Research Article

Bacterial and parasitic etiological agents in HIV seropositive diarrhoea cases

Ranjana Namdeorao Thate[*] and Vijayashree Deotale

Department Medical Microbiology, Maharashtra University of Health Sciences (MUHS) Nasik, India

*Correspondence Info:
Dr. Ranjana Namdeorao Thate
Department Medical Microbiology,
Maharashtra University of Health Sciences (MUHS) Nasik, India
E-mail: ranjanathate26@gmail.com

Abstract

Introduction: Studies in India indicated that diarrhoea has been the third common clinical presentation in AIDS patients. It is a second leading cause of hospital visit in developing nations and makes its place top ten worldwide. A diagnostic spectrum of enteric pathogens causing diarrhoea includes bacteria, parasites, fungi & viruses. Some pathogen tend to occur more frequently to cause more severe disease in person infected with HIV than in HIV uninfected persons with diarrhoea. These include *Salmonella* spp., *Isospora* spp., *Cryptosporidium* spp., *Microsporidia* spp., *Cytomegalovirus.*

Aims and Objectives: Present study was undertaken to find out which etiological agents are most commonly responsible for causing diarrhoea in HIV seropositive patients.

Material Methods: A total of 181 cases were included in this study, of which 150 were HIV seropositive and 31 were HIV seronegative. Diagnosis of HIV infection was done as per NACO guidelines and CD4 count estimation was done. Isolation of bacterial pathogen was done using standard bacteriological procedures and parasite detection was done by direct examination after formal ether concentration technique, using staining as: modified Kinyoun's acid fast staining and modified acid fast trichrome staining.

Results: In this study, most common bacterial pathogen detected was *Escherichia coli (*57.6%), followed by *Proteus spp* (9.4%), *klebsiella* spp (7.7%). Most common protozoal parasites were *Cryptosporidium* (53.6%), & *Microsporidium* (2.9%) and most common helminthic parasite was *S. stercoralis* (1.6%). All the enteric pathogens were predominantly detected with CD4+ count < 200 cells/ul.

Conclusion: *Cryptosporidium* was the most common (53.6%) parasitic enteropathogen detected while diarrheogenic *E. coli* (18.2%) was the most common bacterial enteropathogens isolated. *Cryptosporidium* was the most common (58%) pathogen isolated in patients with CD4+ count <200 cells/ul.

Keywords: Kinyoun, Trichrome, HIV, CD4 count, diarrhoea, Parasitic, bacterial

1. Introduction

Opportunistic enteric infections are major source of diarrhoeal disease in developing countries mainly in HIV infected patients. Intestinal pathogens and the etiological agents vary from patient to patient depending upon the immune status of the patients and from country to country depending on the geographical distribution, endemicity, seasonal variation of the enteric pathogens.[1-4] A characteristic feature of AIDS infections is progressive destruction of immune system functions, which usually results in morbidity and ultimately death due to opportunistic infections.[5]

Gastrointestinal infections are very common in patients with HIV infection or patients with AIDS.[6] Diarrhoea is a common clinical presentation of these infections. Diarrhoea is defined as passage of abnormally liquid or unformed stool at an increased frequency. Diarrhoea may be defined as acute if it occurs for a duration of less than 2wks, persistent if it occurs for a duration of 2-4 wks and chronic if it occurs for a duration of greater than 4 wks.[7] Reports indicate that diarrhoea occurs in 30-60 % of AIDS patients in developed countries and in about 90 percent of AIDS patients in developing countries.[8] Episodes of diarrhoea may be acute, intermittent or recurrent and in some cases, may be chronic and severe. Diarrhoea may significantly diminish patient's quality of life and if it persists may cause dehydration, poor nutrition, and weight loss.[9,10] Diarrhoea has been associated with 50% of HIV/AIDS patients in the developed world and in up to 100% of patients residing in developing countries.[11-13]

HIV/AIDS may not be curable but most of the opportunistic infections can be effectively treated. Prophylaxis against some of these infections will not only prolong the life of an HIV infected individual but also improve the quality of life. Effective prevention, diagnosis and management of accompanying infections are critical for improving the health and wellbeing of people infected with HIV.[5]

2. Material and Methods

2.1 Study group

The hospital based cross sectional study was carried out in a 760 bedded tertiary care rural hospital of central India, between September 2009 and September 2011. Ethical approval was taken from Institutional ethical committee prior to study. A total of 150 HIV seropositive patients with and without diarrhoea and 31 HIV seronegative with diarrhoea cases randomly selected from medicine ward were enrolled in the study after obtaining consent and provided two consecutive stool samples. Before collecting the samples, patient's information such as name, age, sex, occupation, clinical history as well as history of diarrhoea, antibiotic and antiparasitic treatment history was obtained. Patient who had received antiparasitic and antibiotic treatment within past seven days were excluded from the study.

2.2 Diagnosis of HIV infection

Blood samples were collected in plain and ethylene diaminetetraacetic acid (EDTA) vials with 5 ml each from all enrolled patients. Serum samples were tested for rapid test for HIV with Triline ICT rapid test device (Acon Biotech, (Hangzhou), Co., Ltd (China)). Positive sera were sent to National AIDS Research Institute (NARI), Pune, for confirmation of HIV seropositivity.

2.3 CD4 estimation

EDTA blood samples were used for CD4 cell counts and measured by using Flow cytometry (Partec, GmbH, Germany), which works on principle of automated analysis of optical properties of individual particles in fluidic system. The physical and chemical properties of the cells or particles are analysed while cells pass through the measuring apparatus in fluid stream.

2.4 Diagnosis of etiological agents

Fresh stool samples were collected in clean wide mouthed, leak proof plastic sterile containers from each patient and for bacterial culture inoculated on selective and differential media was as per standard protocol.[8,14] Parasite detection was done by direct examination after formalin-ether-concentration of stool specimens as wet saline and iodine mount techniques for the detection of protozoan trophozoites, cysts, helminthic eggs and larva. Additionally, all samples were subjected to modified Kinyoun's acid fast staining for coccidian oocysts and modified acid-fast trichrome staining for microsporidial spores.[10]

For modified Kinyoun's acid fast staining, smear was fixed with absolute methanol for 1 min then flooded with kinyoun's carbol fuchsin for 5min then rinsed with 50% ethanol & then decolourized with 1% sulphuric acid for 2min & counterstained with methylene blue for 1 min.[10] For modified acid-fast trichrome staining, smear was fixed in absolute methanol for 5-10 min, then placed in carbol fuchsin solution for 10 min, then decolourized with 0.5% acid alcohol, then placed in trichrome stain for 30 min then rinsed with acid alcohol for 10 sec & then rinsed in 95 % alcohol several times then placed in 95% alcohol for 30sec then dried and observed under microscope.[10]

Escherichia coli isolates, isolated from stool samples were sent to Central Research Institute (CRI) Kasauli, for identification of serotype of diarrheogenic *E. coli*.

3. Results

This study included total 181 cases of which 150(82.9%) were HIV seropositive and 31(17.1%) were HIV seronegative cases. Among them, predominant age group was between 20-45 years of age. (Table1)

Table 1: Age and sex distribution of study subjects (HIV seropositive patients) (N=150)

Age	Males No. (%)	Females No. (%)	Total No.(%)
Upto25	5(3.3)	6(4)	11(7.3)
26-35	35(23.3)	33(22)	68 (45.3)
36-45	27(18)	16(10.7)	43(28.7)
46-55	8(5.3)	8(5.3)	16(10.7)
>55	10 (6.7)	2(1.3)	12(8)
Total	85	65	150

Patients with Acute diarrhoea, chronic diarrhoea: Majority (42%) of the HIV seropositive cases had chronic diarrhoea (Table 2) while most (87.1%) of the HIV seronegative subjects had acute diarrhoea. (Table2)

Table 2 : Duration of diarrhoea in patients with diarrhoea

HIV status	Acute (<2wks) No.(%)	Persistent (2-4wks) No.(%)	Chronic (>4wks) No.(%)	Total
HIV positive	21(30.4)	19(27.5)	29(42)	69
HIV negative	27(87.1)	1(3.2)	3(9.7)	31
Total	48	20	32	100

Table 3 shows the frequency of intestinal pathogens in relation to the HIV status of the study cases. *C. parvum*, *E.coli,* and *C. albicans* were found to be significantly more common in the HIV-seropositive study cases than the HIV-negative control group (*P* value < 0.05).

Table 3: pathogens isolated from HIV seropositive patients and HIV seronegative patients.

Pathogens	HIV positive with diarrhoea (N=69) No. (%)	HIV positive without diarrhoea (N=81)No. (%)	HIV negative with diarrhoea (N=31) No. (%)	Total (N=181) No. (%)
Bacterial				
E.coli	37 (53.6)	49 (60.5)	18 (50.1)	104 (57.4)
Proteus spp	14 (20.2)	3 (3.7)	0	17(9.4)
Klebsiella spp	6(8.7)	5(6.2)	3(9.6)	14(7.7)
Pseudomonas spp	10(14.5)	1(1.2)	1(3.2)	12(6.6)
Citrobacter spp	4(5.8)	2(2.5)	0	6(3.3)
Aeromonas spp	2(2.9)	0	1(3.2)	3(1.6)
Vibrio cholerae	0	0	2(6.4)	2(1.1)
Salmonella paratyphi A	0	0	1(3.2)	1(0.5)
Morganella marganii	1(1.4)	0	0	1(0.5)
Protozoa				
Cryptosporidium	47(68.1)	48(59.2)	2(6.4)	97 (53.6)
Giardia lamblia	6(8.7)	2(2.5)	2(6.4)	10(5.5)
E. histolytica	3(4.3)	1(1.2)	1(3.2)	5(2.7)
Microsporidia	2(2.9)	0	0	2(1.1)
E. coli	2(2.9)	0	0	2(1.1)
Helminths				
S. stercoralis	2(2.9)	1(1.2)	0	3(1.6)
A. duodenale	1(1.4)	0	0	1(0.5)
H. nana	1(1.4)	0	0	1(0.5)
Fungal				
Candida albicans	7(10.1)	2(2.5)	2(6.4)	11(6)

Overall the rate of isolation of pathogens causing diarrhoea in HIV was higher in HIV seropositive individuals with CD4 counts < 200 cells/μL as compared to HIV seropositive individuals with CD4 counts > 200 cells/μL. However, the isolation rate was significantly higher for *C. parvum*, *E.coli,* and *C. albicans* only (*P* value < 0.05) in patients with CD4 count < 200 cells/μL as shown in Table 4.

Table 4: Correlation of enteric pathogen with CD4+ cells counts.

Pathogen	CD4+ count <200 cells/ul (N=81) No. (%)	CD4+ count 200-500cells/ul (N=42) No. (%)	CD4+ count > 500 cells/ul (N=27) No. (%)	Total (N=150) No. (%)
Protozoa				
Cryptosporidium parvum	58(71.6)	24(57.1)	13(48.1)	95(63.3)
G. lamblia	7(8.6)	0	1(3.7)	8(5.3)
E. histolytica	2(2.46)	2(4.76)	0	4(2.6)
Microsporidia	2(2.46)	0	0	2(1.3)
Entamoeba coli	1(1.2)	1(2.3)	0	2(1.3)
Helminths				
S. stercoralis	1(1.2)	2(4.76)	0	3(2)
H. nana	1(1.2)	0	0	1(0.6)
A.duodenale	1(1.2)	0	0	1(0.6)
Bacterial pathogens				
Diarrheogenic E.coli	18(22.2)	12(28.6)	2(7.4)	32(21.3)
Proteus spp	7(8.6)	5(11.9)	2(7.4)	14(9.3)
Pseudomonas spp	8(9.9)	3(7.1)	0	11(7.3)
Klebsiella spp	9(11.1)	1(2.3)	1(3.7)	11(7.3)
Citrobacter spp.	5(6.2)	1(2.3)	1(3.7)	7(4.6)
Morganella morganii	1(1.2)	0	0	1(0.6)
Aeromonas hydrophila	2(2.46)	0	0	2(1.3)
Candida albicans	7(8.6)	2(4.76)	0	9(6)

4. Discussion

Opportunistic spore forming protozoan parasites such as *I. belli, C. parvum, C. cayetanensis* and *Microsporidia* play major role in causing chronic diarrhoea, accompanied with weight loss in them. 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.[15]

In our study, the age group of patients ranged from 17-67 years with a mean age of study subjects was 37.8 years ± 11.2 SD. The predominant age group in this study among males as well as females was 26-35 years (Table No. 1). This section of population is more affected because they are sexually active. Similar study from Ethiopia had 25-34 years as predominant age group.[16]

Diarrhoea is common complication of HIV infections, 30-90% of patients with AIDS suffer from diarrhoea at some points of their illness and detection of etiological agents varies from 40-83%.[11] In our study, in HIV seropositive patients, persistent and chronic diarrhoea was more common as compared to HIV seronegative patients with diarrhoea. Among the HIV seropositive patients with diarrhoea, 21(30.4%) had acute diarrhoea while 19(27.5%) had persistent diarrhoea and 29(42%) had chronic diarrhoea. While in HIV seronegative patients with diarrhoea, 27(87.1%) had acute diarrhoea, 1(3.2%) had persistent and 3(9.7%) had chronic diarrhoea (Table No. 2). In study of Prasad et al from northern India, 44% of HIV positive patients with AIDS had a history of chronic diarrhoea and etiological agents detected in 73% of them.

Bacterial enteric pathogen has been emerged as significant cause of diarrhoea in HIV infected cases.[11] In our study, *E. coli* was the most common bacterial pathogen isolated in HIV seropositive with and without diarrhoea and HIV seronegative diarrhoea patients. Among the diarrheogenic *E.coli* isolated, Enteropathogenic *E.coli* was the most common isolate. In HIV seropositive diarrhoea patients, *E.coli* was the most common bacterial isolate 37 (53.6%) followed by *Proteus* spp 14(20.2%) and *Pseudomonas* spp 10(14.5%). In HIV seropositive patients without diarrhoea *E. coli* 49(60.5%) & *Klebsiella* spp 5(6.2%), whereas in HIV seronegative patients *E.coli* 18(50.1%), *Klebsiella* spp 3(9.6%), *Vibrio cholerae* 2(6.4%) were the most common isolates (Table No.3). Isolation rate of enteropathogen including *V. cholerae* and *Salmonella* spp was more in HIV seronegative patients whereas these pathogens were not isolated from HIV seropositive diarrhoea patients. In HIV patients due to low resistance to infections acquire large number of infections which results in use of large quantities of antibiotics such as norfloxacilin and trimethoprim-sulfamethoxazole for the treatment of diarrhoea. This may result in the suppression of bacteria sensitive to these antibiotics. Secondly, several reports indicated that histological changes occur in the mucosa of HIV patients which result in rare bacterial pathogens causing diarrhoea.[17,18] These alterations might be unfavorable for colonization by non-invasive pathogens. But these patients are not susceptible to *V. cholerae* infection.[12] Various authors have depicted their finding as a study conducted by Uppal *et al.*[13] showed 2%, Ramakrishan *et al*[19] found 18.5% *Vibrio cholerae* positivity.

In our study, *Cryptosporidium* had showed highest infection rate 97(53.6%) and was the most commonly isolated protozoan followed by *Giardia lamblia* 10(5.5%). Our finding was matching with studies conducted by Sadraei *et al*, New Delhi, (56.5%)[6], Olukemi *et al.* from Nigeria 52.7%[4] whereas Tuli *et al* from BHU showed 39.5%[3] and Gupta S *et al*. 23.6%.[20]

In our study, *Microsporidia* was detected in 2 specimens, 2.9% in HIV seropositive diarrhoea cases. In study of Kumar *et al.*, *Microsporidia* was detected in 1.69% in patients with chronic diarrhoea.[1] One recent study conducted by Tuli *et al*. 2008, from North India (BHU), *Microsporidia* was detected in 26.7%.[3]

In our study, *Isospora belli* and *Cyclospora cayetanensis* were not detected. It may be due to use of Sulphamethoxazole + Trimethoprim prophylaxis in patients with CD4 count < 200 cells/ul given to prevent the *Pneumocystis jirovecii* pneumonia. *S. stercoralis* was detected 3(1.6%) of total patients. Other parasitic infections i.e. *E. histolytica, Ancylostoma duodenale, H. nana*, was detected in few cases. These are not found to be opportunistic in HIV infected patients; there is no evidence for increased prevalence in this population. Kulkarni *et al.*, from NARI, Pune, found *Cyclospora* 0.7% and *Isospora belli* 8%.[21] In study of Gupta M *et al.* from Gujrat, *Cyclospora* was detected in 1%.[2]

CD4 + T cell counts are considered as surrogate markers of immune status of an individual.[9] In this study, *Cryptosporidium* was most common pathogen associated in patients with CD4+ count < 200 cells/ul, 58(71.6%) and 200-500 cells/ul 24(57.1%). While in patients with CD4+ count > 500 cells/ul, *Cryptosporidium* 13(48.1%). (Table No.4).

In our study, prevalence of enteric pathogen was more in HIV seropositive patients with CD4+ count level < 200 cells/ul. There is strong association of enteric pathogens with severe immunodeficiency, particularly with CD4+ lymphocytes counts below 500 cells/ul. The risk to develop diarrhoea or the rate of enteric infections was found to be low in patients with CD4+ cells count > 200 cells/ul. One of the reasons could be regional immunosuppression as suggested by Schneider *et al.* they found, loss of CD4+ cells in intestinal mucosa of the patients with diarrhoea, which were more pronounced than peripheral CD4+ levels and their relation is quite variable.[12]

In study of Kulkarni *et al.*, from NARI, Pune, India, the proportion of opportunistic pathogens in patients with CD4 count < 200 cells/µl was significantly higher than that in the other two groups of patients with CD4 count > 200 cells/µl.[21]

There are some limitations in this study, as the study was performed with small sample size, due to resource constraints we were not able to isolate some bacterial pathogens like *Campylobacter jejuni, Clostridium difficile* and viruses or to perform sensitive tests like PCR for confirmation of parasites such as *Microsporidia*. In addition the participants were unwilling to participate in follow up so no attempt was made to obtain the follow up information regarding diarrhoeal episodes, effectiveness of prophylactic regimens. Also there is no ART centre in our hospital so getting symptomatic HIV seropositive patients with diarrhoea was also unpredictable.

Cryptosporidium was the most common (53.6%) parasitic enteropathogen detected while diarrheogenic *E. coli* (18.2%) was the most common bacterial enteropathogens isolated. *Cryptosporidium* was the most common (58%) pathogen isolated in patients with CD4+ count <200 cells/ul.

References

1. Kumar SS, Ananthan S., Lakshmi P. Intestinal parasitic infections in HIV infected patients with diarrhoea in Chennai. *IJMM* 2002; 20(2):88-91.
2. Gupta M, Sinha M, Raizada N. Opportunistic intestinal protozaon parasitic infection in HIV positive patients in Jamnagar, Gujrat. SAARC. *Journal of Tuberculosis Lung Disease and HIV/AIDS* 2008; 1.
3. Tuli L, Gulati AK, Sundar S. Correlation between CD4 counts of HIV patients and enteric protozoan in different seasons – An experience of a tertiary care hospital in Varanasi (India). *BMC Gastrenterology*. 2008; 8:36.
4. Adesiji YO, Lawal RO, Taiwo SS, Fayemiwo SA, Adeyeba OA. Cryptosporidiosis in HIV infected patients with diarrhoea in Osun State Southwestern Nigeria. *Eur. J. Gen. Med* 2007; 4(3):119-122.
5. Becker ML, Cohen CR, Cheang M, Washington RG, Blanchard JF and Moses S. Diarrhoea is a disease among the HIV infected adults in Karnataka, India: Evaluation of risk factors and etiology. *Am J Trop Med and Hyg* 2007; 76(4):718-722.
6. Sadraei J, Rizvi AM and Baveja UK. Diarrhoea, CD4+ cells counts and opportunistic protozoa in Indian HIV- infected patients. *Parasitol Res* 2005; 97:270–3.
7. Kasper DL, Braunwald E, Houser SL, Fouci AS, Longo DL, Jameson JL *et al.* editors. Harrison's principle of internal medicine 16th ed. vol-1. New York: McGraw-Hill Medical publishing Division*;* 2005.
8. Atilli S, Gulathi AK, Singh VP, Rai M, Sundar S. Diarrhoea, CD4 counts and Enteric infection in hospital based cohort of HIV infected patients around Varanasi, India. *BMC Infect Dis* 2006; 6:39.
9. Farmer JJ III. Enterobacteriacae : Introduction and identification. In: Murray P, Baron EJ, Pfaller M, Tenover F, Yolken RH, editors. Manual of clinical Microbiology. 6th edition. Washington, DC: American society for Microbiology 1995; 438-47.
10. Garcia LS. Diagnostic Medical Parasitology. 4th ed. Washigton D.C: ASM press; 2003.
11. 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.
12. Suthienkul O, Aiumlaor P, Siripanichgon K, Eampokalap B, Likhanonsakul S, Utrarachkij F and Rakue Y. Bacterial causes of AIDS-associated diarrhoea in Thailand. *Southeast Asian J Trop Med Public Health* 2001; 32(1):158-170.
13. Uppal B, Kashyap B, Bhalla P. Enteric pathogen in HIV/AIDS from a tertiary care hospital. *Indian journal of community medicine* 2009:34(3); 237-242.
14. Collee JG, Marmion BP, Fraser AG, Simmons A. Practical Medical Microbiology. 14th ed. New Delhi : Elsevier ; 2008.
15. Mitra AK, Hernandez CD, Hernandez CA, Siddiq Z. Management of diarrhoea in HIV infected patients. *Int J STD AIDS* 2001; 12:630-9.
16. Awole M, Selassie S Gebre, Kassa T, Kibru G. Prevalence of intestinal parasites in HIV-infected adult patients in southwestern Ethiopia. *Ethiop J Health Dev* 2003; 17(1):71-78.
17. Greenson JK, Belitson PC, Yardley JH, Bartlett JG. AIDS Enteropathy: Occult enteric infections and duodenal mucosal alterations in chronic diarrhoea. *Ann Intern Med* 1991; 114:366-72.
18. Clayton F, Rekha S, Cronin WJ, Torlakovic E, Sigal SH, Kotlar DP. Rectal mucosal pathology varies with human immunodeficiency virus antigen content and disease stage. *Gastroenterology* 1992; 103:919-33.
19. Ramakrishna B.S. Prevalence of intestinal pathogen in HIV patients with dirrhoea: Implication for treatment. *Indian J of Paedatric* 1999; 66:85-91.
20. Gupta S, Narang S, Nunavath V, Singh S. Chronic diarrhoea in HIV patients; prevalence of coccidian parasites. *Ind. J of med microbial.* 2008; 172-5.
21. Kulkarni S.V., Kairon E., Sane S.S., Padmawar P. S., Kale V.A., Thakar M.R., Mehendale S.M. & Risbud A.R. Opportunistic parasitic infections in HIV/AIDS patients presenting with diarrhoea by the level of immunosuppression. *Indian J Med Res* 2009 Jul; 130:63-66.