International Journal of Advances in Medicine
Sahoo D et al. Int J Adv Med. 2018 Dec;5(6):1491-1497
http://www.ijmedicine.com
pISSN 2349-3925 | eISSN 2349-3933
DOI: http://dx.doi.org/10.18203/2349-3933.ijam20184763

Original Research Article

Prevalence of enteric pathogens in HIV patients with chronic diarrhoea and their relationship with CD4 count

Debasish Sahoo[1], Purabi Baral[2], Soumya Ranjan Mahapatra[3], Anshuman Sarangi[4]*, Kiran Babu K.[4]

[1]Department of Microbiology, IMS and SUM Hospital, Bhubaneswar, Odisha, India
[2]Department of Microbiology, KIMS Hospital, Secunderabad, Telangana, India
[3]Department of Cardiology, Aster Prime Hospital, Hyderabad, India
[4]Dnadvent Lab, Lifesciences Pvt. Ltd, KIIT-Technology Business Incubator, Bhubaneswar, Odisha, India

Received: 09 October 2018
Revised: 27 October 2018
Accepted: 01 November 2018

***Correspondence:**
Dr. Anshuman Sarangi,
E-mail: anshumanbbsr2@gmail.com

ABSTRACT

Background: HIV presently accounts for the highest number of deaths attributable to any single infective agent. Opportunistic infections (OIs) and associated complications account for a considerable proportion of such mortality. Diarrhoea is among the most common symptom of HIV infection. During the natural course of HIV infection, there is a progressive loss of CD4 T cells. Autors' aim is to study the prevalence of enteric pathogens in HIV patients with chronic diarrhea and their relationship with CD4 count.
Methods: We analysed 186 HIV positive patients out of which 126 had chronic diarrhea and 60 patients presenting without diarrhea as controls. The faecal samples were subjected to microscopic examination. Zeihl Neelson's Stain for mycobacterium, Kinyoun's acid fast stain for microsporodia, stool culture for salmonella and shigella. All patients had barium meal contrast studies, USG of abdomen and pelvis. FNAC of peripheral lymph nodes and finally CD4+ Tcell count using BD FACS.
Results: Total of 68,10,01 patients of HIV diarrhoea with identifiable cause had CD4 <200, 200-250 and >350 respectively. Among HIV diarrhoea without any identifiable cause 22, 17, 08 patients had CD 4 <200, 200-250 and >350 respectively. All diarrhoeal stool samples with *Isopora* had CD 4 <200, *M. tuberculosis* 26 patients CD4 <200, 12 had 200-350 and 03 had >350. Microsporodium 03 patients had CD4 >350. Finally, among stool samples with *Cyclospora*, *E. histolytica*, *Giardia* and *Strongyloides* all patients had CD 4<200.
Conclusions: The present study highlights the importance of testing intestinal pathogens in HIV. Chronic diarrhoea was more common in patients with low CD4 counts.

Keywords: CD4 count, Diarrhoea, HIV, Intestinal pathogens

INTRODUCTION

From 1983 when HIV-1 was defined as primary cause of AIDS to the present HIV infection has changed from a fatal condition to a manageable chronic illness. HIV continues to be a major global public health issue. In 2016, an estimated 36.7 million people were living with HIV (including 1.8 million children) - with a global HIV prevalence of 0.8% among adults.[1,2] Around 30% of these same people do not know that they have the virus.[3] Since the start of the epidemic, an estimated 78 million people have become infected with HIV and 35 million people

have died of AIDS-related illnesses. In 2016, 1 million people died of AIDS-related illnesses.[3]

According to the Health and Family Welfare Department sources of Government of Odisha, Ganjam district tops the list of both HIV positive cases and AIDS death cases, followed by Cuttack and Koraput. In fact, Ganjam district happened to be the major hub of this killer virus. Most HIV cases were reported from Polosara, Hinjili, Digapahandi and Belguntha blocks.

HIV presently accounts for the highest number of deaths attributable to any single infective agent. The threat to their life is not from the virus alone. Opportunistic infections (OIs) and associated complications account for a considerable proportion of such mortality. Opportunistic infections, which occur only among immunosuppressed individuals, infections such as tuberculosis (TB), amoebiasis and leishmaniasis occur more frequently in HIV-infected persons.

Diarrhoea is among the most common symptom of HIV infection and is experienced by over 90% of patients with AIDS. In fact, it is the second leading cause of hospital visits in developing nations and makes its place in the top ten worldwide. Expectedly infectious aetiologies lead the list in developing nations in contrast to non-infectious aetiologies in developed nations. During the natural course of HIV infection, there is a progressive loss of CD4 T cells; the rate of this loss being variable in patients but averaging around 60-100 cells/uL per year.

This drop in CD4 T cells leads to a severely immunocompromised state in the infected host. Thereupon, a reduction in CD4 T cells to below 200cells/uL makes the host highly susceptible to opportunistic infections and increases overall AIDS related morbidity and mortality. Most of these studies demonstrated a strong negative association between diarrhea and CD4 counts. The etiologic spectrum of enteric pathogens is broad, including bacteria, parasites, fungi, and viruses. Pathogens tend to occur more frequently of cause more severe disease in persons infected with HIV than in HIV-uninfected persons with diarrhea. These include, but are not limited to *Isospora spp*, *Cryptosporodium spp*, *Cytomegalovirus*, *Mycobacterium avium* complex, *M. Tuberculosis*, *Salmonella spp* etc. Authors' hospital is a tertiary care centre and a large number patients have been reported as PLHA (People living with HIV/AIDS). There have been only few studies regarding the aetiological agents of chronic diarrhea in HIV due to which we decided to study the prevalence of enteric pathogens in HIV patients in chronic diarrhea and its relationship with CD4count.

METHODS

A prospective case control study was carried out at Medicine Department of Maharaja Krushan Chandra Gajapati Medical College and Hospital, Berhampur, in collaboration with Department of Microbiology from September 2008 to October 2010. The subjects for this study were chosen from the inpatient and outpatient Departments of Medicine, and those attending ART center.

Inclusion criteria

Selection of cases

- HIV seropositive patients
- >14 years of age
- Presenting with chronic diarrhoea (3 or more liquid stools for >14 days).

Selection of controls

- HIV seropositive patients
- Not complaining of diarrhoea, in last one month
- Matched as per age, sex and other parameters.

Exclusion Criteria

- Patients failing to supply 3 stool samples
- Persons who had received antibiotics/anti-parasitic treatment for diarrhea within the past 14 day.

Collection of faecal specimens

Faecal samples were collected in clean wide mouthed sterile container with a spoon in the underside of lid. Patients were instructed to collect 5-10ml of stool when it was liquid consistency and 5-10gms when soft and formed. 3 stool samples were collected form each patient. Samples were transported as soon as possible to the laboratory.

Investigations to confirm microbial infection from stool samples were done as follows.

Direct microscopic examination

- Wet mount preparations of fresh samples were prepared in normal saline and Lugol's iodine. Iodine staining is useful for temporary staining of cysts of amoebae and intestinal protozoa in faeces.
- Zeihl Neelson's stain: This is useful for diagnosis of all Mycobacterium species
- Modified Cold Kinyoun's Acid-Fast Method for *Coccidian Oocysts*
- Modified trichrome - bluestain for microsporidia.
- Stool culture for *Cyclospora cayetanensis*, salmonella and shigella.

All patients (cases and controls) not having acute abdominal condition were subjected to barium meal contrast studies and USG Abdomen and pelvis to search for any evidence of abdominal tuberculosis. All patients with evidence of intestinal TB underwent sputum examination for AFB and x-ray of chest for any evidence

of active or healed pulmonary tuberculosis. FNAC of the peripheral lymph nodes was performed to identify coexisting TB lymphadenitis.

Finally, CD4+T cell counting was done using BD FACS Calibur. (Fluorescence Activated Cell Sorter).

RESULTS

Majority of patients with diarrhoea were in age group of 30-39 years with mean age 33.19years (SD 7.127) and majority of patients without diarrhoea were in age group of 14-24years with mean age 33.33years (SD 9.632) (Figure 1).

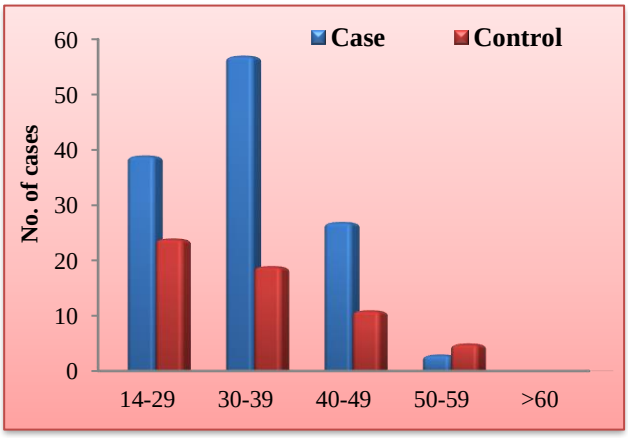

Figure 1: Age distribution of both HIV (+)ve diarrhoeal and non diarrhoeal patients.

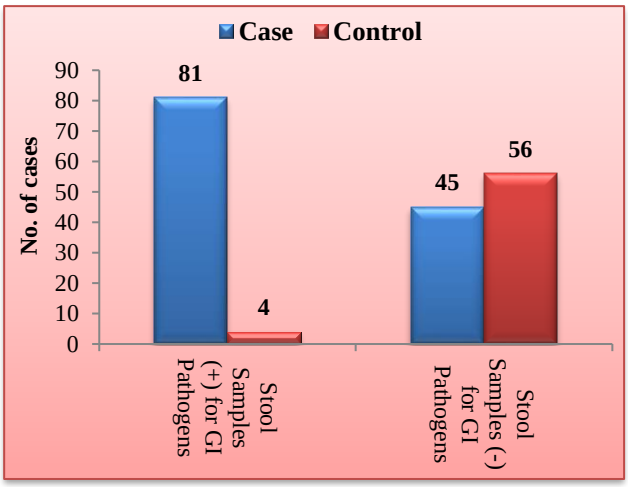

Figure 2: Prevalence of enteric pathogens in stool of HIV patients.

Among HIV patients with diarrhoea, gastroenteric pathogens were detected in 81 (64.29%) stool samples and 45 (35.71%) stool samples could not reveal and identifiable cause. Among HIV patients without diarrhoea only 4 (8.33%) stool samples showed gastroenteric pathogens commonly found in HIV diarrhoeal patients where in 55 stool samples, relevant gastroenteric pathogens could not be detected (Figure 2).

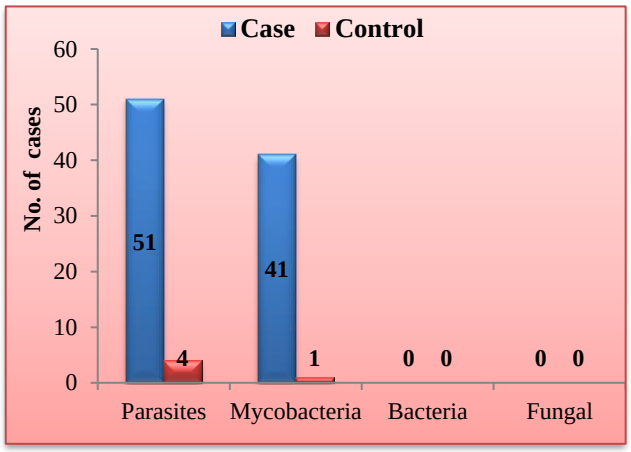

Figure 3: Distribution of pathogens in HIV patients.

Parasites were detected in 51 (40.48%) diarrhoeal patients and 04 (6.47%) non-diarrhoeal patients. However, in 41 (32.53%) diarrhoeal patients and 01 (1.67%) non-diarrhoeal patient mycobacteria were detected from the stool samples. These values were statistically significant ($p<0.001$). Bacterial and fungal pathogens responsible for chronic diarrhoea in a setting of HIV were not detected in both cases and controls (Figure 3).

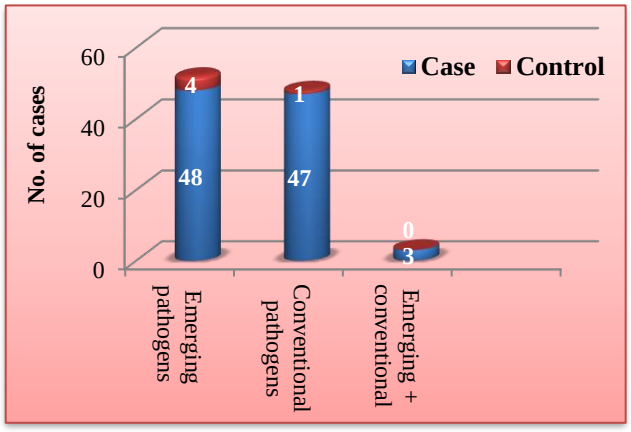

Figure 4: Prevalence of emerging and conventional enteric pathogens in HIV patients.

Amongst the enteric pathogens detected in HIV diarrheal stool samples 48 (38.10%) cases harboured emerging pathogens, 47 (37.3%) cases harboured conventional pathogens and 03(2.38%) cases harboured both. Among non-diarrhoeal HIV patients stool samples only 4 (6.67%) patients had emerging pathogen and conventional pathogen was detected in only 1 (1.67%) patient (Figure 4). *M. tuberculosis* was found in 41 (32.53%) HIV patients with diarrhoea and 1 (1.67%) HIV patient without diarrhoea.

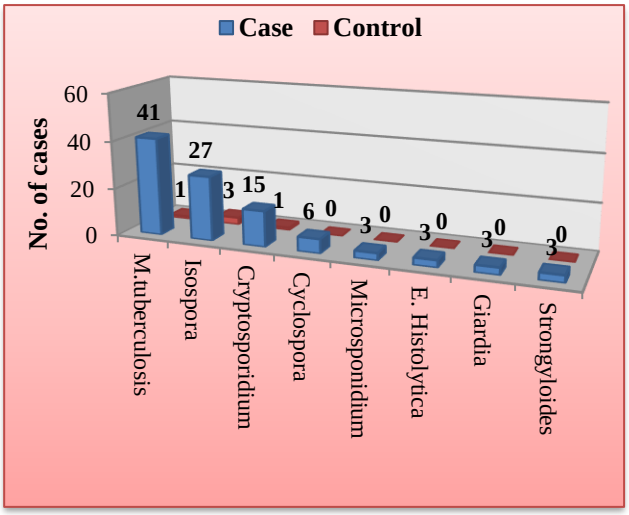

Figure 5: Pathogen profile.

The association of *M. tuberculosis* infection which chronic diarrhoea was statistically significant. (P<0.05) *Isospora* was found in 27 (21.49%) HIV patients with diarrhoea and 3 HIV patients without diarrhoea. The association of *I. belli* which chronic diarrhoea was statistically significant (P <0.05). *Cryptosporidium* was detected in 15 (11.9%) patients with diarrhoea and 1 HIV patient without diarrhoea. The association of *Cryptosporidium* with diarrhoea was statistically significant (P <0.05). Among diarrhoeal patients, *Cyclospora* was detected in 6 (4.76%) patients and *Microsporidum*, *E. histolytica*, *Giardia* and *Strongyloides* were detected in 3 (2.38%) patients each and none of them were identified in HIV patients without diarrhoea (Figure 5).

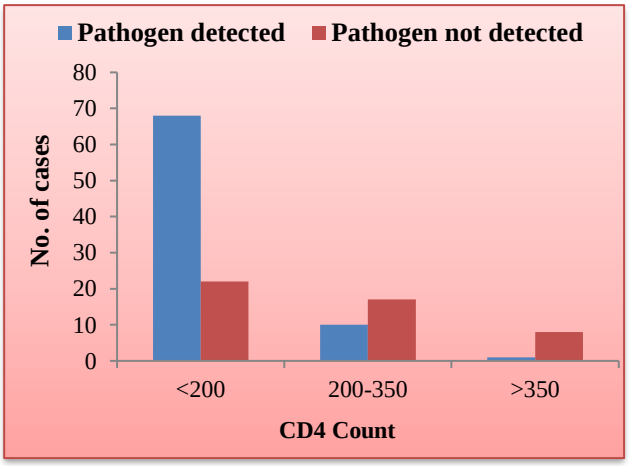

Figure 6: Relation between CD 4 count and pathogen detection.

Among HIV diarrhoeal patients with an identifiable cause, majority i.e.68 cases had CD4 <200, 10 patients had CD4 within 200-350 and 01 patient had CD4 >350. Among HIV diarrhoeal patients without any identifiable cause, 22 cases had CD4 <200, 17 cases had CD4 within 200-350 and 08 patients had CD4 >350 (Figure 6).

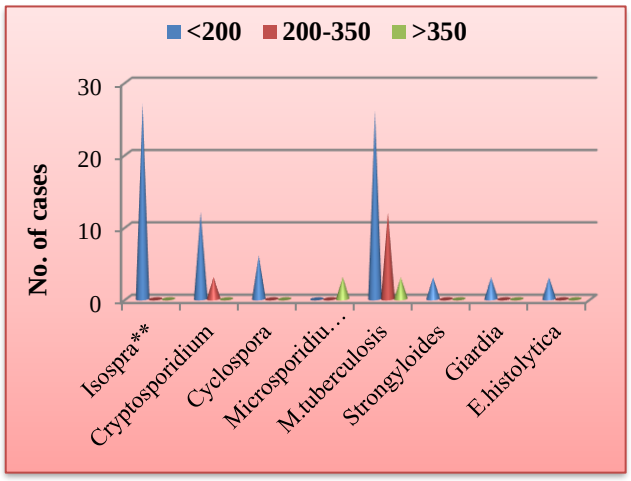

Figure 7: Relation of individual pathogen with CD4 count.

All diarrhoeal stool samples with *Isospora* had CD4 <200. Correlation between *Isospora* and CD4 count was significant (<0.05). Among diarrhoeal stool samples with *Mycobacterium tuberculosis*, 26 patients had CD4 <200, 12 patients had CD4 200-350 and 03 patients had CD4 >350. In 03 patients with *Microsporidium*, CD4 >350. Among diarrhoeal stool samples with *Cyclospora*, *E. histolytica*, *Giardia* and *Strongyloides*, all patients had CD4 <200 (Figure 7).

DISCUSSION

The sample size consisted of 186 HIV positive patients out of which 126 patients presenting with chronic diarrhoea and 60 patients presenting without diarrhoea were taken for the study as cases and controls respectively.

Among cases majority were in age group of 30-39 years with mean age group 33.19years (SD 7.27) and among controls majority were in age group 17-24 years with mean age group 33.33years (SD 9.632). The present study showed a male preponderance with 91 males and 35 females (male: female ratio of 2.6:1) among cases and 39 males and 21 females (M: F= 1.8:1) among controls. Males had also outnumbered females in similar studies done earlier. Study done by Uppal et al, showed M:F ratio of 4:1 among cases and 3: among controls.[4]

Predominance of male cases may be due to migration to other cities in search of work, staying away from families for longer periods and males being promiscuous by habit resulting in acquiring HIV infection.[5] Generally females particularly of low-socio-economic classes have a tendency to avoid health checkups leading to a low detection rate.

The percentages of isolation of pathogen from stool samples were 64.29% (81 cases) among cases and 8.33% (4 cases) among controls. Evidence of intestinal TB on barium-meal contrast studies and Ultrasonography of Abdomen in patients with diarrhoeal stool samples negative for enteric pathogens were in 7 (15.57%) and 4 (8.89%) patients respectively. Study conducted by Gupta et al, detected intestinal pathogens in 28.3% cases whereas in a study conducted by Sathaporn et al, definite cause of diarrhoea was detected in 64.4% HIV patients.[6,7] Similar study done by Prasad et al, showed a higher pathogen isolation rate similar to present study i.e. 73%.[8] Despite using best possible diagnostic techniques, the isolation rate is 34-73% in chronic diarrhea, and present study results are towards higher range in compared to other studies.[9]

Higher isolation rate in present study were due to strict exclusion criteria

- If less than 3 samples are collected
- If patient has taken antibiotics and antiprotozoals in last 14 days
- Long duration of the study
- Patients undergoing barium-meal contrast studies and USG of abdomen.

Negative stool samples can be accounted for-

- Unavailability of diagnostic facilities for viral causes and clostridium difficle toxin.
- Use of cotrimoxazole prophylaxis.
- Cases due to HIV enteropathy and to lesser extent due to malignancies.[5]

Intestinal parasites were detected in 40.48% cases and *Mycobacteria* in 23.8% cases. Present study did not reveal any bacterial or fungal pathogen as a cause of chronic diarrhoea in HIV patients. Among the pathogens 48 (38.1%) cases belonged to emerging pathogens and 4 7(37.3%) cases belonged to conventional pathogens and 3 (2.38%) belonged to the combination of both reflecting the importance of emerging opportunistic pathogens in setting of chronic diarrhoea in HIV patients.

An infectious agent can be identified in about 50% of patients with AIDS-associated diarrhoea.[10] Patients with AIDS are susceptible to many infections that rarely afflict individuals who have a normal immune system. They contact opportunistic infections because HIV has severely weakened or destroyed their immune system. A study done by Gupta et al, showed intestinal parasites in 28.3% cases with coccidian parasites comprising of 25.6% cases.[6] Another study done by Prasad et al, showed emerging enteric pathogens and conventional protozoan, bacterial pathogens in 13 (50%) and 5 (19%) patients respectively as a single agent and remaining 1% patients harbouring both.[8] Enteric parasites were detected in 48 (35%) stool samples by Kulkarni et al, of which 30 (62.5%) were opportunistic and 18 (37.5%) were non opportunistic.[11]

The most commonly found enteric pathogen in our study was *Mycobacterium tuberculosis* (32.53%) as evidenced in stool samples and imaging studies followed by *Isospora* in 21.49% cases and *Cryptosporidium* in 11.9% cases. As compared to the controls, the observed incidence of these organisms in HIV patient with chronic diarrhoea was significantly higher ($p < 0.05$). Less frequently found pathogens were *Cyclospora* in 4.76% cases and *Microsporidium*, *Entamoeba histolytica*, *Giardia* and *Strongyloides* in 2.38% cases each. In control group, *Isospora* was detected in 3 cases (5%) each.

Studies from various parts of the world show contrasting prevalence rates with marked geographical variations.[4] Some studies also demonstrated regional variability of pathogen as well as changing trends of aetiology in the same population (from infectious to non-infectious). A study done by Gupta et al, showed *Isospora* (29.2%).[6] Prasad et al, it was *Cryptosporidium* (11.5%) and Joshi et al, *Isospora belli* was the most frequently encountered parasite in 17% of patients, followed by *Entamoeba histolytica* in 14.9% and *Cryptosporidium* in 8.5%.[8,12] Attili et al, demonstrated *E. histolytica* as the commonest cause (57.14%) followed by *Cryptosporidium* (19.05%) and *Isospora* (9.52%).[5] Uppal et al[4] showed bacterial aetiology as the predominant cause (*E. coli*, 24%, *Cl difficle* 10%, *Salmonella* 4%, *V. cholera* 4% and *Shigella* 2%) followed by *Candida* 36% and enteric parasite only 20% in a study done by Mohandas et al, *Cryptosporidium parvum* was the most common (10.8%), followed by *Giardia lamblia* (8.3%) *Cyclospora cayetanensis* and *Blastocystis hominis* each were detected in 3.3% of the patients, while *Isospora belli* and *Enterocytozoon bieneusi* were each detected in 2.5% of the patients.[13] Cryptosporidium parvum as the predominant pathogen in the stool of HIV patients with chronic diarrhoea were also seen in the studies done by Al-megrin et al, and Kulkarni et al.[11,14]

The common parasites detected in study at Zambia by Khumolo et al, in HIV patients with diarrhoea were ascaris lumbricoides, hookworm, *Entamoeba coli*, and *Cryptosporidium spp*.[15]

Present study differed strongly form other similar studies with respect of mycobacterium tuberculosis as a predominant cause of chronic diarrhoea in HIV/AIDS. A study done at Nairobi Kenya by Mwchari et al, showed *Cryptosporidium* as the leading cause (17%) followed by mycobacterium tuberculosis (13%) But none of the Indian studies showed similar kind of results. These differences can be explained by i] Geographic variation.[4] and ii] most of the studies focus on intestinal protozoa as a cause of chronic diarrhoea. In HIV patients as the immunosuppression progresses, extra pulmonary tuberculosis becomes increasingly common and sputum

smears are seldom positive for acid fast bacilli (AFB).[16] In present study out 41 cases detected as intestinal TB, only 9 patients were positive for sputum AFB, 2 patients had radiological evidence of pulmonary tuberculosis and 12 patients had imaging and pathological evidence of extrapulmonary TB (other than intestinal TB). Thus, present study substantiates the theory of decrease in sputum AFB detection rate and increase in incidence of extra pulmonary tuberculosis with progression of HIV infection.

Our study demonstrated a higher pathogen detection rate at a low CD4 count. Among cases 68 patients had CD4 <200 and remaining i.e. 10 patients had CD4 within 200-350 and 1 patient had CD4 >350. Similar results were seen in studies done by Tuli et al, and Atilli et al.[5,17] It was observed that the CD4 cell count influenced the cause of diarrhoea as well as the diagnostic yield. The diagnostic yield of stool analysis is low in patients with higher CD4 cell counts. The probable reasons were i] effective HAART helps eradicating opportunistic protozoal infection and associated with the influx of CD4 positive cells into the lamina propria. ii] As the opportunistic infections causing diarrhoea in AIDS become less common, other gastrointestinal diseases, which are common in young age group, like inflammatory bowel disease and celiac disease, irritable bowel syndrome and idiopathic steathrrhoea are presently leading the list of etiological agents. iii] variety of unknown/unidentified infection of HAART-related toxicities.[5]

All diarrhoeal stool samples with *Isospora* had CD4 <200 and correlation between *Isospora* and CD4 count was significant (<0.05). 80% cases of *Cryptosporidium* had CD4 <200 and remaining 20% cases of *Cryptosporidium* had CD4 within 200-350. A similar study by Fredrick et al, showed CD4 <200 consistent for both *Isospora* and *Cryptosporidium*.[18] The most commonly found enteric pathogen in our study *Mycobacterium tuberculosis* did not show any significant correlation with CD4 count. Unlike other opportunistic infections which occur at CD4+ counts below 200/mm^3, active TB occurs throughout the course of HIV disease.[19]

In the present study *Mycobacterium tuberculosis* (32.52%) was found to be the most common pathogen causing chronic diarrhoea in this part of the world. Among parasitic infections, *I. belli* (21.49%) followed by *Cryptosporidium* (11.9%) were the most the common pathogens isolated in present study. Chronic diarrhoea as a manifestation in HIV patients was more common with low CD4 counts. Gastro enteric pathogen detection was more common in patients with low CD4 counts.

The present study highlights the importance of testing for intestinal pathogens in HIV/aids patients and emphasizes the necessity of awareness among clinicians regarding the occurrence of these pathogens in this population and health education of the population for food hygiene and adherence to proper treatment. The frequency and the danger of these opportunistic infections require their efficient diagnosis which should also include CD4 count and appropriate management that may ultimately lead to reduction in morbidity and untimely mortality for patients with HIV/AIDS in India.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Fact sheet. Latest global and regional statistics on the status of the AIDS epidemic. UNAIDS. 2017. Available at: http://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf.
2. Global AIDS update. UNAIDS. 2016. Available at: http://www.unaids.org/sites/default/files/media_asset/global-AIDS-update-2016_en.pdf
3. Ending AIDS: Progress towards the 90-90-90 targets. UNAIDS. 2017. Available at: http://www.unaids.org/en/resources/documents/2017/20170720_Global_AIDS_update_2017
4. Uppal B, Kashyap B, Bhalla P. Enteric pathogens in HIV/AIDS from a tertiary care hospital. Indian J Community Med. 2009 Jul;34(3):237-42.
5. Attili SV, Gulati AK, Singh VP, Varma DV, Rai M, Sundar S. Diarrhea, CD4 counts and enteric infections in a hospital–based cohort of HIV-infected patients around Varanasi, India. BMC Infectious Dis. 2006 Dec;6(1):39.
6. Gupta S, Narang S, Nunavath V, Singh S. Chronic diarrhoea in HIV patients: prevalence of coccidian parasites. Indian J Med Microbiol. 2008 Apr 1;26(2):172-5.
7. Manatsathit S, Tansupasawasdikul S, Wanachiwanawin D, Setawarin S, Suwanagool P, Prakasvejakit S, et al. Causes of chronic diarrhea in patients with AIDS in Thailand: a prospective clinical and microbiological study. J Gastroenterol. 1996 Aug 1;31(4):533-7.
8. Prasad KN, Nag VL, Dhole TN, Ayyagari A. Identification of enteric pathogens in HIV-positive patients with diarrhoea in northern India. J Health Population Nutrition. 2000 Jun 1:23-6.
9. Joshi M, Chowdhary AS, Dalar PJ, Maniar JK. Prevalence of intestinal parasitic pathogens in HIV-seropositive individuals in Northern India. Natl Med J India. 2002;15(2):72-4.
10. Guidelines for Management and treatment of OI's and Malignancies in HIV/AIDS. NACO; 2007. Available at: http://naco.gov.in/sites/default/files/Guidelines.
11. 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 Jul 1;130(1):63-6.
12. Joshi MA, Chowdhary AS, Dalai PJ, Maniar JK. Parasitic diarrhoea in patients with AIDS. National Med J India. 2002 Mar 1;15(2):72-4.
13. Mohandas K, Sehgal R, Sud A, Malla N. Prevalence of intestinal parasitic pathogens in HIV-seropositive individuals in Northern India. Japanese J Infectious Dis. 2002 Jun 1;55(3):83-4.
14. Al-Megrin WA. Patients in Riyadh, Saudi Arabia. Pak J Biol Sci. 2010;13(8):390-4.
15. Khumalo-Ngwenya B, Luo NP, Chintu C, Sunkutu R, Sakala-Kazembe F, Baboo KS, et al. Gut parasites in HIV-seropositive Zambian adults with diarrhoea. East African Med J. 1994 Jun;71(6):379-83.
16. Sharma SK, Mohan A, Kadhiravan T. HIV-TB co-infection: epidemiology, diagnosis and management. Indian J Med Res. 2005 Apr 1;121(4):550-67.
17. Scott B. Diagnostic Microbiology. 12th Ed, Mosby; 2006.
18. Akinbo FO, Okaka C, Omoregie R. Prevalence of intestinal parasitic infections among HIV patients in Benin City, Nigeria. Libyan J Med. 2010 Jan 1;5(1):5506.
19. HIV and Tuberculosis. API; Medicine update. 2005;147:726-30.

Cite this article as: Sahoo D, Baral P, Mahapatra SR, Sarangi A, Babu KK. Prevalence of enteric pathogens in HIV patients with chronic diarrhoea and their relationship with CD4 count. Int J Adv Med 2018;5:1491-7.