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Original Research Article

Opportunistic Coccidian Intestinal Parasitic Infections in Asymptomatic HIV Patients with Special Reference to Its Correlation to Immune Status and HAART

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ABSTRACT

Objective: The present study was undertaken to detect opportunistic parasites in HIV/AIDS patients without diarrhoea and its correlation with different levels of immunity (CD4 Count) and status of HAART.

Materials and Methods: Sixty stool samples from HIV seropositive patients with various other chief complains, but without diarrhea, attending BLDE University's Shri B.M. Patil Medical College Hospital & Research Centre, Bijapur, were included in the present study. Stool samples were examined by standard parasitological methods. HAART treatment status and CD4 counts of these patients were noted.

Results: Thirteen of sixty samples studied were positive for coccidian parasites. *Cryptosporidium species* was predominant (18.33%) followed by *Cyclospora* infection and mixed infection (1.67% each). Modified Kinyoun's acid fast staining could identify maximum number of coccidian parasites. All positive cases (13) were with CD4 count<200 cells/µl. Moreover 32.43% and 4.35% of cases were found to be positive for Coccidian parasites among patients on HAART and not on HAART respectively.

Conclusion: The present study shows that asymptomatic HIV/AIDS patients have colonization of opportunistic enteric coccidian parasites and are associated with low CD4 count. The present study highlights the fact that routine examination of the stool samples for coccidian parasites must be done even in the non-diarrhoeal HIV/AIDS infected individuals and especially in those with CD4 count< 200 cells/µl. Furthermore present study reveals that HIV-infected patients on HAART can still present with coccidian infection.

Keywords: Coccidian parasites, CD4+ T cell count, HIV, HAART

INTRODUCTION

Human immunodeficiency virus (HIV), causative agent in acquired immunodeficiency syndrome (AIDS), is fast becoming a major threat in the Indian subcontinent.[(1)] India has the third largest number of people living with HIV/AIDS. As per the 2008-09 HIV estimates, there are an estimated 23.9 lakh people currently living with HIV/AIDS in India.[(2)] Infections by opportunistic intestinal parasites have been the hallmark of AIDS since the beginning of

the epidemic. In recent years, numerous studies have outlined the emergence of important gastrointestinal coccidian parasites like *Cryptosporidium species, Cyclospora cayetanensis, Microsporidia species and Isospora belli*.[3]

The progressive decline and the ultimate collapse of the immune system functions, as measured by CD4 count, are characteristic of AIDS. Opportunistic infections take advantage of the deficient cell mediated and the humoral defense mechanisms causing morbidity and ultimately death.[4]

The dramatically reduced price of highly active antiretroviral therapy (HAART) medications due to its production by Indian generic manufacturers since the year 2000 has significantly increased access to treatment in resource limited regions.[5] The widespread use of HAART has resulted in a significant decrease in the incidence of opportunistic enteric pathogens as a consequence of immune recovery. However patients with advanced HIV disease who were recently diagnosed or have poor response to HAART can still suffer from opportunistic coccidian infections.[6] The data on which is limited in Indian settings.

There is a paucity of data on intestinal asymptomatic infections by these coccidian parasites, therefore the present study was done to detect the opportunistic enteric coccidian parasites in non-diarrhoeal HIV seropositive patients. Nevertheless, in India, few studies have tried to investigate the extent of intestinal coccidian parasitic infections in relation to HAART and CD4 count. The present study was aimed at correlating the asymptomatic opportunistic enteric coccidian parasitic infection with the immune status of the patients, determined by the CD4 count, as well as with HAART.

MATERIALS AND METHODS

After obtaining an approval from the institutional ethical committee and an informed consent from the patients, a total of 60 stool samples from patients of both sexes irrespective of age groups who were HIV seropositive with various other chief complains but without diarrhea attending BLDE University's Shri B.M. Patil Medical College Hospital & Research Centre, Bijapur, from May 2012 to September 2013 were included in the study. Study patients were interviewed using the structured questionnaire. Information was obtained on demographic characteristics, present and past history of diarrhea and anti-parasitic treatment, HAART. CD4 +Tcell count was obtained from patients records.

The HIV serostatus of the patients was determined according to NACO guidelines. Strategy IIB & III were used for diagnosis of HIV infection. All patients were tested for HIV using three separate kits, commercially available ELISA/Rapid Tests (Genetic system, Bio-Rad Labs, USA and Tridot, J Mitra & Co, New Delhi), having different system in order to confirm the diagnosis.[7]

The stool samples were collected in sterile leak proof plastic containers with a wide mouth and a tight - fitting lid without any preservative. The patients were asked to collect their stool sample preferably in the morning. They were instructed to avoid contamination of the stool specimen with urine or water. A disposable plastic spoon was kept inside each container for their convenience while collecting the sample. Patients were instructed to drop the spoon along with the sample inside the container and close the lid tight. The containers were labelled properly and transported to the laboratory without delay. The specimens were processed within 1-3 hours of collection.

The specimens were examined by naked eye for Colour, Consistency, and

Presence of blood, mucus, adult worms or segments of worms. Standard procedure for stool examination was carried out. Samples were examined microscopically directly and following concentration methods like formalin-ether concentration method and Sheather's sugar floatation technique. Direct microscopic examination of faeces in saline (0.85%) and 1:5 dilution of Lugol's iodine in distilled water was done to detect trophozoites, ova, cysts, larvae and oocysts. Ethanol fixed stool smears were stained with Modified Kinyoun's Acid-Fast Staining Method (Cold method) by using 1% H_2SO_4 as decolourizer for detecting coccidian parasites (*Cryptosporidium, Cyclospora, and Isospora species*) in the faeces specimens.[8]

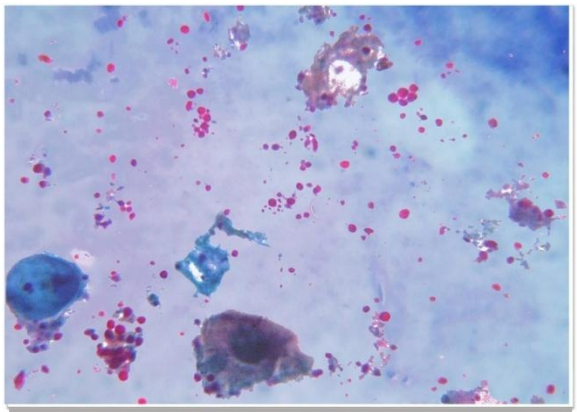


Figure 1: ***Cryptosporidium parvum*** **(Modified Kinyoun's acid fast stain)**

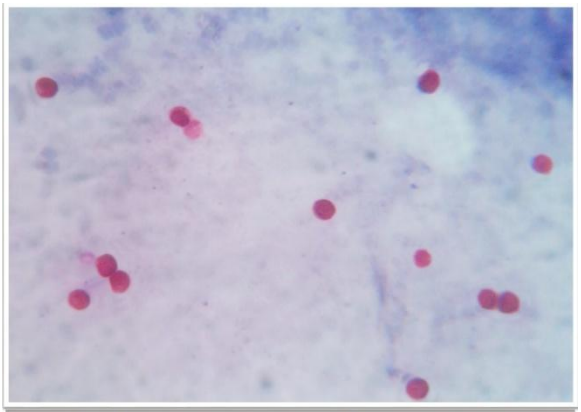
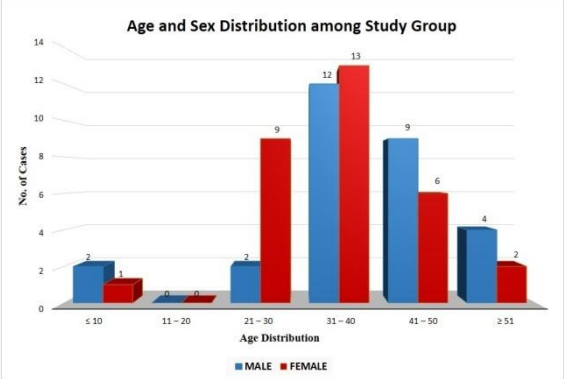


Figure 2: ***Cyclospora cayetanensis*** **(Modified Kinyoun's acid fast stain)**

Statistical analysis was done by using Graph Pad Prism version 6. Chi-square test was done to evaluate any correlation in HIV positives between parasitic infections, CD4 count and HAART. Observed differences in data were considered significant and noted in the text if $P < 0.05$ was obtained

RESULTS



Graph 1 : Age and sex distribution among study group.

In the present study out of 60 HIV positive patients without diarrhea, 29 were Male and 31 were Female. The mean ages of the male and female patients were 39.14 ± 12.87 and 36.26 ± 11.41 years respectively. The age and sex distribution of the study subjects is presented in graph 1. The age range of 31 to 40 years of both male (20%) and female (21.67%) sexes were the predominant age group.

Out of 60 samples examined 13 (21.67%) showed coccidian enteric parasitic infection. *Cryptosporidium parvum* oocyst appeared as pinkish spherical bodies measuring 4-6µm and was the most common parasite isolated in 11 (18.33%) samples. *Cyclospora cayetanensis* was detected in only one (1.67%) case and appeared as pinkish spherical bodies measuring 8 - 10µm in diameter. Mixed infection with *Cryptosporidium and Cyclospora species* was observed in 1 (1.67%) case.

The study population consisted of 11 patients with CD4 count <100 cells/µl, 15 patients with CD4 count 100 - 200 cells/µl and 34 patients with CD4 count < 200 cells/µl. Among 11 patients with CD4 count less than 100 cells/µl, parasites could be identified in 8 (72.72%) cases. Cryptosporidium parvum was the most common parasite identified in 7(63.64%), whereas 1(9.09%) case of mixed infection with *Cryptosporidium and Cyclospora species* was observed. Opportunistic intestinal parasites were detected in 5 (33.33%) out of 15 HIV-positive patients without diarrhea having CD4+ T cells count 100 - 200 cells/µl, *Cryptosporidium*(26.67%) was the most common pathogen followed by *Cyclospora*(6.67%). While in 34 non-diarrhoeal HIV-positive patients having CD4+ T cells count ≥200 cells/µl, no coccidian intestinal parasites were detected (Table 1).

Table 1: Correlation of CD4 counts and no. of cases positive for parasites.

PARASITES	CD4 COUNTS (cells/µl)			Total n=60
	< 100 n=11	100 – 200 n=15	>200 n=34	
Cryptosporidium parvum	7 (63.64%)	4 (26.67%)	0	11 (18.33%)
Cyclospora cayetanensis	0	1 (6.67%)	0	1 (1.67%)
Cryptosporidium parvum + Cyclospora cayetanensis	1 (9.09%)	0	0	1 (1.67%)
Total	8 (72.72%)	5 (33.33%)	0	13 (21.67%)
χ^2 (Chi square) = 21.70, d.f.=1, p< 0.0001, highly significant for enteric parasites in HIV positive patients without diarrhea having CD4+ T cell count <200 cells/µl and CD4+ T cell count ≥200 cells/µl				

Among 60 asymptomatic HIV positive patients 37 were on HAART, out of which 32.43% (12/37) were infected with intestinal coccidian. Whereas 4.34% (1/23) of patients not on HAART were positive for enteric coccidian infection. The highest infection prevalence of 66.67% was at CD4 count<100 cells/ µl followed by 33.33% at CD4 count 100- 200 cells/ µl in patients on HAART. Out of 23 HIV positive patients without HAART enteric infection with coccidian was identified in 1 patient whose CD4 count was less than 200 cells/ µl, whereas the other 22 patients not on HAART, with no enteric coccidian infection, had CD4 counts greater than 200 cells/ µl. (Table 2).

Table 2: Correlation of HAART status & CD4 Count with asymptomatic coccidian enteric infection.

CD4 COUNT (cells/µl)	ON HAART N=37		NOT ON HAART N=23	
	+VE for parasite	-VE for parasite	+VE for parasite	-VE for parasite
<100	8 (66.67%)	3 (12%)	0	0
100 - 200	4 (33.33%)	10 (40%)	1 (100%)	0
>200	0	12 (48%)	0	22 (100%)
Total	12	25	1	22
χ^2 (Chi square) = 6.59, d.f.=1, p= 0.01, significant for enteric parasites in HIV positive patients without diarrhea on HAART and HAART naïve group.				

DISCUSSION

Opportunistic infections are one of the most commonly identified causes that aggravate the condition of HIV-infected patients. Of these, parasites play an important role. Among parasitic

opportunistic infections coccidian parasites – *Cryptosporidium parvum, Cyclospora cayetanensis, Microsporidia species and Isospora belli*, are the main cause of enteric/intestinal infections in HIV infected patients. Cryptosporidiosis is one of the key parasitic diseases which have been included in the Centres for Disease Control and prevention (CDC) case definitions for AIDS.[9] Decline in the immunological and the mucosal defensive mechanism, evident with decreasing CD4 count (< 200 cells/µl), predisposes the HIV positive individuals to gastrointestinal infections. Most of the morbidity and mortality in such patients is due to opportunistic infections.[10]

Highly active antiretroviral treatment (HAART) increases the length and quality of life and productivity of patients by improving survival and decreasing the incidence of opportunistic infections in people with HIV.[11] The therapy leads to effective suppression of plasma viral loads and restoration of CD4 +T cells in peripheral blood in HIV-infected patients. Whereas CD4+ T cell restoration in gut associated lymphoid tissue (GALT) during therapy was shown to be modest and delayed compared to that in peripheral blood.[12] Very few studies has been done to compare the prevalence of intestinal parasites with CD4 count and HAART experience of HIV positive persons.

In our study out of 60 non-diarrhoeal HIV seropositive patients 29 were male and 31 were female. Our study shows a female preponderance in HIV infected patients similar to previous studies.[1] However, others have reported more males than females in this group. [13] Overall age group 31 - 40 years accounted for a maximum number of cases of both sexes [males: 12 cases (20%) and females: 13 cases (21.67%)]. Previous studies by Raytekar et al have also indicated HIV prevalence to be most common in the 31-40 year old age group.[13] Whereas Beena Uppal et al and Ramana et al has 21 - 30 years and 21 - 40 years as the most predominant age group respectively.[14,1]

In the present study, the overall prevalence of the coccidian parasites was 21.67% (13/60). It was higher as compared to the findings of similar Studies by other workers, who reported rates of 0.81% - 16.4% in non-diarrheal cases, which were conducted in different regions of India.[15-17, 10] Whereas the prevalence of the infection with the coccidian parasites in the non-diarrhoeal patients was 57.1% in a study by Sucilathangam G. et al.[18] The variation may be perhaps because of difference in endimicity of these parasites in different regions

The predominant enteric infection in this study was with *Cryptosporidium parvum* (18.33%). Similar study by Sachin Deorukhkar et al and Venkatesh Naik et al showed lower rate of infection contrary to a study from South India, which reports *Cryptosporidium parvum* prevalence of 46%.[10,17,18] A case of *Cyclospora cayetanensis* (1.67%) and a case of dual infection with *C. cayetanensis and C. parvum* was also identified in non-diarrhoeal patients studied by us which had also been reported by other study from south India.[18]

Other similar studies conducted showed infection with *Microsporidia spp*. and *Isospora belli* which was not detected in our study.[15,18] This discrepancy in the findings may be attributed to geographical variation, as well as differences in the populations, socioeconomic status and access to potable water, different screening, staining methods and molecular methods for detection of parasites used by several studies leading to variation in percentage and pattern of parasites identified.[15,18]

These opportunistic parasites can be acquired at any time during the course of the HIV infection. However, previous reports

have suggested high rate of prevalence of these infections in patients with CD4 count less than 200 cells/µl. In present study, among 13 enteric parasite isolated, 72.72% patients with CD4+ T cell count<100 cells/µl were positive for enteric parasites. While 33.33% patients were positive for enteric parasites of the patients with CD4+ T cell count in the range between 100-200 cells/µl. In patients with CD4+ T cell count>200 cells/µl no parasite was detected. Two studies from south India also reports high rates of coccidian infection in non-diarrhoeal HIV patients with CD4 count < 200 cells/µl.[13,18] Contrary to our finding Dwivedi et al reports a mean CD4 count of 301 cells/ µl in non-diarrhoeal cases positive for coccidian infection.[19]

It was observed that the CD4 cell count influenced the cause of diarrhea as well as the diagnostic yield.[20] *Cryptosporidium* infection is an important cause of diarrhea in the developing world.[21] The correlation between the CD4+ T cells and the *Cryptosporidium* infection in this study shows high rate of infection (11/26, 42.31%) in patients with CD4 count < 200 cells/ µl which correlates well with other similar studies.[18]

At higher CD4+ T-cell levels, generally spontaneous clearing of the parasite takes place. In resource poor settings like ours, patients usually go undiagnosed for long periods and present late in the course of the disease. Consequently, the patients usually present with profound, persisting and multiple intestinal infections and a low CD4+ T-cell counts.[15]

Of the 60 HIV positive patients included in the study, a total of 37 were on HAART and 23 were not on HAART. The overall prevalence of intestinal parasites among the study participants was significantly higher in the HAART group (32.43% (12/37)) compared to those not on HAART [4.34% (1/23)]. Basnet A. et al from Nepal reported coccidian parasites in 9.0% of patients without ART and 2.0% in patients on ART which is contrary to our study.[22] This may be likely because of variation in the CD4 +T cells count levels in the study groups at initiation of therapy.

The patients on HAART positive for infection in our study had mean CD4 count < 200 cells/ µl (91.67 ± 47.96). In patients on HAART, 11 had CD4 count <100 cells/ µl with parasites positivity rate of 72.72% (8/11), CD4 count 100 - 200 cells/ µl was in 14 patients with 4/14 (28.57%) positive for parasite, whereas out of 12 patients with CD4 >200 cells/ µl none were positive for coccidian enteric infection. In the group of 23 patients not on HAART, 22 patients had CD4 count >200 cells/µl (423.09 ± 121.92) and all had no enteric infection with coccidians. This shows that with higher CD4 counts the infection rate decreased in patients both on HAART and not on HAART. In this study the duration of initiation of HAART has not been included. For a good immune response i.e. a rise of 20-30% in the CD4+ T-cell number from baseline value, HAART for a period of 6 to 12 months is needed. However the restoration of CD4 + T cells does not occur in all individuals, this might be due to thymus failure or defect at the bone marrow precursor levels.[23]

The real problem is that, despite several hypothetical mechanisms proposed so far, we do not know exactly why CD4 T cells decrease and the causes of progressive immune system depletion. This may be perhaps due to HAART acting positively on some mechanisms involved in the CD4 + T cell depletion associated with HIV-1 disease, but not in all individuals. In other subjects with decrease in CD4+T cell, the anti-retroviral treatment also fails to partially reconstitute the immune system. The present study may probably be having non responder patients on HAART with

drastically reduced CD4 counts were found to be at higher risk of coccidian opportunistic infection. Several factors are associated with impaired CD4+ T-cell reconstitution, including older age, previous therapeutic failure, previous duration of antiretroviral therapy, low level of CD4+ T-cells before HAART, persistent HIV-1 replication at lymphoid tissues, low adherence to HAART, and previous treatment interruption.[23]

In the present cross sectional study the number of cases recruited is limited, and control group was not included. In this study the duration of initiation of HAART and history of prophylactic co-trimoxazole administration has not been included. A further prospective study is required detailing the progress of opportunistic parasitic infections on more number of samples. However the present study underlines the importance of initiation of HAART may be more beneficial in earlier course of the disease with higher CD4 count.

CONCLUSION

A significant number of intestinal parasites were also seen in HIV positive patients without diarrhoea in our study, thus indicating that there may be asymptomatic infections which may be going undiagnosed, thereby increasing the morbidity and mortality which are associated with them. The rate of infection with a particular enteric parasite in HIV/ AIDS patient will depend upon the endemicity of that particular parasite in the region. Laboratory support is essential to determine the asymptomatic enteric infection with coccidian parasites. Simple stool examination with modified acid fast staining technique on stool samples may reveal the existence of these parasitic infections. A high index of suspicion in such individuals should be born in mind of physicians to advice appropriate and timely such simple diagnostic technique. The technique is economical, rapid and good for differentiating infective agents of intestinal coccidian parasites.

The routine examination of the stool samples for coccidian parasites must be done even in the non-diarrhoeal HIV-infected individuals and with CD4 count which are < 200 cells/µl. As low CD4 counts at the initiation of therapy predicts bad therapeutic response for HAART.

Parasitic opportunistic infection remain a major concern in HIV- infected individuals and are unlikely to be eradicated from these patients. Existing parasitic infections still occur in those not yet diagnosed with HIV or not in medical care, those not receiving prophylaxis, and those not taking or not responding to HAART.

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