

Research Article

HAART and Immune status as predictors of opportunistic enteric coccidian infections in HIV/AIDS patients

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Abstract

Cryptosporidiosis, Isosporiasis and Microsporidiosis are the major intestinal coccidian parasitic infections, reported in HIV-infected patients and are the key parasitic diseases included in the Centers for Disease Control and prevention (CDC) case definitions for AIDS. The present study seeks to find the association between immune status, treatment status and duration of diarrhoea in HIV infected patients and risk of acquiring enteric coccidian infections.

Isospora belli was the commonest parasite (73% of parasites) followed by *Cryptosporidium* (13%), *Cyclospora* (7%) and *Blastocystis hominis* (7%); one case of mixed infection with *Isospora belli* and *Blastocystis hominis*. More parasites were isolated from patients with chronic diarrhoea than those without (p<0.01). Coccidian parasites were more commonly detected in HIV positive patients with CD4+ T cell counts between 50 to 200cells/μl (p<0.01). Isolation of enteric parasites was significantly more common in patients before the administration of antiretroviral therapy (p<0.05).

Keywords: parasitic infections, HIV, Isospora, CD 4 counts, HAART

1. Introduction

The major target for HIV is CD4+ T cell; a vital component of Cell mediated Immunity for combating intestinal parasitic infections. Hence a reduction in CD4+ T cell count predisposes HIV infected patients to various opportunistic coccidian enteric parasitic infections. Administration of Highly Active Anti Retroviral Therapy would lower the HIV viraemia; boost the immune response thus leading to clearance of these parasites. Several studies were carried out in India^{1,2} and abroad^{3,4,5} to correlate the immune status and treatment status of HIV patients with the prevalence of these parasitic infections. The present study was carried out to evaluate the role of the treatment status, immune status and duration of diarrhoea as predictors of acquiring coccidian infections in HIV/AIDS patients.

2. Material and Methods

Study subjects included 100 HIV positive patients with diarrhea (cases) and 50 HIV positive patients without diarrhea (controls). Single stool samples were collected from HIV positive patients attending the ART centre in Gandhi hospital. After taking written consent, each patient was interviewed using a questionnaire to collect information such as age, sex, clinical illness and treatment exposure.

2.1 Samples were screened for ova, cysts, motile trophozoites and oocysts of coccidian parasites by wet mount and Iodine mount preparations.

2.2 Smears were stained by Modified acid fast method and examined under 1000x for red coloured acid fast oocysts of *Cryptosporidium*, *Isospora* and *Cyclospora* with typical morphological features.

2.3 Modified Safranin method was used to screen reddish orange oocysts of *Cyclospora*, 8-10μm in size with a crinkled cyst wall.

2.4 Chromotrope staining was used to screen for Microsporidial spores.

2.5 All the samples were subjected to Formalin ethyl acetate concentration method and the sediment was screened for the parasites by the above methods.

2.6 CD4 Counts were determined by Flow cytometry using FACS calibur

Statistical analysis was done using SPSS software and P value <0.05 was considered significant.

3. Results

Isospora belli was the commonest parasite (73% of parasites) followed by *Cryptosporidium* (13%), *Cyclospora* (7%) and *Blastocystis hominis* (7%) with one case of mixed infection with *Isospora belli* and *Blastocystis hominis*.

The mean age of male and female patients in the study group was 33.3 yrs and 26.3 respectively. The mean age of male and female patients in the control group was 32.4 and 29.9 yrs respectively.

Table 1: Parasites associated with Acute and chronic diarrhoea

Parasite	Acute diarrhoea n=70 (%)	Chronic diarrhoea* n=30 (%)
<i>Cryptosporidium</i> (n=2)	2 (2.85)	0 (0)
<i>Cyclospora</i> (n=1)	1 (1.42)	0 (0)
<i>Isospora belli</i> (n=11)	2 (2.85)	9 (30)
<i>Blastocystis hominis</i> (n=1)	0 (0)	1(3.33)

Table 1 show the various parasites isolated in acute and chronic diarrhoea. *Isospora belli* was the only parasite detected in patients with chronic diarrhoea. All the patients with *Cryptosporidium* and *Cyclospora* infection had acute diarrhoea. 13 of the 14 patients (92.8%) infected with parasites had watery stools.

Table 2: Distribution of CD4 + T cell counts and their correlation with enteric parasites

CD ₄ counts	Total pts (n=100)	Total parasites (n=15) (%)	<i>C.parvum</i>	<i>I.belli</i>	<i>Cyclospora</i>	<i>B.hominis</i>
<50	9	1(11.11)	1	0	0	0
50-200*	49	13(26.53)	1	10	1	1
201-500	32	1(3.125)	0	1	0	0
>500	10	0 (0)	0	0	0	0

Table 2 shows the distribution of CD4 + T cell counts and their correlation with enteric parasites. Mean CD4+ T cell counts of HIV positive patients with and without diarrhoea was 224.14 cells/μl and 313.04 cells/μl respectively. Patients with chronic diarrhoea had lower mean CD4+ T cell counts (156.6) compared to those with acute diarrhoea (253.1). 13 of the 15 parasites (86.6%) isolated were observed at CD 4+ T cell counts of 50-200 cells/μl. For the purpose of statistical analysis, 2 ranges of CD 4+ T cell counts were analysed - CD 4+ T cell counts between 50-200 and the rest. Maximum parasite yield was seen in patients with CD 4+T cell counts between 50-200 cells/μl and it was statistically significant (P value<0.01).

Figure 1: Enteric parasites and CD+ T Cell count (cells/mm³)

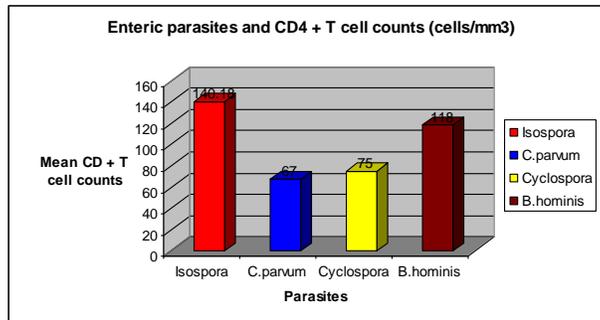


Figure 1 shows Mean CD 4+ T cell counts of patients with various parasitic infections. Patients with *Isospora* infection had mean CD4+ T cell counts of 140.18 cells/μl, *Cryptosporidium* - 67 cells/μl, *Cyclospora* - 75 cells/μl and the one patient with mixed infection had a CD4+ T cell count of 118 cells/μl. Mean CD 4+ T cell counts of the patients with enteric parasites was 125.07cells/μl.

Table 3: Association of enteric parasites and antiretroviral therapy

Parasites	On ART (%)	Not on ART* (%)
<i>Cryptosporidium</i> (n=2)	0 (0)	2 (100)
<i>Isospora belli</i> (n=11)	1 (9.09)	10 (90.9)
<i>Cyclospora</i> (n=1)	0 (0)	1 (100)
<i>Blastocystis hominis</i> (n=1)	1 (100)	0 (0)

Table 3 shows the association of enteric parasites and antiretroviral therapy. In 6 (42.8%) of the 14 patients with intestinal parasites, mean duration of HIV infection was 11.5 days with diarrhoea being the presenting symptom. 31% of the patients in the study group were on Antiretroviral therapy while 19 (38%) patients in the control group were on Antiretroviral therapy. All the patients with *Cryptosporidium* and *Cyclospora* infection were not started on antiretroviral therapy. Only one patient with mixed infection with *Isospora* and *Blastocystis hominis* was on antiretroviral therapy since 2 months.

Figure 2: Distribution of Parasites in HIV positive patients with and without anti retroviral therapy

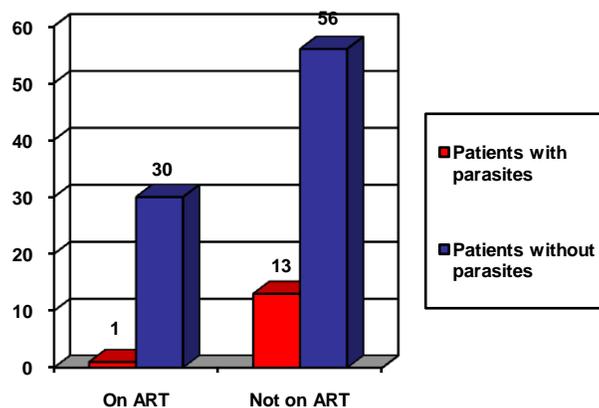


Figure 2 shows the number of enteric parasites found in patients with and without antiretroviral therapy. 92.8% (n=13) of the patients with parasitic infection were not on Antiretroviral therapy. Isolation of enteric parasites was seen more commonly in patients without antiretroviral therapy and it was statistically significant (P value<0.05).

4. Discussion

Isospora belli was the only parasite detected in patients with chronic diarrhoea. *Cryptosporidium* and *Cyclospora* were associated with acute diarrhoea. Similar observation was made by Kumar et al⁶. A contradictory finding was revealed by Certad et al⁷ who noted that a significant proportion of patients with Isosporiasis had acute diarrhoea. Gupta et al⁸ found *Isospora belli* in 41.1% of chronic diarrhoea while in the present study *Isospora belli* was detected in 30 % of HIV positive patients with chronic diarrhoea.

It was found in the present study that the prevalence of intestinal parasites was significantly higher in patients with chronic diarrhoea than in those with acute diarrhoea ($P < 0.05$). A similar observation was made by Tarimo *et al*⁹. 92.8% of the patients infected with parasites had watery stools which are in agreement with the observation made by Dalvi *et al*⁶.

One case of mixed infection with *Isoospora belli* and *Blastocystis hominis* was seen in the present study. Certad *et al*⁷ observed that the most common co infection in patients with Isosporiasis was *Blastocystis hominis* (18%) followed by *Strongyloides stercoralis* (14%), and *Cryptosporidium parvum* (5%). Dwivedi *et al*¹ noted in their study that chronic diarrhoea cases had polyparasitic infections. The various factors contributing to mixed infections are the presence of chronic diarrhoea, lack of personal hygiene, and the deteriorated immune status of the patients.

Fisseha *et al*¹⁰ found that the more "common" parasites, *Ascaris*, *Strongyloides*, *Giardia lamblia*, *T. trichura*, *Taenia* and *Entamoeba histolytica* are not opportunistic in AIDS. Awole *et al*¹¹ also made a similar observation that there is no evidence for an increased prevalence of *Giardia lamblia*, *Entamoeba histolytica* in HIV positive patients and exposure to these 2 parasites is likely to occur independently of HIV infection. Lindo *et al*¹² found that the prevalence of *G. lamblia*, *A. lumbricoides* and *T. trichura* were significantly higher in HIV negative patients. In the present study too, none of the conventional parasites were isolated.

Mean CD 4+ T cell counts of the HIV positive patients with diarrhoea (224.14 cells/ μ l) were lower when compared to those without diarrhoea (313.04 cells/ μ l). Further, patients with chronic diarrhoea had lower mean CD 4+ T cell counts (156.6 cells/ μ l) when compared to patients with acute diarrhoea (253.1 cells/ μ l). Similar findings were noted by Dwivedi *et al*¹ and Zali *et al*³.

Maximum parasite yield was noted in patients with CD 4+ T cell counts between 50-200cells/ μ l and this was statistically significant ($P < 0.01$). Similar observation was made by Dalvi *et al*² in 2006. De Oliveira Silva *et al*⁴ in their study in Brazil in 2007 made a similar observation that CD4 + T cell count > 200 cells/ μ l is a limiting factor for opportunistic infections in HIV/AIDS patients.

It was observed in the present study that a significant proportion of patients not on antiretroviral therapy were harboring intestinal parasites ($P < 0.05$). This finding was also noted by Bachur *et al*⁵ in 2008, which compared the prevalence of enteric parasites in HIV positive patients in 2 eras, pre Highly active anti retroviral therapy era and the era of Highly active anti retroviral therapy. They found that there was a significant reduction in the prevalence of enteric parasites between the 2 eras.

Hence all HIV positive patients with chronic diarrhoea need to be investigated for Isosporiasis and promptly treated as effective treatment and chemoprophylaxis are available. Periodic monitoring of CD4 + T cell counts are valuable as empirical treatment based on latest CD 4 count can be promptly instituted since most of coccidian diarrhoeal infections occur at CD4 + T cell counts less than 200cells/ μ l. Initiation of Antiretroviral therapy to eligible patients should not be delayed as most of coccidian parasitic infections occur before the administration of Antiretroviral therapy.

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