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 200 cells/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 India4,2 and abroad1,3 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.

1. Samples were screened for ova, cysts, motile trophozoites and oocysts of coccidian parasites by wet mount and iodine mount preparations.
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.
3. Modified Safranin method was used to screen reddish orange oocysts of Cyclospora, 8-10µm in size with a crinkled cyst wall.
4. Chromotrope staining was used to screen for Microsporidial spores.
5. All the samples were subjected to Formalin ethyl acetate concentration method and the sediment was screened for the parasites by the above methods.
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.

| Parasite               | Acute diarrhoea n=70 (%) | Chronic diarrhoea n=30 (%) |
|------------------------|---------------------------|----------------------------|
| Cryptosporidum (n=2)   | 2 (2.85)                  | 0 (0)                      |
| Cyclospora (n=1)       | 1 (1.42)                  | 0 (0)                      |
| Isospora belli (n=11)  | 2 (2.85)                  | 9 (30)                     |
| Blastocystis hominis (n=1) | 0 (0)                  | 1 (3.3)                     |
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.

| CD4+ T cell counts | Total pts (n=100) | Total parasites (n=15) (%) | *C. parvum* | *I. belli* | *Cryptosporidium* | *B. hominis* |
|-------------------|-------------------|---------------------------|-------------|-------------|------------------|------------|
| <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 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 2:** Distribution of Parasites in HIV positive patients with and without antiretroviral therapy

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 antiretroviral 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 al. A contradictory finding was revealed by Certad et al who noted that a significant proportion of patients with Isosporiasis had acute diarrhoea. Gupta et al 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. 92.8% of the patients infected with parasites had watery stools which were in agreement with the observation made by Dalvi et al.

One case of mixed infection with *Isospora belli* and *Blastocystis hominis* was seen in the present study. Certad et al. observed that the most common coinfection in patients with Isosporiasis was *Blastocystis hominis* (18%) followed by *Strongyloides stercoralis* (14%), and *Cryptosporidium parvum* (5%). Dwivedi et al. noted in their study that chronic diarrhoea cases had poly parasitic 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. found that the more “common” parasites, *Ascaris, Strongyloides, Giardia lamblia, T. trichura, Taenia and Entamoeba histolytica* are not opportunistic in AIDS. Awole et al. 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. 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/µl) were lower when compared to those without diarrhoea (313.04 cells/µl). Further, patients with chronic diarrhoea had lower mean CD 4+ T cell counts (156.6 cells/µl) when compared to patients with acute diarrhoea (253.1 cells/µl). Similar findings were noted by Dwivedi et al. and Zali et al.

Maximum parasite yield was noted in patients with CD 4+ T cell counts between 50-200 cells/µl and this was statistically significant (P<0.01). Similar observation was made by Dalvi et al. in 2006. De Oliveira Silva et al. their study in Brazil in 2007 made a similar observation that CD4 + T cell count >200 cells/µl 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 before and after the highly active antiretroviral therapy. Periodic monitoring of CD4 + T cell counts are valuable as empirical treatment based on latest counts would not be delayed as most of coccidian parasitic infections occur before the administration of Antiretroviral therapy.

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 200 cells/µl. 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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