Research Article

Study of intestinal parasites in HIV infected patients with chronic diarrhoea and their association with CD4 T-cell count and Anti-Retroviral Therapy

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Abstract

Introduction: Chronic parasitic diarrhoea is a major problem in HIV patients. The frequency of enteric parasites varies at different immune level and from region to region. Against this background an attempt was made to identify the pattern of enteric parasites in HIV positive patients with chronic diarrhoea and their association with CD4 counts and ART. **Materials and Methods:** A total 208 stool samples were collected and examined for enteric parasites by microscopy and special staining methods. HIV testing was carried out as per NACO guidelines and CD4 T-cell counts were estimated by FACS count system. **Observations and Results:** Out of total 208, enteric parasites were detected in 108 (51.92%) stool samples. Protozoan parasites (46.63%) predominated over helminths (5.28%). Opportunistic parasites such as *Cryptosporidium* spp., *Cyclospora* spp., *Isospora* spp. were detected in 80 (38.46%) and non-opportunistic parasites such as *E. histolytica, G. lamblia, S. stercoralis*, *A. lumbricoides, A. duodenale* and *Taenia* spp. were detected in 28 (13.46%) stool samples. Most common parasite found was *Cryptosporidium* spp. in 57 (27.40%) stool samples. Opportunistic parasites were significantly high in patients with CD4 count < 200 cells/µl and who were not taking ART. **Conclusion:** Chronic diarrhoea in HIV positive patients with CD4 counts <200 cells/µl has high probability of association with intestinal parasitic infections. Cryptosporidial diarrhoea is the major problem in these patients in our area. Pattern of infections often guide the clinician about appropriate management of opportunistic infections and is as important as antiretroviral therapy (ART) in preventing mortality and morbidity among HIV-infected persons.

Keywords: HIV patients, Chronic diarrhoea, Intestinal parasites, CD4, ART

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INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) caused by Human Immunodeficiency Virus (HIV) is the most important public health problem of 21st century. The total number of people living with HIV/AIDS in India is estimated at 21 lakh in 2012.[1] Since the beginning of the AIDS pandemic, opportunistic infections have been recognized as common complications of HIV infection. Among these opportunistic infections, gastrointestinal tract (GIT) infections are very common in patients with HIV infection or AIDS.[2] Chronic diarrhoea is a major problem in patients with HIV, especially those with advanced disease. It has been estimated that 30-50% of patients with AIDS in USA and 63-93% in developing countries like India suffer from chronic diarrhoea.[3] The etiological spectrum of enteric pathogens causing diarrhoea includes parasites, bacteria, fungi, and viruses.[4] Parasitic infections of the GIT are a major cause of

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morbidity in developing countries and are increasingly important in certain populations from developed countries.[5] HIV seropositive patients with CD4 T-cell count < 200 cells/µl had reported an excess risk of diarrhoea compared with those having ≥ 500 cells/µl[6] and majority of the opportunistic infections are not observed until CD4 count falls below 200 cells/µl. Immune status of HIV infected patients improves with Antiretroviral Therapy (ART), they becomes less prone to opportunistic infections and infectious etiologies of diarrhoea are parallel to that of immunocompetent patients.[4] Patterns of enteric parasites causing chronic diarrhoea in developing countries like India, show contrasting prevalence rates with marked geographical variation.[2,3,6] Only a limited data regarding enteric parasites causing chronic diarrhoea and their association with CD4 T-cell count and ART is available from our region. Against this background an attempt was made to identify enteric parasites in HIV positive patients presenting with chronic diarrhoea and to elucidate their association with CD4 counts and ART.

MATERIAL AND METHODS

A total 208 HIV seropositive patients with chronic diarrhoea (three or more liquid stools daily) for more than 14 days were included in this study. After taking detailed history and written informed consent, Blood and stool samples were collected from each patient. HIV testing was carried out using commercially available kits (HIV Comb, Parikshak, and Tridot Kits) by HIV testing strategy II B as per NACO guidelines.[7] CD4 T-cell count was performed by automated flow cytometry analyzer (FACS Calibur, Becton Dickinson) and the study subjects were categorized in three categories (< 200 cells/µl, 200-499 cells/µl, ≥ 500 cells/µl) depending on their immune status. A single fresh stool sample was collected in leak proof, labeled and sterile plastic container. Samples were concentrated by formalin-ether sedimentation and Seather's sucrose floatation method[8] and were examined as a wet saline mount and iodine preparation for the detection of ova, larvae and cysts (both direct and concentrated specimens). The samples were subjected to Modified Kinyoun's acid fast staining[9] for *Cryptosporidium* spp., *Cyclospora* spp., and *Isospora* spp. and Modified trichrome (Weber) staining[8] for *Microsporidium* spp. The statistical analysis was performed to test the significance of association between enteric parasites and CD4 T-cell count and ART by using chi-square test and software Graph pad prism 5. Values were considered to be statistically significant when the *P* value was less or equal to 0.05.

Table 1: Parasites detected in the stool samples (n=208)

Parasites			Stool samples
Protozoa	Intracellular protozoa (Coccidian parasites)	*Cryptosporidium* spp.	57 (27.40%)
		Isospora spp.	18 (8.66%)
		Cyclospora spp.	05 (2.40%)
	Extracellular protozoa	*G. lamblia*	12 (5.77%)
		E. histolytica	05 (2.40%)
Helminths	Nematodes	*S. stercoralis*	05 (2.40%)
		A. lumbricoides	03 (1.44%)
		A. duodenale	02 (0.96%)
	Cestodes	*Taenia* spp.	01 (0.48%)
	Total		**108 (51.92%)**

Table 2: Association of parasites with CD4 T cell count

Parasites	No. of subjects with CD4 T cell count			
	< 200 cells /µl (n=143)	200-499 cells/µl (n=56)	≥ 500 cells/ µl (n=9)	Total (n=208)
Protozoa				
Cryptosporidium spp.	46 (32.17%)	11 (19.65%)	-	57 (27.40%)
Isospora spp.	14 (9.79%)	04 (7.15%)	-	18 (8.66%)
Cyclospora spp.	04 (2.80%)	01 (1.78%)	-	05 (2.40%)
G. lamblia	07 (4.89%)	05 (8.93%)	-	12 (5.77%)
E. histolytica	03 (2.10%)	01 (1.78%)	01 (11.11%)	05 (2.40%)
Helminths				
S. stercoralis	05 (3.49%)	-	-	05 (2.40%)
A. lumbricoides	01 (0.70%)	02 (3.57%)	-	03 (1.44%)
A. duodenale	01 (0.70%)	01 (1.78%)	-	02 (0.96%)
Taenia spp.	-	01 (1.78%)	-	01 (0.48%)
Total	**81 (56.64%)**	**26 (46.42%)**	**01 (11.11%)**	**108 (51.92%)**

Table 3: Distribution of the parasites in subjects taking ART and not taking ART

Parasites	No. of subjects taking ART(n=94)	No. of subjects not taking ART (n=114)	Total (n=208)
Protozoa			
Cryptosporidium spp.	16 (17.02%)	41 (35.96%)	57 (27.40%)
Isospora spp.	06 (6.38%)	12 (10.53%)	18 (8.66%)
Cyclospora spp.	01 (1.06%)	04 (3.51%)	05 (2.40%)
G. lamblia	05 (5.31%)	07 (6.14%)	12 (5.77%)
E. histolytica	02 (2.13%)	03 (2.63%)	05 (2.40%)
Helminths			
S. stercoralis	01 (1.06%)	04 (3.51%)	05 (2.40%)
A. lumbricoides	02(2.13%)	01 (0.88%)	03 (1.44%)
A. duodenale	02 (2.13%)	-	02 (0.96%)
Taenia spp.	01 (1.06%)	-	01 (0.48%)
Total	**36 (38.29%)**	**72 (63.16%)**	**108 (51.92%)**

OBSERVATIONS AND RESULTS

Out of total 208 patients, 136 (65.39%) were males and 72 (34.61%) were females. The Majority (46.16%) of the patients belongs to age group 31-40 years.

In the present study, Enteric parasites were detected in 108 (51.92%) stool samples [Table 1 Protozoan parasites (46.63%) predominated over helminths (5.28%). Opportunistic parasites such as *Cryptosporidium* spp., *Cyclospora* spp., *Isospora* spp. were detected in 80 (38.46%) and non-opportunistic parasites such as *E. histolytica, G. lamblia, S. stercoralis*, *A. lumbricoides, A. duodenale* and *Taenia* spp. were detected in 28 (13.46%) stool samples. Most common parasite found was *Cryptosporidium* spp. in 27.40%, followed by *Isospora* spp. in 8.66%. Mixed enteric parasites were observed in six stool samples. These six samples had *Cryptosporidium* spp. along with *Isospora* spp. and *G. lamblia* in two samples each, while *Cyclospora* spp. in one sample. Association of enteric parasites with CD4 cell count was assessed and shown in table 2.

Enteric parasites detected were significantly higher in subjects with CD4 count < 200 cell/µl than the CD4 count > 200 cell/µl (P=0.033). A total 64 (44.75%) opportunistic parasites were detected in patients with CD4 count < 200 cell/µl, 16 (28.57%) with CD4 count 200-499 cell/µl and no opportunistic parasite was detected in patients with CD4 count ≥ 500 cell/µl. This shows opportunistic parasites were significantly higher in subjects with CD4 count < 200 cell/µl than CD4 count > 200 cell/µl (P=0.003). 17 (11.88%) non-opportunistic parasites were detected in patients with CD4 count < 200 cell/µl, 10 (17.85%) with CD4 count 200-499 cell/µl and 1 (11.11%) with CD4 count ≥ 500 cell/µl. There was no significant predominance of non-opportunistic parasites in HIV positive patients at any level of immunosuppression (P=0.296). Enteric parasites were significantly higher in subjects who were not taking ART (P<0.0001) [Table 3].

DISCUSSION

Diarrhoea is a common complication of HIV infection; 30-90% of patients with AIDS suffer from diarrhoea and detection of enteric pathogens varies from 40% to 83%.[3]Also there are many reports regarding the frequency of various pathogens causing diarrhoea from different parts of India.[2,3,6,10,11] Many studies have highlighted the emergence of important protozoan parasites and helminths as major cause of morbidity and mortality in patients with AIDS. A total 108 (51.92%) enteric parasites were detected in our study. Various studies from India have reported high prevalence of intestinal parasites, ranging from 30-60%.[2,6,10] Deorukhkar *et al*[12] from Western Maharashtra in the year 2011 reported high intestinal parasite prevalence (68.97%) in HIV positive patients from rural area while Uppal *et al* [13] from New Delhi in the year 2009 reported 20% intestinal parasites in HIV seropositive subjects with diarrhoea. In the present study, protozoan parasites (46.63%) predominated over helminths (5.28%) comparable to studies conducted by Kumar *et al*[10] in 2002 and Gupta *et al*[14] in 2008. The opportunistic parasites such as *Cryptosporidium* spp., *Cyclospora* spp., *Isospora* spp. and Microsporidia are documented in patients with AIDS. Other than opportunistic parasites, *E. histolytica, G. lamblia, S. stercoralis, A. lumbricoides, A. duodenale* and *T. trichiura* are commonly encountered in developing countries but are not currently considered opportunistic in AIDS patients.[2] We also found that opportunistic parasites were most commonly associated with chronic diarrhoea in HIV patients as compared to non-opportunistic parasites in our area. *Cryptosporidium* spp. was the most common enteric parasite detected in this study followed by *Isospora* spp. Cryptosporidiosis is

distributed all over the world and is generally tends to vary from one locality to another and from one country to another depending on the level of contamination in water, food, and contacts with the animals, which are important factors in dissemination of the parasite.[13] The frequency of *Cryptosporidium* spp. and *Isospora* spp. infection in HIV/AIDS patients is reported widely between 2.9- 33% and 2.5- 26.1% respectively in various studies conducted in India.[2,6,14] *Cyclospora* spp. was found in 2.40% stool specimens in our study. Kumar *et al*[10] in 2002, Gupta *et al*[14] in 2008 and Basak *et al*[15] in 2010 reported *Cyclospora* spp. in 1.69%, 2.9% and 2.02% HIV seropositive patients respectively. Another coccidian parasite, microsporidium was not detected in our study similar to few other studies.[16,17] This could be due to endemicity of a particular enteric parasite in the community is likely to govern the incidence and prevalence of a particular parasitic infection in HIV/AIDS. Among the non-opportunistic parasites, *G. lamblia* was detected in 5.77% of stool samples and *E. histolytica* was detected in 2.40% stool specimens comparable to Gupta *et al*[14] and Kumar *et al*[10] Among the helminths, *S. stercoralis* was found in 2.40% in our study similar to other studies.[14,15] Other helminths such as *A. lumbricoides, T. trichiura*, *A. duodenale*, *E. vermicularis, S. mansoni, Taenia* spp. and *Hymenolepis nana* have shown lesser frequency and importance in AIDS patients.[19] In our study, *A. lumbricoides, A. duodenale, Taenia* spp. were found in 1.44%, 0.96%, and 0.48% patients respectively. Deorukhkar *et al*[12] in 2011 reported *A. duodenale* in 8.73%, *A. lumbricoides* in 3.61%, *Taenia* spp. in 1.51% and *S. stercoralis* in 0.60% patients. Concurrent infections with parasites in HIV infected patients are not uncommon. In the present study, mixed infections were detected in 2.89% study subjects similar to other studies.[3,20] Most of the opportunistic infections are not observed until the CD4 count falls below 200 cells/µl. In various studies, parasites associated with HIV were more likely encountered as the CD4 cell count falls below 200 cells/µl.[14,20,21] This may be because immunocompromised patients were either more susceptible to acquire particular parasites and/ or unable to clear once infection is established. In agreement with previous studies, [14,20,21] opportunistic parasites were significantly higher in subjects with CD4 count < 200 cell/µl than CD4 count > 200 cell/µl (P=0.003). No such significant predominance was observed in case of non-opportunistic parasites like in other studies.[20,21] This result shows the importance of the association of opportunistic intestinal parasites with chronic diarrhoea in the HIV infected patients particularly with CD4 count < 200 cells/µl. While non-opportunistic intestinal parasite infections are still a problem in HIV-infected patients at any level of CD4 count. Appropriate management of opportunistic infections is as important as antiretroviral therapy (ART) in preventing mortality and morbidity among HIV-infected persons. Enteric parasites were found in 38.29% (36/94) subjects who were on ART and 63.16% (72/114) in those not taking ART. Enteric parasites were significantly higher in subjects who were not taking ART (P<0.0001). This elucidate that the patients taking ART are less susceptible to develop an opportunistic infections as it is effective in raising the CD4 count and lowering HIV RNA levels.

CONCLUSION

Cryptosporidial diarrhoea is the major problem in HIV seropositive patients in our area. Enteric parasites were found to be more common in HIV seropositive patients with CD4 cell count < 200 cells/µl. The introduction and rapid implementation of highly active anti-retroviral therapy (HAART), has profoundly affects the incidence and complications AIDS including diarrhoea. Various studies from different parts of the world show contrasting patterns of enteric pathogens with marked geographical variations. This emphasizes the need for thorough investigations of these patients to identify pathogens for proper management in particular region. This will not only prolong the life of HIV infected individuals but also improve quality of life.

REFERENCES

1. UNAIDS. Report on the Global AIDS Epidemic. Geneva: UNAIDS; 2013. http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf
2. Kulkarni SV, Kairon R, Sane SS, Padmawar PS, Kale VA, Thakar MR, *et al*. Opportunistic parasitic infections in HIV/AIDS patients presenting with diarrhoea by the level of immunesuppression. Indian J Med Res 2009; 130:63-6.
3. Prasad KN, Nag VL, Dhole TN, Ayyagari A. Identification of enteric pathogens in HIV positive patients with diarrhoea in Northern India. J Health Popul Nutr 2000; 18(1):23-6.
4. Wilcox CM. Etiology and prevention of diarrhea in AIDS: a global perspective at the millennium. World J Gastroenterol 2000; 6(2):177-86.
5. Kang G. Opportunistic protozoan parasitic infections of gastrointestinal tract. Ind J Med Microbiol 2000; 18(2):50-4.
6. Attili SV, Gulati AK, Singh VP, Verma DV, Rai M, Sundar S. Diarrhoea, CD4 counts and enteric infections in hospital – based cohort of HIV-infected patients around Varanasi, India. BMC Infect Dis 2006; 6:39.
7. National AIDS Control Organisation, Ministry of Health and Family Welfare, Govt of India. Laboratory diagnosis of HIV infection. In: laboratory manual for technicians

(ICTCs, PPTCTCs, Blood banks and PHCs). New Delhi 2007. p. 23-29.
8. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC Jr, Procop GW, *et al*, editors. Parasitology. In: Colour atlas and textbook of diagnostic microbiology. 6th ed. Philadelphia: Lippincott Williams and Wilkins; 2006. p. 1244-1326.
9. Garcia LS. Macroscopic and microscopic examination of fecal specimens. In: Diagnostic Medical Parasitology. 5th ed. Washington DC: ASM press; 2007. p. 782-830.
10. Kumar SS, Ananthan S, Lakshmi P. Intestinal parasitic infection in HIV infected patients with diarrhoea in Chennai. Ind J Med Microbiol 2002; 20(2):88-91.
11. Mohandas, Sehgal R, Sud A, Malla N. Prevalence of intestinal parasitic pathogens in HIV-seropositive individuals in Northern India. Jpn J Infect Dis 2002; 55:83-4.
12. Deorukhkar S, Katiyar R, Saini S, Siddiqui AU. The Prevalence of Intestinal Parasitic Infections in HIV Infected Patients in a Rural Tertiary Care Hospital of Western Maharashtra. (A 5 year study). JCDR 2011; 5(2):210-2.
13. Uppal B, Kashyap B, Bhalla P. Enteric pathogens in HIV/AIDS from tertiary care hospital. Indian J community med 2009; 34(3):237-42.
14. Gupta S, Narang S, Nunavath V, Singh S. Chronic diarrhoea in HIV patients – Prevalence of coccidian parasites. Indian J Med Microbiol 2008; 26(2):172-5.
15. Basak S, Bose S, Mallik SK, Ghose AK. Intestinal parasitic infections in HIV seropositive patients- A study. JCDR 2010; 4:2433-7.
16. Chhin S, Harwell JI, Bell JD, Rozycki G, Ellman T, Barnett JM, *et al*. Etiology of chronic diarrhea in antiretroviral-naïve patients with HIV infection admitted to Norodom Sihanouk Hospital, Phnom Penh, Cambodia. Clin Infect Dis 2006; 43:925–32.
17. Akinbo FO, Okaka CE, Omoregie R. Prevalence of intestinal parasitic infections among HIV patients in Benin City, Nigeria. Libyan J Med 2010; 5:5506.
18. Chawala R, Ichhpujani RL. Enteric spore-forming opportunistic parasites in HIV/AIDS. Trop Parasitol 2011; 1:15-9.
19. Cimerman S, Cimerman B, Lewi DS. Enteric parasites and AIDS. Sao Paulo Med J 1999; 117(6):166-73.
20. Navin TR, Weber R, Vugia DJ, Rimland D, Robert JM, Addiss DG, *et al*. Declining CD4+ T lymphocyte count are associated with increased risk of enteric parasitosis and chronic diarrhea: results of three year longitudinal study. J Acquir immune Defic Syndr Hum Retrovirol 1999; 20(2):154-9.
21. Wiwanitkit V. Intestinal parasitic infections in Thai HIV infected patients with different immunity status. BMC Gastroenterol 2001; 1:3.

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