

Original article

Evaluation of enzyme linked immunoassay for detection of cryptosporidium antigen in stool specimens of HIV infected patients of diarrhoea

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Abstract:

Introduction: Myriad causes of diarrhoea, with geographical variations seen with parasitic etiologies, make it important to understand the presentation of enteric parasitic infections in HIV infected patients. This study was undertaken to determine the parasitic infections in HIV infected patients and correlate with their CD4 counts in the local context.

Methods: This study enrolled 220 HIV infected patients presenting with diarrhoea, 100 HIV negative patients with diarrhoea as control group. Stool samples were examined microscopically by saline and iodine mount to look for parasites. Modified Kinyoun acid fast staining technique was used to detect coccidian parasites. Whole procedure was repeated after concentration for samples not showing any parasite by direct microscopy methods.

Keywords: Diarrhoea, Coccidian Parasites

Keynotes: Coccidian parasites the commonest enteric parasites in HIV infected Diarrheal Patients with less CD4 counts. In these patients among the coccidian parasites *Cryptosporidium spp* was the commonest.

Introduction:

Enteric opportunistic parasitic infections are noted as major source of diarrheal disease in developing countries mainly in Human Immunodeficiency virus (HIV) infected patients. Enteric parasites were recovered in more than 20% of HIV infected individuals, with coccidian parasites comprising 76.3%.^{1, 2} *Cryptosporidium spp* are major cause of diarrhea in developing countries mainly affecting HIV infected individuals with low CD4 lymphocyte counts.³ Hence the present study is aimed to detect the prevalence of intestinal parasitic infections in HIV infected individuals presenting with diarrhoea and its correlation with CD4+Tcell count.

Aim & Objectives:

Aim: To identify the parasites from stool samples of HIV infected patients with diarrhea.

Objectives:

1. To identify the parasites in stool samples of HIV infected patients.
2. To compare the result with HIV negative patients.
3. To correlate the obtained results in HIV positive patients with CD4+T cell count.

Material & Methods:

The present cross-sectional type study approved by institutional ethical committee comprises of 220 HIV positive patients presented with diarrhoea, attending the tertiary care hospital during period of one year from January 2012 to December 2012. Hundred HIV sero-negative patients presented with diarrhoea attending medicine OPD included in control group. Diarrhoea defined as 2 or more fluid or 3 or more soft stools per day.⁴

Stool samples were collected from these patients and these samples were processed to examine for various

enteric parasites. Macroscopic and microscopic examination of stool samples was performed. Microscopic examination included saline & iodine mount. Modified Kinyoun acid fast staining was done by modified Kinyoun cold technique. Special staining for detection of *Microsporidium spp* was not performed. Microscopic examination was repeated after concentration for samples which were not

showing any parasite by direct microscopy methods. Concentration of the stool was done by formal ether sedimentation method.

The CD4+ T cell counts were noted of HIV seropositive patients, which were determined by BD FACS count system. Comparison of results in study and control group was done.

Observations & results:

Prevalence of Enteric Parasites in Patients of Diarrhoea.

	Study group	Control group
Patients with parasitic diarrhoea	57 (25.90%)	18 (18%)
Patients without parasitic diarrhoea	163 (74.09%)	82 (82%)
Total	220	100

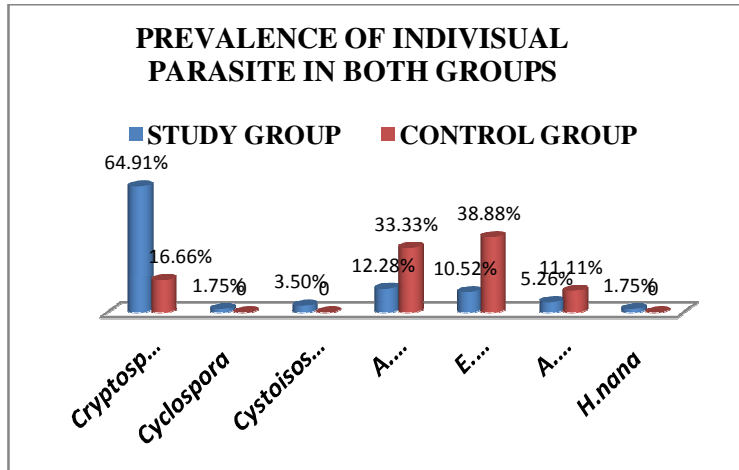
Difference in prevalence of parasitic diarrhoea in study & control group was not significant. (Chi square=1.976, df=1, P=0.160)

Prevalence Of Individual Parasite Detected In Study & Control Groups.

Parasites	Study group	Control group	Total
Coccidian			
<i>Cryptosporidium spp.</i>	37 (64.91%)	03 (16.66%)	40
<i>Cyclospora spp.</i>	01 (01.75%)	00	01
<i>Cystoisospora belli</i>	02 (03.50%)	00	02
Noncoccidian			
<i>Ancylostoma duodenale</i>	07 (12.28%)	06 (33.33%)	13
<i>Entamoeba histolytica</i>	06 (10.52%)	07 (38.88%)	13
<i>Ascaris lumbricoides</i>	03 (05.26%)	02 (11.11%)	05
<i>Hymenolepis nana</i>	01(01.75%)	00	01
Total	57	18	75

In study group, prevalence of coccidian parasites was highest, (70.17%) whereas in control group, prevalence of coccidian parasites was 16.66%. This difference in study & control group was statistically highly significant. (Chi square=16.6, df=2, P<0.001) In control group, helminthic parasites were at highest rate (44.44%) followed by protozoal (noncoccidian) parasites (38.88%). In study group, *Cryptosporidium spp* was the predominant parasite

(64.91%) followed by, *Ancylostoma duodenale* (12.28%) & *Entamoeba histolytica* 10.52%. In control group, *Entamoeba histolytica* (38.88%) was commonest parasite followed by *Ancylostoma duodenale*. (33.33%).



Correlation of type of parasite with CD4 + T cell count in study group.

CD4+T cell count (cells/μl)	Study group		Total
	Coccidian n=40	Non-coccidian n=17	
<200	29 (72.5%)	06 (35.29%)	35
200-500	06 (15.0%)	08(47.05%)	14
>500	05 (12.5%)	03 (17.64%)	08
	40	17	57

Percentage of coccidian parasite (72.5%) in patients with CD4 + T cell count <200 cells/μl was higher than other two groups of patients with CD4 + T cell count 200-500 cells/μl & >500 cells/μl. This difference of CD4 +T cell count with percentage of coccidian parasites was statistically highly significant. (Chi square=7.225, P=0.007).Percentage of noncoccidian parasite was similar in patients of all groups of CD4+T cell count which was statistically

not significant. Hence there is no correlation of percentage of noncoccidian parasite with CD4+T cell count. (Chi square=0.941, P=0.332)

Discussion:

A wide variety of gastrointestinal manifestations mainly opportunistic enteric parasitic infections are described in patients with HIV infection.⁵ The presence of opportunistic parasites mainly *Cryptosporidium parvum*, *Cyclospora cayetanensis*,

Cystoisospora belli, and *Microsporidium spp* is documented in patients of HIV/AIDS.⁶

In present study, out of 220 patients in study group, 57 (25.90%) had parasitic diarrhoea while in control group, 18% of patients had parasitic diarrhoea. Prevalence of enteric parasitic infection in HIV infected patients in present study was comparable to the study by Tiwari *et al*⁷ and Gupta *et al*.⁸

Difference in prevalence of coccidian parasite in study (70.17%) & control (16.66%) group was found to be statistically highly significant (P<0.001). Various studies noted the higher prevalence of coccidian parasite compared to noncoccidian parasites in HIV infected patients of diarrhoea.^{1, 8,9,10}

In HIV seronegative patients, helminthic parasites (44.44%) were commonest. In present study, out of 57 cases (study group) of parasitic diarrhoea, prevalence of *Cryptosporidium spp* was highest 64.91% followed by *Ancylostoma duodenale* 12.28% & *Entamoeba histolytica* 10.52%. Higher prevalence of *Cryptosporidium* infection (56.5%) was also noted by Sadraei *et al*.¹¹ In control group, *E.histolytica* (38.88%) was commonest parasite followed by *A.duodenale* (33.33%). In present study, *Cystoisospora* was detected in lower number of HIV infected cases (3.50%) which was comparable with study by Mohandas¹² *et al* in which prevalence of *Cystoisosporidiosis* was 2.5%.

Among the study group, percentage of coccidian parasites(72.5%) is significantly higher in patients with CD4+T cell count less than 200 cells/ μ l than other two groups i.e.CD4+T cell count 200-500 cells/ μ l and CD4 >500 cells/ μ l (P=0.007). Bala *et al*¹ studied distribution of enteric parasites with CD4+T cell count of HIV infected patients of diarrhoea. They also found that maximum number of cases of

diarrhoea due to coccidian parasite had CD4+T cell count less than 200 cells/ μ l. Similarly, Dwivedi *et al*¹⁵ observed that enteric coccidian parasites are significant agents associated with diarrhoea, in HIV infected patients especially among those with lower CD4+T cell count.

Conclusion:

Out of 220 patients, enteric parasites were found in 25.90% of patients in study group and in 18% of control groups. In HIV seropositive patients of diarrhoea coccidian parasites (70.17%) were most frequently detected compared to helminthic (19.29%) and other protozoal parasites (10.52%) whereas, in control group, helminthic parasites (44.44%) were predominant compared to other two groups. This difference was statistically significant. (P<0.001). Parasites most commonly detected in HIV infected patients with diarrhoea was *Cryptosporidium spp* (64.91%) followed by *A.doudenale* (12.28%), *E.histolytica* (10.52%), *A. lumbricoides* (5.26%), *Cystoisospora* (3.50%), *Cyclospora* and *H.nana* were detected in single case each. In study group, majority of the cases having parasitic diarrhoea had CD4+T cell count less than 200 cells/ μ l. Out of 40 cases of study group, from which coccidian parasites detected, 72.5% of the patients had CD4+T cell count less than 200 cells/ μ l. Prevalence of noncoccidian parasite was similar in patients of all groups of CD4+T cell count i.e. <200 cells/ μ l, 200-500 cells/ μ l, >500 cells/ μ l.

Enteric coccidian parasitic infection especially due to *Cryptosporidium spp* is one of the major health problems among HIV infected patients of diarrhoea. This emphasizes the need for stool examination including special staining of these patients to identify these parasites for appropriate management.

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