora belli</i>	0
<i>Cyclospora cayetanensis</i>	0
Total	41 (41%)

Percentages of coccidian parasites were found to be 41 in asymptomatic HIV positive patients. All were of *Cryptosporidium spp* species. Not a single case of *Cyclospora cayetanensis* or *Isospora belli* was found in the study subjects.

Table 4: Correlation of parasites detected with CD4 count

CD4 Range	HIV positive asymptomatic patients n=100
	<i>Cryptosporidium</i> species
<200 cells/ $\mu$ L	22/34 (64.7%)
200-500 cells/ $\mu$ L	13/41 (31.7%)
>500 cells/ $\mu$ L	06/25 (24.0%)
Total	41/100

Colonization of *Cryptosporidium* species in study subjects was more in patients with low CD4 count (<200 cells/ $\mu$ L) {22/34 (64.7%) Vs 19/66(28.8%) (Chi-square =10.53, df=1, P=0.001 Sig)}

## Discussion

Due to deterioration of immune status, HIV infected individuals are susceptible to panorama of opportunistic infections during their lifetime. HIV infection and opportunistic infections have found to have deleterious effects in both directions.<sup>16</sup> Gastrointestinal infections are one of the commonly encountered diseases in HIV infected patients. Out of 100 HIV asymptomatic seropositive patients, 41 were found to harbor coccidian parasites. Dwivedi *et al*<sup>9</sup>, in their study has reported a prevalence of 44% coccidian parasites in asymptomatic HIV positive patients which is similar to the present study. In a similar study by Sucilathangam G *et al*<sup>14</sup> reported even higher prevalence of coccidian parasites (57.1%). Sherchen JB *et al*<sup>19</sup> did not find any asymptomatic coccidian infection in their study. Other workers have reported a prevalence of enteric parasites 1.4 to 30.08% in asymptomatic HIV positive patients.<sup>5-7, 10-12, 20</sup> Wide variations in the prevalence of coccidian parasites in various studies published could be due to differences in geographical distribution of parasites.<sup>19</sup>

In present study, *Cryptosporidium spp* was the only Coccidian parasite detected in study subjects. A study done in Cambodia<sup>21</sup> reported a prevalence of 52.5 % of *Cryptosporidium spp* in asymptomatic HIV positive patients. Although most of the recent studies shows *Cryptosporidium spp* as predominant among coccidian parasites, they have reported a lower prevalence of *Cryptosporidium spp* among asymptomatic group.<sup>5, 7-11, 13, 14, 20</sup> The probable reason for such a high prevalence colonization rate in present study could be due to the fact that

the mean CD4 T cell counts of the subjects in asymptomatic HIV positive patients was low (347.15 cells/ $\mu$ L). Reason for high prevalence of *Cryptosporidium* species in asymptomatic HIV positive groups could also be due to poor sanitation and contaminated water supply in this geographical area. Some studies also reported *Cyclospora cayetanensis* and *Isospora belli* in asymptomatic HIV patients<sup>5, 14</sup> but these were not detected in the present study.

To see any relationship between the coccidian parasites detected and CD4 T cell counts, correlation was done between the coccidian parasites and CD4 T cell counts of HIV positive asymptomatic patients. In asymptomatic HIV positive patients with CD4 count less than 200 cells/ $\mu$ L, 64.7% were harboring *Cryptosporidium* species. In HIV positive asymptomatic patients with CD4 count less than 200 cells/ $\mu$ L colonization of *Cryptosporidium* species was more as compared to patients with higher CD4 T cell counts. This finding is similar to studies by other workers.<sup>5, 6, 14</sup> Thus it reinstates that lower the CD4 T cell counts more is the probability of detecting coccidian parasites in asymptomatic HIV seropositive patients.

Higher prevalence of *Cryptosporidium* species in the HIV asymptomatic seropositive cases leads to patients excreting the infective oocysts in the environment which in turn may infect other patients with impaired immunity, thus spreading the infection. Moreover asymptomatic carriage may precede wasting and diarrhoeal illness leading to profound morbidity.<sup>2</sup>

Recently K Gupta *et al*<sup>15</sup> suggested that pathogenic burden accelerates disease progression and contributes to early morbidity in

HIV positive individuals. Therefore asymptomatic HIV positive patients, particularly with low CD4 T cell counts, should be screened for coccidian parasites on regular basis and treated accordingly to minimize the spread and reduce the morbidity associated with it. Coccidian parasites like *Cyclospora cayatanensis* and *Isospora belli* have effective treatment available whereas *Cryptosporidium spp* shows negligible innate drug susceptibility.<sup>22</sup> The only drug which show some efficacy against *Cryptosporidium spp* is Nitazoxamide<sup>15</sup> but there are no effective prophylactic chemotherapy available.<sup>23</sup>

The findings of this study could guide the clinicians to determine appropriate prophylactic measures in dealing with the coccidian parasitic load in HIV infected patients. Since infection with coccidian parasites occur largely by ingestion of food and drink contaminated with oocysts of parasites,<sup>24</sup> patients should be counseled regarding use of safe and boiled drinking water and maintaining personal hygiene to prevent these parasitic infections.

Community level measures like adequately treated water supply to houses is of paramount importance so that contamination of the environment with oocysts of these parasites

would be greatly reduced. Control of parasitic infestations by these measures will reduce the transmission of these parasites in the environment and will bring down the burden of morbidity in HIV patients.

This institution based study had several limitations like small sample size and limited diagnostic methods. These factors could well underestimate the results obtained. Nevertheless this study emphasizes that we should screen for coccidian infection in asymptomatic HIV positive individuals, particularly with low CD4 T cell counts.

### Conclusion

The present study reports high prevalence of *Cryptosporidium* species in asymptomatic HIV seropositive patients. *Cryptosporidium* species was the only coccidian parasite detected in HIV infected patients. *Cryptosporidium* species were more common in asymptomatic HIV positive patients with CD4 T cell counts less than 200 cells/ $\mu$ L.

Based on above findings we suggest that coccidian parasites should be looked for in the routine microscopic examination of the stool samples of HIV positive asymptomatic patients particularly with low CD4 T cell counts.

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