Intestinal parasitic infections in ART naive and on-ART AIDS patient

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Introduction

- Intestinal parasitic infections in Human immunodeficiency (HIV) seropositive patients vary with geographical topology and are associated with socioeconomic variables.
- A consistent association between HIV infection and intestinal parasitosis has been reported in tropical regions [1] and more so in patients with CD4+ T-cell counts less than 200 cells/mm^3
- The clinical spectrum caused by intestinal parasites among HIV positive patients ranges from asymptomatic to severe infection leading to chronic diarrhea, dehydration and mal-absorption [2}.
- Almost 80% of the AIDS patients die because of AIDS related infections including intestinal parasitic infections rather than HIV infection itself [3].
- Present hospital based study was conducted to determine the spectrum of intestinal parasitosis in adult HIV/AIDS (Acquired Immunodeficiency Syndrome) patients in our tertiary care setting.

Materials and methods

❖A total of three hundred and forty two (n=342) individuals were enrolled and were screened for intestinal parasitosis..
❖Of these study population one hundred and forty two (n=142) were adult HIV seropositives and were further subdivided into ART naive (n=80) without diarrhea (CD4+T-cell count > 350 cells/µl) and sixty two (n=62) on-ART with diarrhea.
❖The rest two hundred (n=200) were non-HIV individuals comprising of hundred each with diarrhea (n=100) and without diarrhea.
❖Modified Ziehl-Neelsen (MAF) staining was performed on fecal smears to detect oocysts of *Cryptosporidium* spp., *C. cayetanensis* and *C. belli* [4] and modified Trichrome staining for microsporidia [5].
❖Diagnostic PCR assay was carried out targeting 18S rRNA gene of the genus *Cryptosporidium* [6, 7], *C. cayetanensis* [7], *C. belli* [8] and *Enterocytozoon bieneusi* (*E. bieneusi*) [9] using previously published primers, and PCR products were visualised in 2% gels stained with ethidium bromide.

Ethical statement

The ethical approval for this study was obtained from institutional ethical committee of our institute, before the commencement of the study. The participants were informed that the procedure used did not pose any potential risk and their identities and personal particulars will be kept confidential. During the meetings, enrolled individuals were informed that their participation is absolutely voluntary and they can withdraw from the study at any point of time without giving any reasons.

Results

❖A total of one hundred and thirty one (n=131) intestinal parasites could be identified from the study population [HIV seropositive (n=142) and non-HIV (n=200) individuals].
❖Amongst the intestinal parasites, 64% (84/131) were identified in HIV seropositives and 36% (47/131) in non-HIV individuals (OR=4.7; 95% CI 2.95 to 7.52; p< 0.0001.
❖Of these eighty four parasites identified in HIV seropositives (74%, 62/84) were found in patients with diarrhea and (26%, 22/84) without diarrhea (p<0.001).
❖Out of the total parasites identified (n=131), 25% (33/131) were coccidia and microsporidia, that includes *Crypstosporidium* spp. (13/131, 9.9%), *Cystoisospora belli* (13/131, 9.9%), *Cyclospora cayetanensis* (3/131, 2.2%), *Enterocytozoon bieneusi* (4/131, 3%), 42% (55/131) were non-coccidian pathogenic and helminthic parasites that includes *Giardia intestinalis* (27/131, 20.6%), *Entamoeba histolytica/Entamoeba dispar* (14/131, 10.6%), *Ascaris lumbricoides* (1/131, 0.7%), *Strongyloides stercoralis* (4/131, 3%), *Hymenolepis nana* (9/131, 6.8%). 33% (46/131) other parasites were identified including *Blastocystis hominis* (26/131, 19.8%), *Entamoeba coli* (9/131, 6.8%) and *Endolimax nana* (8/131, 6.1%).
❖The higher frequency of infection as well as multiple parasitic infections were observed at CD4+ T-cell counts of less than 200 cells/µL and was two-fold higher compared to individuals having counts more than 350 cells/µL (p<0.0001).

Microscopic examination

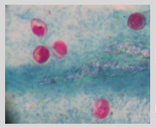

Fig. 1 Oocysts of *Cryptosporidium* spp.

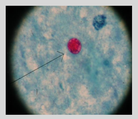

Fig. 2 Oocysts Of *C. cayetanensis.*

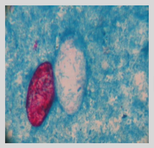

Fig. 3 Oocysts of *C. belli*

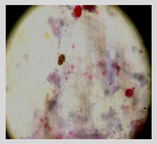

Fig. 4 Spores of *E.. bieneusi*

PCR assay

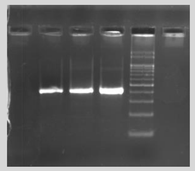

Fig. 5 PCR for 18S rRNA gene of Cryptosporidium spp.
Lane 1: Negative control
Lane 2: Positive control
Lane 3 &4: Positive samples
Lane 5: 100 bp molecular marker
Lane 6: Blank

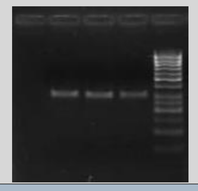

Fig. 6 PCR for 18S rRNA gene of *Cyclospora cayetanensis*
Lane 1: Negative control
Lane 2: Positive control
Lane 3 &4: Positive samples
Lane 5: 100 bp molecular marker

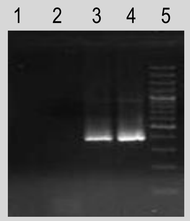

Fig. 7 PCR for 18S rRNA gene of *Cystoisospora belli*
Lane 1: Negative control
Lane 2: Positive control
Lane 3 &4: Positive samples
Lane 5: 100 bp molecular marker

Fig. 8 PCR for 18S rRNA gene of *Enterocytozoon bieneusi*
Lane 1: Positive control
Lane 2: Positive sample
Lane 3 :100 bp molecular marker
Lane 4: Negative control
Lane 5: Blank

	On- ART HIV patients with diarrhea (n=62)	ART naive HIV patients without diarrhea (n=80)
Opportunistic Parasites (n=30)	No. (%)	No. (%)
Cryptosporidium spp. (n=12)	10 (16)	2 (3)
Cyclospora Cayetanensis (n=1)	1 (2)	-
Cystoisospora belli (n=13)	9 (15)	4 (5)
Enterocytozoon bieneusi (n=4)	4 (6)	-
Non opportunistic, Pathogenic parasites (n= 33)		
Giardia intestinalis (n=16)	10 (16)	4 (5)
Entamoeba histolytica/E. dispar (n=8)	5 (8)	2 (3)
Ascaris lumbricoides (n=1)	1 (2)	-
Strongyloides stercoralis (n=4)	4 (6)	-
Hymenolepis nana (n=4)	3 (5)	1 (1)
Non opportunistic, non pathogenic parasites (n=22)		
Entamoeba coli (n=4)	3 (5)	1 (1)
Endolimax nana (n=3)	3 (5)	-
Blastocystis hominis (n=15)	9 (15)	3 (4)

Figure 9: Distribution of parasites based on CD4+ T-cell count'

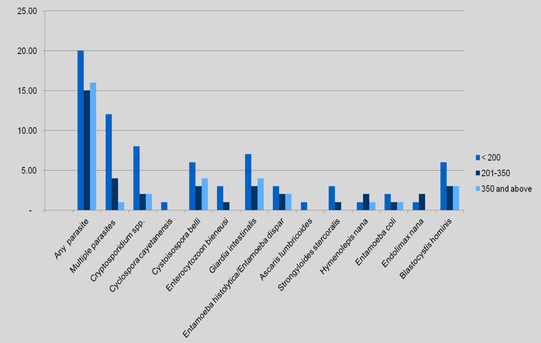

Figure 9: Distribution of parasites based on CD4+ T-cell count

Discussion

•Most pf the parasites identified in HIV sero-positive patients, were coccidian and microsporidia implicating a clear association of these parasites in HIV sero-positive patients.
•*Cryptosporidium* as a cause of diarrhea in HIV seropositive patients has been reported from India , and more importantly in patients with CD4+ T-cell counts of less than 200 cells/µl .At a higher CD4+ T-cell count, generally spontaneous clearing of the parasites take place. In a resource poor setting like ours, patients usually go undiagnosed for long periods and present late in the course of disease. Consequently patients usually presents with profound, persistent and multiples parasitic infections.

•Poor personal hygiene, low socioeconomic status and contaminated drinking water are the other additional factors responsible for high frequency of Cryptosporidiosis .
•Diarrhea is an important clinical problem among HIV sero-positive individuals and is associated with significant impairments in health and quality of life.
•The present study shows that diarrhea is a concern among the participants regardless of their HIV status though it more likely takes chronic course among HIV infected participants than HIV uninfected group.

References

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