



ISOLATION OF ENTERIC PATHOGENS FROM HIV POSITIVE PATIENTS AND THEIR CORRELATION WITH CD₄⁺ T LYMPHOCYTE COUNT

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Abstract: Human immunodeficiency virus (HIV) infection is a serious problem throughout the world. Diarrhea is one of the most common gastrointestinal complications and affects up to 80 percent of patients with HIV and is the presenting symptom of approximately a third of patients with HIV. This study was conducted to define the microbial etiologies of diarrhea and to compare the magnitude and distribution of pathogens isolated from stool samples of HIV positive patients with diarrhea by using different diagnostic techniques and to determine their relationship with CD₄⁺ T lymphocyte cell count. Stool samples from seventy HIV positive patients with diarrhea and seventy HIV negative patients with diarrhea were examined for presence of parasites and bacteria by different diagnostic techniques. CD₄⁺T lymphocytes count of HIV positive patients was done by BDFACS Calibur flow cytometry. Parasites were isolated from 54 (77.14%) of HIV positive patients with diarrhea. *Cryptosporidium parvum* (32.86%) was the most common parasite followed by Microsporidia sp. (14.29%) and *Isospora belli* (10%) which was significantly higher than the control group. *Candida species* (30%) were isolated more from HIV positive patients. Opportunistic parasites were isolated from the patients with CD₄⁺ T lymphocyte count between 200-499/ μ l. It is important to understand the microbial etiologies of diarrhea among these cases so that appropriate medical investigations, specific therapy and adequate nutritional counseling can be started on time which will help to reduce the socioeconomic and medical costs of this disease in our country.

Key words: Acquired Immunodeficiency Syndrome; AIDS; CD₄⁺ T lymphocyte count; Diarrhea; HIV; HIV positive patients; Opportunistic infection

INTRODUCTION

Human immunodeficiency virus (HIV) infection is a serious problem throughout the world. Globally, an estimated 35.3 million people were living with HIV in 2013. There were 2.3 million new HIV infections globally. India has the third-highest number of people living with HIV¹.

HIV causes progressive degradation of the immune system. Patients with HIV are at a high risk of developing recurrent infections that can include gastrointestinal complications. Diarrhea is one of the most common gastrointestinal complications and affects up to 80 percent of patients with HIV and is the presenting symptom in approximately a third of patients with HIV.² The causes of diarrhea in HIV patients can be infectious or noninfectious.³ Infectious diarrhea in HIV is caused by a variety of pathogens including parasites, bacteria, viruses, and fungi. Noninfectious diarrhea could be due to antiretroviral therapy (ART) related adverse effects and HIV enteropathy. The etiological agents vary from patient to patient and from country to country depending on the geographical distribution, endemicity, seasonal variation of the enteric pathogens, and immune status of the patient.^{4,5}

Intestinal parasites remain a major cause of morbidity and mortality in HIV patients worldwide. Intestinal parasites are widely distributed in India largely due to the low level of environmental and personal hygiene, contamination of food and drinking water that results from improper disposal of human excreta and lack of awareness of simple health promotion practices.⁶ It is not only the spectrum of bacterial organisms that differ between the HIV-immuno-deficient population and the immuno-competent population but also their frequency, severity and persistence. Bacterial infection such as *Salmonella species*, *Campylobacter species* and *Shigella species* occur more frequently in HIV positive than in the HIV negative population.^{7,8,&9}

CD₄⁺T lymphocyte count estimation has a major role in the management of person with HIV infection. CD₄⁺T lymphocyte count is a predictor for HIV disease progression in patients not only on therapy, but also for monitoring a patient's response to therapy. In HIV infected patients, a progressive decline in their immunological responses makes them extremely susceptible to a variety of common and opportunistic infections. The lower the CD₄⁺T lymphocyte cell count, the higher the risk of HIV-related illnesses.

It is important to understand the etiologies of diarrhea cases so that appropriate medical investigations, specific therapy and adequate nutritional counseling can be started in time which will reduce the socioeconomic and medical costs for this disease.

Therefore this study was conducted to define the microbial etiologies of diarrhea and to compare the magnitude and distribution of pathogens isolated from stool samples of HIV positive and negative patients with diarrhea by using different diagnostic techniques. With this the study also determined the relationship between CD₄⁺ T lymphocyte cell count and opportunistic intestinal parasites.

MATERIAL AND METHODS

This study was conducted in Department of Microbiology, Sawai Man Singh Medical College Jaipur from April 2013 to June 2014. This was a hospital based observational descriptive study. Seventy HIV positive patients with diarrhea were enrolled for study. HIV status of these patients was confirmed at Integrated Counseling & Testing Center (ICTC) by three different antibody tests as per National AIDS Control Organization (NACO) guidelines. Seventy HIV negative patients with diarrhea who came for routine examination of their stool samples to the

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Department of Microbiology were enrolled as control group. It was ensured that in both the group patients had not received any specific anti-diarrheal treatment in the last two weeks. After obtaining informed consent, socio-demographic, risk factors and relevant clinical history was recorded on a structured proforma.

Stool sample were collected in a clean, wide mouth container and processed within 2 hours of collection. Iodine and saline wet mount preparations were made from both un-concentrated and formol-ether concentrated stool specimens and were observed under the microscope for presence of ova and cyst. From a part of the stool, smears were prepared, heat fixed, and stained by the Kinyoun's (modified acid fast stain), and modified trichrome stain.¹⁰

All stool samples were routinely cultured on MacConkey agar, Blood agar, Xylose Lysine Deoxycholate agar and Thiosulphate Citrate Bile Salt Agar to isolate known bacterial enteropathogens. Routine enrichment was done on Selenite F Broth and Alkaline Peptone Water for isolation of *Salmonella species*, *Shigella species* and *Vibrio species*. For isolation of Campylobacter species samples were inoculated on Butzler's Media with Campylobacter growth supplement (Himedia, India) and was incubated in a microaerophilic environment at 42°C for 48-72 hrs. The organisms were identified upto species level using standard microbiological techniques.¹¹ Serotyping of *Escherichia coli* isolates was carried out using antisera obtained from Denka Seiken Co. Ltd., Japan and tests were performed as per the manufacturer's instructions. CD₄ +T lymphocytes count was done by FACS Calibur flow cytometry (Becton Dickinson Immunocytometry system, San Jose, CA., USA).

Statistical analyses

Statistical analyses were done using computer software (SPSS version 20 and primer). The qualitative data were expressed in proportion and percentages and the quantitative data expressed as mean and standard deviations. The difference in proportion was analyzed by using chi square test and the difference in means were analyzed by using student T Test and Correlation analyses were performed using Pearson correlation coefficient. Significance level for tests was determined as 95% (P< 0.05).

RESULT

Table 1 shows the demographic details of patients. There was significant difference in distribution of patients in different age groups. In present study majority of HIV patients were laborer or self-employed.

Table 1: Demographic profile of patients with diarrhea enrolled in the study

Character	HIV positive patients with diarrhea (n=70)	HIV negative patients with diarrhea (n=70)
Sex		
Male	42 (60%)	39(55.71%)
Female	28(40%)	31(44.29)
Age group		
0-10 years	2 (2.86%)	16 (22.86%)
11-20 years	4 (5.71%)	12 (17.14%)
21-30 years	21 (30%)	17 (24.28%)
31-40 years	32 (45.71%)	4 (5.71%)
41-50 years	6 (8.57%)	5 (7.14%)
>50 years	5 (7.14%)	16 (22.86%)
Mean age	33.14 ± 10.98Years	28.92 ± 20.88 Years
Occupation		
Laborer	16(22.8%)	13(8.6%)
Student	3 (4.29%)	18(25.7%)
Service (govt. /pvt.)	4 (5.71%)	7(10%)
Business/self-employed	12 (17.14%)	9(12.8%)
Truck – driver/helper	8 (11.43%)	0(0%)
House wife	24 (34.29%)	23(32.8%)

The incidence of acute diarrhea in HIV positive patients was 50.00% while in HIV negative patients was 64.29%. Watery diarrhea was more common in HIV negative patients. Weakness and anorexia were significantly more associated with HIV positive patients while vomiting and nausea were more in HIV negative patients. (Table 2).

Table 2: Clinical presentations of patients with diarrhea enrolled in the study

Features	HIV positive patients with diarrhea (n=70)	HIV negative patients with diarrhea (n=70)
Duration of diarrhea		
Acute diarrhea (up to 14 days)	35(50.00%)	45(64.29%)
Persistent diarrhea (15-28 days)	17(24.29%)	9(12.86%)
chronic diarrhea (>28 days)	18(25.71%)	16(22.85%)
Mean duration of diarrhea	20.74±23.35 days	14.09±13.51 days
Appearance		
Loose/ Watery	38(54.29%)	46(65.71%)
Semiformed	25(35.71%)	20(28.57%)
Formed	7(10%)	4(5.71%)
Clinical symptoms		
Abdominal pain	45(64.28%)	51(72.58%)
Weakness	51(72.58%)	19(27.14%)
Vomiting	16(22.85%)	41(58.57%)
Nausea	21(30%)	39(55.71%)
Anorexia	32(45.71%)	11(15.71%)
Fever	18(25.71%)	42(60%)

In the present study parasites were isolated from 54 (77.14%) HIV positive patients and 27 (38.57%) of HIV negative patients with diarrhea. More than one parasite was isolated from 23(32.85%) of HIV patients as compared to 10 (14.28%) from HIV negative patients. *Entamoeba histolytica* & *Giardia lamblia* were isolated from both HIV positive and negative patients however *Cryptosporidium parvum* (32.86%), *Microsporidia sp.* (14.29%) and *Isospora belli* (10%) were found to be significantly more common in the HIV positive patients. (Table 3)

Table 3: Presence of intestinal parasites among patients with diarrhea enrolled in the study

Parasites detected	HIV positive patients with diarrhea (n=70)		HIV negative patients with diarrhea (n=70)		P value
	Number of isolates	% of isolation	Number of isolates	% of isolation	
Protozoa					
<i>Entamoeba histolytica</i>	19	27.14	15	21.42	0.426
<i>Giardia lamblia</i>	11	15.71	13	13.87	0.823
<i>Cryptosporidium parvum</i>	23	32.86	1	1.43	<0.001S
<i>Isoospora belli</i>	7	10	0	0	0.02S
<i>Microsporidia sp.</i>	10	14.29	0	0	0.003S
Helminthes					
<i>Ascaris lumbricoides</i>	3	4.29	3	4.29	NA
<i>Ancylostoma sp.</i>	5	7.14	2	2.85	0.211
<i>Hymenolopsis nana</i>	0	0	1	1.43	NA
<i>Taenia species</i>	0	0	1	1.43	NA

The mean CD4 + T lymphocyte count of HIV positive patients with diarrhea was 334.71±154.07 / μl ranging from 38 to 594/ μl. The majority of HIV positive patients (50%) had a CD4 + T lymphocyte counts between 200-499/μl. (Table-4)

Table 4: CD4+ T cell count distribution in study group

CD4 range (cells/μl)	No. of patients	(%)
CD4 < 200 /μl	15	21.43
CD4 200 – 500 /μl	35	50
CD4 > 500 /μl	20	28.57
Total	70	100

Table 5 shows the isolation of parasites and their association with CD4 +T lymphocyte counts. Isolation of parasites was more in patients who had CD4 +T lymphocyte counts between 200-499 cells/μl as compared to HIV positive patients with CD4 +T lymphocyte counts ≥ 500 cells/μl. Isolation of *Cryptosporidium parvum* was significantly higher (53.33%) in patients with CD4 +T lymphocyte counts < 200/ul.

Table 5: Parasites and their association with CD4+ T Cell counts in HIV positive patients

Parasites	CD4 <200 /μl(N=15)		CD4 = 200 – 500 / μl(N=35)		CD4 >500 /μl(N=20)		P Value
	No	%	No	%	No	%	
<i>Cryptosporidium sp</i>	8	53.33	14	40	1	5	0.005
<i>Isoospora sp</i>	6	40.00	1	2.86	0	0	0.002
<i>Microsporidia sp</i>	5	33.33	5	14.28	0	0	0.24
<i>E.histolytica sp</i>	3	20.00	10	28.57	6	30	0.77
<i>Giardia sp</i>	2	13.33	5	14.29	4	20	0.82
<i>Ancylostoma sp</i>	1	6.67	1	2.86	3	15	0.242NS
<i>Ascaris</i>	0	0.00	1	2.86	2	10	NA

Pathogenic *Escherichia coli* were isolated from both HIV positive and HIV negative patients while *Candida* species were isolated from 21 (30%) of HIV positive patients. (Table-6)

Table 6: Bacterial isolates and *Candida* species among patients with diarrhea enrolled in the study

Pathogens	HIV positive patients with diarrhea (n=70)		HIV negative patients with diarrhea (n=70)		P value
	Frequency	% of isolation	frequency	% of isolation	
<i>Candida species</i>	21	30	4	5.71	<0.001
<i>EPEC</i>	12	17.14	14	20.00	0.82
<i>EIEC</i>	5	7.14	3	4.29	0.71
<i>ETEC</i>	4	5.71	1	1.43	0.36
<i>Pseudomonas species</i>	8	11.43	4	5.71	0.365
<i>Brevundimonas species</i>	1	1.43	0	0.00	1.0
<i>Enterococcus species</i>	6	8.57	1	1.43	0.121
<i>Salmonella species</i>	1	1.43	0	0.00	1.0
<i>Shigella species</i>	1	1.43	0	0.00	1.0

DISCUSSION

Intestinal parasites are common causes of public health problems in India. In HIV patient’s intestinal infections exemplified by diarrhea are the leading causes of morbidity and mortality in developing countries but their association with causative agents has received only cursory attention.

In present study 75% of HIV positive patients with diarrhea were in age group 21-40 years. This is the most common group affected by HIV as it is the sexually active group. The mean age of HIV positive patients was 33.14 ± 10.98 years. Our results are similar to that of other studies reported from India ^{12, 13,&14}. The higher mean age of clinical presentation of HIV positive patients may be because in our country HIV infection is suspected when

some clinical signs and symptoms start appearing or when illness is quite advanced.

In present study male patients were more than females with male female ratio 1.5: 1. Our findings are consistent with that shown by other studies^{15,16&17}. Predominance of male cases may be due to their migration in search of work. Staying away from their spouse for longer periods may result in acquiring HIV infection. Moreover, the male preponderance might be due to the fact that in the existing social milieu in India, females do not seek medical care fearing isolation and loss of family support.

In present study majority of HIV positive patients were laborer, self-employed, truck-driver/helper and housewives. This may be due to the fact that laborers

migrate in search of work. Migrant laborers stay away from their families for long periods and tend to be promiscuous. Long working hours, relative isolation from the family and geographical mobility may prop casual extra marital sexual relationships and make them highly vulnerable to HIV. Migrant workers tend to have little access to HIV information, voluntary counseling and testing and health services. Returning or visiting migrants, many of who do not know their status, may infect their wives which considerably increase the rate of infections among housewives.

Truck drivers itself is a highly vulnerable group of HIV transmission. They stay away from their families for extended periods of time and are in close proximity to "high-risk" sexual networks. HIV prevalence patterns in truckers have tended to mirror the local epidemics. They transfer HIV from urban to rural settings.

In present study 50% of HIV positive and 64.29% of HIV negative patients had acute diarrhea. The mean duration of diarrhea was 20.74 ± 23.3 days in HIV positive patients while 14.09 ± 13.51 days in HIV negative patients. Statistically significantly higher mean was observed in HIV positive patients in comparison to HIV negative patients with diarrhea. In HIV positive patients' diarrhea is often of longer duration. Our findings are similar to that of Dwivedi *et al.*,¹⁸. The consistency of stool sample is associated with etiological agent. In the present study loose/watery stool was present in 54.29%, of cases followed by semi-formed in 35.71% and formed in 10%. Our findings are similar to that reported by Jha *et al.*,¹⁹ and Cornelius *et al.*,²⁰. Watery stool is due to infection with the more invasive and virulent enteropathogens causing more inflammation and increased shedding. In present study most common clinical finding in HIV positive patients were weakness 51(72.58%), abdominal pain 45(64.28%) and anorexia 32 (45.71%). Our findings are in accordance with other study from India.¹⁹ In present study statistical significant difference was found in various clinical symptoms in HIV positive and negative patients with diarrhea as weakness and anorexia was more in HIV positive patients and fever, nausea and vomiting in HIV negative patients.

The etiology of diarrhea in HIV positive patients is multifactorial. We examined the stool samples for parasites and enteric bacteria. In present study parasites were isolated from 54 (77.14%) of HIV positive patients with diarrhea and 27 (38.07%) of HIV negative patients with diarrhea. In HIV positive patients with diarrhea more than one parasite was isolated from 32.8% of cases. Statistically significant difference was there in isolation of parasites in both the groups. Our findings are in accordance with that of studies conducted by Prasad *et al.*,²¹ and Chhin *et al.*,²² where parasites were isolated from 73% and 75% patients respectively. Intestinal parasites are widely distributed due to the low level of environmental and personal hygiene, contamination of food and drinking water due to improper disposal of human excreta. In addition, lack of awareness of simple health promotion practices is also a contributing factor.

Opportunistic infections constitute a major health problem in patients infected with HIV. The coccidian parasites are the leading enteric parasites in these patients. These organisms usually cause a self-limiting illness in immunocompetent individuals but as the immune status of the patient falls they cause life-threatening profuse watery diarrhea.

In present study, *Cryptosporidium* was found to be predominant enteric parasite (32.86%) associated with diarrhea in HIV positive patients. Isolation rate of parasite in acute, persistent and chronic diarrhea was 25.7%, 35.29% and 44.44%, respectively. Isolation rate increased with duration of diarrhea. It was similar to the other studies reported from India^{14,17,&18}. *Cryptosporidium parvum* is an intestinal pathogen having a zoonotic nature. It is a common cause of severe diarrhea in immunocompromised humans. It is now an AIDS -defining illness as it is involved with chronic diarrhea and the most common cause of enteric disease in HIV patients. Small environmentally resistant oocysts, low infective dose (10–100 oocysts), and oocysts being resistant to disinfectants are some of the factors which impact the epidemiology of *Cryptosporidium* infection. *Cryptosporidium* species are highly resistant to standard dose of chemical disinfectants such as chlorine that is used in the treatment of drinking water. As no effective cure is available for cryptosporidiosis especially in immunocompromised individuals, it usually has a poor prognosis.

In present study *Microsporidia* was isolated from 14.29% of cases. In studies conducted by Brandonisio *et al.*,²³, *Microsporidia* was isolated from 9.23% of cases and Dwivedi *et al.*,¹⁸ reported *Microsporidia* in 6.7% of HIV positive patients with diarrhea however Tuli *et al.*,¹⁴ reported *Microsporidia* from 26.7% of cases. Risk of getting infected with intestinal microsporidiosis is high due to lack of safe and well-protected drinking water. Once infected with intestinal microsporidiosis, HIV patients that do not have access to antiretroviral therapy will be prone to severe complication.

In present study *Isoospora* was isolated from 10% of cases. Our findings are similar to that of Naik *et al.*,²⁴ however Prasad *et al.*,²¹, Gupta *et al.*,⁷² and Vyas *et al.*,¹⁷ reported higher isolation of *Isoospora* in their studies (31%, 41.1% and 31.3% respectively). In this study slightly lower isolation rate as compared to earlier studies may be due to asymptomatic shedding of oocysts.

In HIV negative patients with diarrhea, *Cryptosporidium* was isolated from one patient only. He was 70 year old man so his immunity status may be compromised however we did not perform CD₄⁺ T lymphocyte count of that patient. No other coccidian parasite was isolated from HIV negative patients with diarrhea.

In present study *Entamoeba histolytica* and *Giardia* species were isolated from 27.14%, & 15.71%, of HIV positive patients with diarrhea and 21.42% & 13.87% HIV negative patients with diarrhea respectively. No statistical

significant difference was found in both the groups. It is similar to that of other studies reported from India.^{18,&22} In few studies^{21,24,&25} low isolation was reported.

Amongst helminthes group *Ancylostoma* species were isolated from 7.14% and *Ascaris* 4.29%, of HIV positive patients with diarrhea. It was similar to the isolation of these parasites in HIV negative patients with diarrhea. The presence of these parasites reflects poor environmental hygiene and sanitation in the community.

CD₄⁺ T lymphocyte count is used for management of HIV infected individuals and is helpful in the decision on when to intervene with the antiretroviral treatment or with prophylaxis for opportunistic infections. In present study isolation of opportunistic parasites were significantly higher in patients with CD₄ + T lymphocyte count < 200 cells/μl (*Cryptosporidium* 53.33%, *Isoospora* 40.0% and *Microsporidia* 33.33%) than > 200 cells/μl. Relation between the CD₄ + T lymphocyte count and presence of opportunistic parasites in HIV positive diarrheal patients was significant. Our findings are in accordance with that of Adamu *et al.*,²⁶ and Asma *et al.*,²⁷ who also reported that intestinal parasites were significantly more common with lower CD₄ + T lymphocyte count.

The isolation rates of parasites decreased with the increase in the CD₄ + T lymphocyte count however in present study coccidian parasites were also isolated from patient with CD₄ + T lymphocyte count between 200-500cell/μl. It may be due to regional immunosuppression as suggested by Schneider *et al.*,²⁹. They found, loss of CD₄ + T lymphocyte count cells in intestinal mucosa of the patients with diarrhea, which were more pronounced than peripheral CD₄ + T lymphocyte count levels and their relation is quite variable. The mucosal immunity, an important factor to prevent diarrhea is therefore variable even in patients with good immunity (i.e. peripheral CD₄ + T lymphocyte count >200). We did not perform the mucosal CD₄ + T lymphocyte count levels thus any comments on the mucosal immune status based on blood CD₄ + T lymphocyte count levels would be in appropriate. But we can presume that probably the low mucosal immunity could be a cause of diarrhea in patients with high CD₄ + T lymphocyte count levels

In present study stool samples were processed for isolation of probable diarrheogenic bacteria. The most common bacteria isolated were pathogenic *Escherichia coli* isolated from 21(30%) of HIV positive patients. Our findings are in accordance with other studies from India.^{13,&30} We did serotyping of *Escherichia coli* isolated from diarrhea patients. *Enteropathogenic E. coli* (EPEC) was the predominant species isolated from 17.14% of HIV positive patients and 20% of HIV negative patients followed by *Enteroinvasive E. coli* and *Enterotoxigenic E. coli*. No statistically significant difference was found in both the groups. These organisms usually cause a self-limiting illness in immunocompetent individuals but in the case of immunocompromised patients they can cause life threatening, profuse watery diarrhea.

Other pathogens isolated in present study were *salmonella sp.*, *shigella sp.*, *Brevundimonas sp.*, *Enterococcus sp.*, and *Pseudomonas sp.* Their isolation rate was low and didn't have statistical significance. In present study samples were also processed for isolation of *Campylobacter* species. The species were not isolated from any of the samples. In a study conducted by Liesenfeld *et al.*,³¹ & Prasad *et al.*,²¹ *Campylobacter* species was isolated from 0.94% & 3.8% of samples respectively. However in a study conducted by Samie *et al.*³² *Campylobacter* species was isolated from 31% of samples. Difference in finding might be due to different location of study and low sample size. Clinical and bacteriological pattern vary not only from region to region but also in the same region from time to time. A periodic assessment of the problem is vital for any region to understand the current bacteriological pattern and reduce its complication.

In present study *Candida species* were isolated from 30.0% of HIV positive patients. *Candida species* were significantly more common in the HIV-positive group than the HIV negative control group. This suggests that immunodeficient state in HIV infected patients makes patients more susceptible to such infections, and once established they are not able to prevent the proliferation or clear the infecting agent. The isolation of *C. albicans* in stools of HIV positive patients varies from place to place^{13, 30,&33}. These pathogens have been reported as opportunistic in the gastrointestinal tract and are considered in some situations the only etiological agent necessary for diarrheic symptoms or relevant only when associated with other microorganisms

CONCLUSION

The criteria utilized to establish specific treatment should include sign and symptoms, the severity of diarrhea which depends on the immunological state of the patient and of the virulence of the pathogen involved. These studies are important tools for interventions in patients with HIV enabling a better understanding of the etiology of diarrhea, so that treatment can be started.

REFERENCES

- UNAIDS report on the global AIDS epidemic. 2013
- Siddiqui U, EJ Bini, K Chandarana. "Prevalence and impact of diarrhea on health-related quality of life in HIV infected patients in the era of highly active antiretroviral therapy". *J Clin Gastroenterol* 41(5) (2007):484-490.
- MacArthur R, D H DuPont. "Etiology and pharmacologic management of noninfectious diarrhea in HIV-infected individuals in the HAART era." *Clin Infect Dis.* 55 (2012): 860-867.
- Blanshard C, N Francis, B. Gazzard. "Investigation of chronic diarrhoea in acquired immunodeficiency syndrome. A prospective study of 155 patients." *Gut.* 39. (1996): 824-832.
- Kulkarni SV, R Kairon, SS Sane, PS Padmawar, VAS Kale, MR Thakar. "Opportunistic parasitic infections in HIV/AIDS patients presenting with diarrhoea by the level of immunosuppression." *Ind J Med Res.*; 130. (2009):63-66.

6. World Health Organization. 1981. Intestinal protozoa and helminthic infections: Reports of a WHO Scientific Group, Geneva..
7. Baer J, D Vugia, A Reingold. "HIV infection as a risk factor for Shigellosis." *Emerg Inf Dis.* 5(1999):820–3.
8. Sorvillo F, L Lieb, S Waterman. "Incidence of campylobacteriosis among patients with AIDS in Los Angeles County." *J AIDS.* 4. (1991):598–602.
9. Weber R, B Lederberger, R Zbinden. "Enteric infections and diarrhea in human immunodeficiency virus-infected persons: a prospective community- based cohort study." *Arch Intern Med.* 159. (1999):1473–80.
10. Baveja UK, J Sokhey. Manual on laboratory diagnosis of common opportunistic infections associated with HIV/AIDS, Government of India National Institute of Communicable Diseases.
11. Collee JG, Miles RS, Watt B. Tests for the identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A (eds.), Mackie & MacCartney Practical Medical Microbiology, 14 th ed. Churchill Livingstone: London; 1996. p. 151-79.
12. Carcamo C, T Hooton, MH Wener. "Etiologies and manifestations of persistent diarrhea in adults with HIV-1 infection: a case-control study in Lima, Peru." *JID.* 191. (2005):11–19
13. Kumar SS, S Ananthan, P Lakshmi. "Intestinal parasitic infection in HIV infected patients with diarrhoea in Chennai". *Ind J Med Microbiol.* 20.2. (2002):88-91.
14. Tuli L, AK Gulati, S Sundar, TM Mohapatra. "Correlation between CD4 counts of HIV patients and enteric protozoan in different seasons - an experience of a tertiary care hospital in Varanasi (India)." *BMC Gastroenterol.* 8.(2008):36
15. John F., M Jose, L Dubon Arba. "Intestinal parasitic infections in human immunodeficiency virus (HIV)-positive and HIV-negative individuals in san Pedro Sula, Honduras." *Am. J. Trop. Med. Hyg.* 58.4 (1998):431–435.
16. Mohammed Awole, Gebre-Selassie Solomon, Kassa Tesfaye. "Prevalence of Intestinal Parasites in HIV-Infected adult Patients in Southwestern Ethiopia." *Ethiop.J.Health Dev* 17 (1).(2003):71-78.
17. Vyas N, S Sood, B Sharma, M Kumar. "The Prevalence of Intestinal Parasitic Infestation and the Related Profile of the CD +4 Counts in HIV/AIDS People with Diarrhoea in Jaipur City." *JCDR.* 7.3. (2013): 454-456.
18. Dwivedi KK, G Prasad, S Saini, S Mahajan, S Lal, UK Baveja. "Enteric opportunistic parasites among HIV- infected individuals: associated risk factors and immune status." *Jpn J Infect Dis* 60 (2007): 76-81.
19. Jha A K, B Uppal, S Chadha. "Clinical and Microbiological Profile of HIV/AIDS Cases with Diarrhea in North India." *Journal of Pathogens* Article ID 971958.(2012): 7 pages
20. Cornelius K. Kipyegen, S Robert, O Shivairo, R Odhiambo. "Diarrhea and Intestinal Parasites among HIV Infected Patients in Baringo, Kenya. *Journal of Biology, Agriculture and Healthcare* 3.14(2013). 12-22.
21. Prasad KN, VL Nag, TN Dhole, A Ayyagari. "Identification of enteric pathogens in HIV-positive patients with diarrhea in northern India." *J Health Popul Nutr* 18. (2000): 23-6.
22. Senya Chhin, I Joseph. Harwell. "Etiology of Chronic Diarrhea in Antiretroviral-Naïve Patients with HIV Infection Admitted to Norodom Sihanouk Hospital, Phnom Penh, Cambodia". *Clinical Infectious Diseases.*43. (2006):925–32.
23. Brandonisio O, P Maggi, M. Panaro." Intestinal protozoa in HIV-infected patients in Apulia, South Italy." *Epidemiol. Infect.* 123.(1999) 457-462
24. Venkatesh Naik R, H Ravichandraprakash, PM Ukey. "Opportunistic intestinal parasitic infections in HIV/AIDS patients presenting with diarrhea and their correlation with CD4+ T-Lymphocyte counts." *IJPBS.* 2.4 (2012).293-299
25. Mohandas K, R Sehghal, A Sud, N Malla. "Prevalence of intestinal parasitic pathogens in HIV-seropositive individuals in northern India." *Jpn J Infect Dis.* 55. (2002):83-89.
26. Adamu H, B Petros. "Intestinal protozoan infections among HIV positive persons with and without Antiretroviral Treatment (ART) in selected ART centers in Adama, Afar and Dire-Dawa, Ethiopia." *Ethiop. J. Health Dev;* 23.2(2009):133 140.
27. Asma, I, S Johari, L.H Benedict, A Sim Yvonne, L. Lim. "How common is intestinal parasitism in HIV-infected patients in Malaysia?" *Tropical Biomedicine;* 128.2.(2011):400–410
28. Tabaseera N, K Anuradha, D Venkatesha. "Diarrhea, CD4 Cell Counts and Intestinal Parasitic Infection in HIV Seropositive Patients in a Tertiary Care Hospital." *International Journal of Health Sciences & Research* 2. (2012):.17-22.
29. Schneider T, H Jahn, W Schmidt, E Riecken, M Zeitz, R Ullrich. "Loss of CD4 cells in patients infected with HIV is more pronounced in mucosa than in blood." *GUT* 37(1995):524-29.
30. Uppal B, B Kashyap, P Bhalla. "Enteric pathogens in HIV/AIDS from a tertiary care hospital." *Ind J Comm Med.* 34.3(2009):237-42.
31. Oliver Liesenfeld. "Culture of Intestinal Biopsy Specimens and Stool Culture for Detection of Bacterial Enteropathogens in Patients Infected with Human Immunodeficiency Virus." *J Clin Microbiol.* 30. 3(1995): 745–747.
32. Samie A, PO Bessong, CL Obi. "Bacterial and Parasitic Agents of Infectious Diarrhoea in the Era of HIV and AIDS - The Case of a Semi Rural Community in South Africa, Microbes, Viruses and Parasites in AIDS" (2011) Available from <http://www.intechopen.com>.
33. Leopold G. Lehman, Kangam Lafortune, Marthe-Liliane Mbenou. "Intestinal parasitic and candida infection associated with HIV infection in Cameroon." *J Infect Dev Ctries* 7.2 (2013):137-143.

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