Correlation between CD4 Counts And Parasitic Intestinal Infection Among Diarrheal and Non-Diarrheal Human Immunodeficiency Virus Positive Indian Patients

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Abstract

Aim: With fall in CD_4 cell counts In Human Immunodeficiency Virus (HIV) positive patients, chances of opportunistic infections increases. The present study was conducted to observe the trends in intestinal parasitic infections in diarrheal and non diarrheal HIV positive patients and their correlation with CD_4 cell counts.

Material and methods: 146 HIV positive diarrheal and 154 HIV positive non-diarrheal patients were enrolled for collection of stool samples for detection of parasites. CD_4 counts of the patients were recorded at the time of sample collection.

Results: Cryptosporidium parvum was detected as the most common parasite in diarrheal (40.41%) and non-diarrheal patients (21.42%). The detection was statistical significant in diarrheal patients ($p < 0.001$) and patients with CD_4 counts $\leq$ 200µl ($p < 0.05$). Other parasites detected in diarrheal group were Isospora (11.64%), Giardia (10.27%), Cyclospora (2.73%), E. histolytica (4.79%), Strongyloides (1.36%) and Ascaris (1.36%).

Conclusion: Gastrointestinal problems due to opportunistic intestinal parasites are one of the hallmarks of HIV infection. These at times can be life threatening. Thus, timely detection helps in proper patient care and treatment of these problems.

Keywords: *HIV positive, Gastrointestinal problems, Opportunistic intestinal parasite, CD_4 count.*

I. INTRODUCTION

Since the beginning of the HIV epidemic in the world, opportunistic infections have been recognized as an important clinical complication. Opportunistic parasitic infections are a hallmark of HIV infection.[1] One of the most common presenting complaints in HIV infected individuals is diarrhea .[2] The infectious etiological agents include both opportunistic agents that consistently cause severe, chronic or frequent gastrointestinal disease and non opportunistic agents that usually cause acute, treatable diarrheal illness.[3] As the gastrointestinal symptoms in HIV postive patients are nonspecific and are a challenge to diagnosis and management,[4] therefore, there is a need to use sensitive and non-invasive diagnostic modalities for the timely diagnosis and to outline the trends observed in opportunistic infections affecting gastrointestinal tract in HIV positive patients so as to plan out our strategies accordingly and more effectively.

II. MATERIALS AND METHODS

The present study was conducted in a tertiary care teaching hospital after clearence from institutional ethics commitee. 10% formolised fresh stool samples from 300 HIV positive patients (146 diarrheal and 154 non-diarrheal), who were diagnosed by 3 Elisa/ Rapid/ Simple (E/R/S) tests after written informed consent, were collected. Relevant data and history was recorded.

CD4 count estimation was done by FACS count at the time of sample collection.

Identification of parasites in stool sample: A part of stool sample was emulsified in a drop of normal saline and lugol's iodine[5] and observed under the microscope before and after formol ether concentration method.[6] Thereafter, the concentrated samples were stained by modified acid fast and modified trichrome staining techniques.[7] Stained smears were then observed under the microscope.

The presence of parasites was correlated with CD4 cell count.

Statistical analysis: Data was analyzed using SPSS software version 14.0. The proportions of opportunistic pathogens were compared between the CD4 groups by using chi square test.

III. RESULTS

Majority of patients (78.33%), in this study were in the age group of 16-45 years. The average age of patients in the study is 35.44 years. Males accounted for 57.67% of HIV seropositive patients while females were 42.33%. Male to female ratio in the current study was 1.36:1. Patients belonging to rural areas accounted for 77.66%. Literacy status

revealed that most of the patients (70.33%) had education upto or below primary level. Housewives constituted 33.33% of the study population followed by daily wagers 28% (mainly manual labourers) and 21.66% salaried workers mainly constituting truck drivers. Heterosexual behavior (with single or multiple partners) was the most common mode of acquiring the disease, in 70% of patients followed by intravenous drug abuse (9.66% of the cases). In 3.33% of cases h/o blood transfusion was present. 5.66% had transmission from parent to child.

Table 1: Showing pattern of infection among diarrheal and non diarrheal group in relation to CD_4 count.

Type of infection	Diarrheal Group (n=146)	Mean CD_4 count (cells/µl)	Non diarrheal group (n=154)	Mean CD_4 count (cells/µl)
Single parasite	93 (63.69%)	227.01	46 (29.88%)	266.76
Multiple (Dual) parasites	7 (4.79%)	116.28	Nil	-
No parasite	46 (31.50%)	363.47	108 (70.12%)	383.84

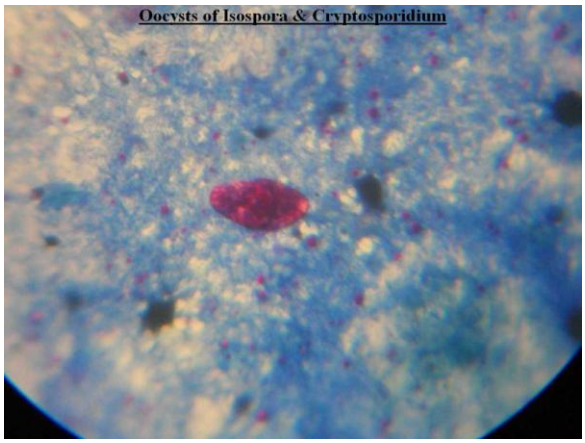

Fig. 1 Oocysts of Cryptosporidium & Isospora

Table 2 : Parasitic profile of diarrheal and non diarrheal group.

Parasite[#]	Diarrheal group (n=146)	Non diarrheal group (n=154)	p value
Cryptosporidium parvum	59 (40.41%)	33 (21.42%)	p < 0.001
Isospora	17 (11.64%)	-	p< 0.001
Giardia lamblia	15 (10.27%)	9 (5.84%)	-
Cyclospora	4 (2.73%)	-	p < 0.05
Entamoeba histolytica	7 (4.79%)	-	p < 0.01
Entamoeba coli	-	2 (1.30%)	-
Strongyloides	2 (1.36%)	-	-
Ascaris lumbricoides	2 (1.36%)	-	-
Hymenolepsis nana	1 (0.68%)	2 (1.30%)	-
Microsporidia	-	-	-

 # The number of parasites may exceed the number of patients, as more than one parasite was observed in some patients

Table 3 : CD_4 counts of the patients

CD4 counts (cells/µl)	Diarrheal group	Non diarrheal group
0-200	69 (47.26%)	47 (30.51%)
201-400	44 (30.13%)	51 (33.11%)
401-800	27 (18.49%)	47 (30.51%)
>800	6 (4.10%)	9 (5.84%)
Total	146	154

Table 4 : Relationship between CD_4 count and parasitic infections in diarrheal and Non-diarrheal HIV seropositive patients

Parasite[#]	CD_4 ≤200		CD_4 201-400		CD_4 401-800		CD_4 >800		Mean CD_4	
	Diarrheal	Non diarrheal	Diarrheal	Non diarrheal	Diarrheal	Non diarrheal	Diarrheal	Non diarrheal	Diarrheal	Non diarrheal
Cryptosporidium parvum	29	18	19	10	8	4	3	1	237.64	244.33
Isospora	11	-	5	-	1	-	-	-	145.82	-
Cyclospora	3	-	1	-	-	-	-	-	162.75	-
Giardia. Lamblia	9	3	6	4	-	2	-	-	181.13	295.66
Entamoeba histolytica	4	-	2	-	1	-	-	-	240.14	-
Entamoeba coli	-	-	-	1	-	1	-	-	-	463
Strongyloides	2	-	-	-	-	-	-	-	76.5	-
Ascaris. Lumbricoides	-	-	2	-	-	-	-	-	251.5	-
Hymenolepsis nana	-	-	1	1	-	-	-	1	358	562.5
Microsporidia	-	-	-	-	-	-	-	-	-	-

 # The number of parasites may exceed the number of patients, as more than one parasite was observed in some patients

IV. DISCUSSION

With the deterioration of immune status due to HIV infection, numerous opportunistic infections occur in the affected individuals. Of these gastrointestinal parasitic infections are universally recognized phenomenon. These largely present with diarrheal symptoms leading to life threatening complications.[8]

Prevalence of parasites in stool was 68.49% in diarrheal group and 29.88% in non-diarrheal patients. However, studies conducted by other workers have reported relatively lower prevalence (53.6 to 57.4%) in diarrheal patients as compared to our finding. This could be because of majority of patients in our study had lower level of education (70.33% had education below primary level) and belonged to rural areas (77.66%) where sanitation system and water supply were poor and open defecation practices were followed. However, prevalence of 12.9 to 40% of intestinal parasites have been reported by some workers in non-diarrheal patients as compared to our finding of 29.88%.[8,9,10]

Cryptosporidium parvum was detected as the most common parasite in our study. Prevalence of *C. parvum* among the diarrheal and non-diarrheal

group of patients was 40.41% and 21.42% respectively. The difference in prevalence of *C. parvum* ($p < 0.001$) was highly significant in diarrheal group in comparison to non-diarrheal group. This observation was consistent with various reports from different parts of country showing *Cryptosporidium* detection ranging from 40% to 60% except for reports from Manipur where detection rate was higher (94.4%).[11,12,13,14] This could be due to poor sanitation, contaminated water supplies, open defecation practices & contact with livestock in rural areas.

Isospora (11.64%), *Giardia lamblia* (10.27%), *E. Histolytica (*4.79%), *Cyclospora* (2.73%), *Strongyloides* (1.36%) and *A. lumbricoides* (1.36%) were the other parasites detected among the diarrheal patients. *G. lamblia* (5.84%), *E. coli* (1.30%) and *H. nana* (1.30%) were detected in non-diarrheal cases. The difference in prevalence of *Isospora* ($p < 0.001$), *Cyclospora* ($p < 0.05$) and *E. histolytica* ($p < 0.01$) was significant in diarrheal group in comparison to non-diarrheal group.

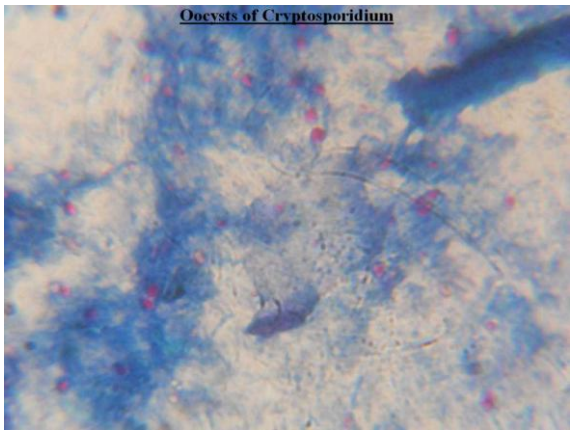

Fig. 2 Oocysts of Cryptosporidium

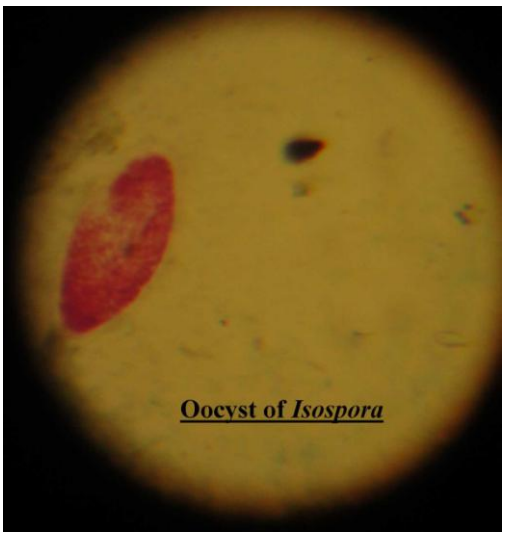

Fig. 3 Oocyst of Isospora

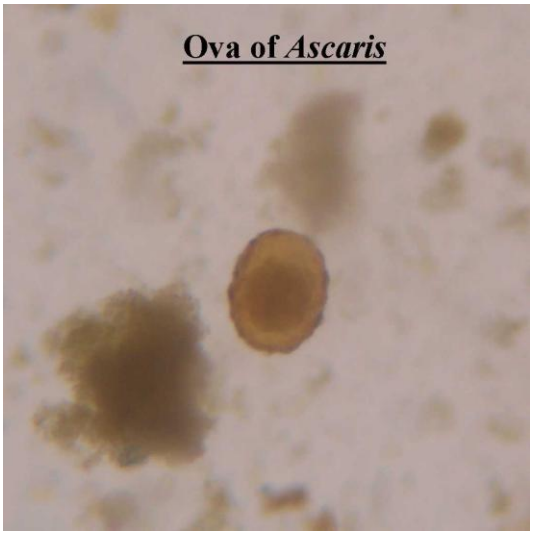

Fig. 4 Ova of Ascaris lumbricoides

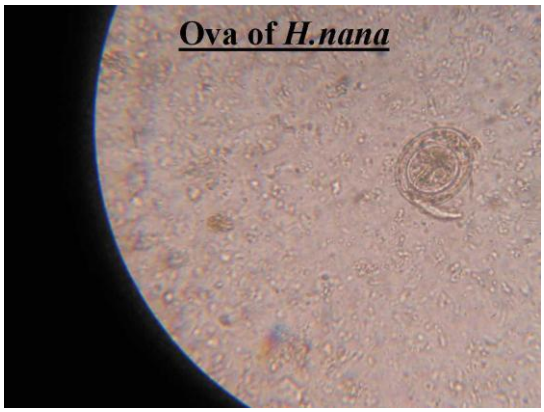

Fig. 5 Ova of H. nana

No case of Microsporidia was detected in our study. This may be due to difficulty in detection of these organisms in fecal samples by conventional staining method.[1]

In our study, it was observed that patients with diarrhea were having lower CD_4 counts in comparison to non-diarrheal group. The mean CD_4 counts of diarrheal group and non-diarrheal group were 266.32/µl and 352.69/µl respectively. It was also observed that the patients with mixed parasitic infection were having much lower CD_4 counts (table 1).

In relation to CD_4 counts, parasites detected in diarrheal patients with CD_4 counts $\leq$ 200/µl were C*ryptosporidium* (29/54), *Isospora* (11/17), *Cyclospora* (3/4), *Giardia* (9/15), *E. histolytica* (4/6) and *Strongyloides* (2/2) (table 4). Among the non diarrheal patients with CD_4 counts $\leq$200/µl, C*ryptosporidium* (18/33) and *Giardia* (3/9) were detected (table 4). The prevalence of *C. parvum* in patients with CD_4 counts < 200/µl was significant ($p < 0.05$) as compared to those with CD_4 counts > 200/µl. No such statistical significance was observed between CD_4 counts and other parasites in our study. Studies carried out by various other workers have also reported significantly higher proportion of

opportunistic pathogens in patients with CD4 count <200 cells/µl.[15,16,17] As the HIV infection progresses, protective CD_4 cell counts begin to decline resulting in increased susceptibility to opportunistic pathogens. This relation has been observed in our study as most of the patients harboring intestinal parasites were having counts $\leq$ 200/µl.

V. CONCLUSION

With decline in CD_4 cell counts $\leq$200/µl in HIV infected patients, the risk of acquiring opportunistic intestinal parasitic infection increases. Thus, routine screening of the stool samples of HIV seropositive patients with diarrhea should be done for prompt patient care so as to prevent the emergence of fulminant form of the disease.

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REFERENCES

[1] Mohandas, Sehgal R ,Sud A , Malla N. Prevalence of intestinal parasitic pathogens in HIV-seropositive individuals in Northern India. Jpn J Infect Dis., 2002; 55: 83-84.

[2] Framm SR, Soave R. Agents of diarrhea. Med Clin North Am. 1997; 81: 427–47.

[3] Smith PD, Lane HL, Gill VG, Manilchewitz JF, Quinnan GV, Fauci AS, *et al* . Intestinal infections in patients with AIDS: Etiology and response to therapy. Ann Intern Med 1988; 108: 328-33.

[4] Bhaijee F, Subramony C, Tang SJ, Pepper DJ. Human Immunodeficiency Virus-Associated Gastrointestinal Disease: Common Endoscopic Biopsy Diagnoses. Patholog Res Int. 2011; 2011: 247923.

[5] Arora DR, Arora BB. Diagnostic procedures. Medical Parasitology. 3rd ed. Delhi (India): CBS Publishers and distributors pvt ltd; 2010. p. 235.

[6] Forbes BA, Sahm DF, Weissfeld AS. Laboratory methods for diagnosis of parasitic infections. Bailey & Scott's Diagnostic Microbiology. 12th ed. St. Louis (Mis): Mosby Inc; 2007. p. 619-20.

[7] Forbes BA, Sahm DF, Weissfeld AS. Laboratory methods for diagnosis of parasitic infections. Bailey & Scott's Diagnostic Microbiology. 12th ed. St. Louis (Mis): Mosby Inc; 2007. p. 622-23.

[8] Hailemariam G, Kassy A, Abebe G. Intestinal parasitic infection in HIV/AIDS and HIV seronegative individuals in a teaching hospital, Ethiopia. Jpn J Infect Dis 2004; 57: 41-43.

[9] Zali MR, Mehr AJ, Rezaian M. Prevalence of intestinal parasitic pathogens among HIV positive individuals in Iran. Jpn J Infect Dis 2004; 57: 268-70.

[10] Carcamo C, Hooton T, Wener MH. Etiologies and manifestations of persistent diarrhea in adults with HIV-1 infection: a case-control study in Lima, Peru. J Infect Dis 2005; 191: 11-19.

[11] Anand L, Dhanachand C; Brajachand N. Prevalence and epidemiologic characteristics of opportunistic and non-opportunistic intestinal parasitic infections in HIV positive patients in Manipur. J Commun Dis 1998 Mar; 30(1): 19-22.

[12] Singh A, Bairy I. *Cryptosporidiosis* in HIV seropositive patients in Kasturba Hospital, Manipal, Karnataka, South India. J Inst Med 2005; 27: 2.

[13] Kang G, Ward HD, Wanke CA, Dheepa M. Molecular epidemiology of *Cryptosporidial* infections in HIV infected individuals in southern India. Proceedings of the 53rd Annual meeting of the American Society of Tropical Medicine and Hygiene; 2004 Nov 7-11; Miami, Florida, USA.

[14] Banerjee P, Chakrabarti T, Banerjee KL. Stool pathogens in diarrhea associated with HIV illness and importance of *Cryptosporidium*--a 7 year study in Calcutta, India. 12th International Conference on AIDS; 1998 Jun 28- Jul 3; Geneva, Switzerland. p. 539-40.

[15] Dwivedi KK, Prasad G, Saini S, Mahajan S, Lal S, Baveja UK . Enteric opportunistic parasites among HIV infected individuals associated risk factors and immune status. Japan J Inf Dis 2007; 60: 76-81.

[16] Kulkarni SV, Kairon R, Sane SS, Padmawar PS, Kale VA, Thakar MR et al. Opportunistic parasitic infections in HIV/AIDS patients presenting with diarrhea by the level of immune suppression. Indian J Med Res 2009 Jul; 130: 63-66.

[17] Sadraei J, Rizvi MA, Baveja UK. Diarrhea, CD4+ cell counts and opportunistic protozoa in India HIV-infected patients. Parisitol Res 2005; 97: 270-73.