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Opportunistic Intestinal Parasites in HIV Infected Individuals and Its Correlation with the CD4 Counts.

Amol Zope*, Anand Pai, Anuradha De, and Sujata M Baveja.

Department of Microbiology, Lokmanya Tilak Municipal Medical College, Mumbai – 400022, Maharashtra, India.

Research Article

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***For Correspondence**

Department of Microbiology, Lokmanya Tilak Municipal Medical College, Mumbai – 400022, Maharashtra, India.

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ABSTRACT

The association between intestinal parasitic infections and human immunodeficiency virus (HIV) infection is well documented. These infections are often correlated with the CD4+T cell counts of the individuals. The prevalence of intestinal parasitic infections in HIV seropositive patients was compared with CD4 counts < 200 cells/µl, CD4 counts between 200- 500 cells/µl, and CD4 counts > 500 cells/µl. During one and a half years period, a total of 220 HIV seropositive patients were included in the study. One hundred and nineteen HIV seropositive patients were with CD4 counts < 200 cells/µl, 73 HIV seropositive patients were with CD4 counts between 200- 500 cells/µl and 28 HIV seropositive patients were with CD4 counts >500 cells/µl. The stool samples from these patients were examined for enteric parasites using saline and iodine mounts. Modified Z-N staining was carried out for enteric coccidian parasites and Modified trichrome stain for the microsporidia. The prevalence of intestinal parasites was 41.36%. Enteric parasitic infections with CD4 counts <200 cells/µl included *Cryptosposidium spp* 20.17%, *Isopsora belli* 6.72%, *Entamoeba histolytica* 5.88%, *Giardia lamblia* and *Ascaris lumbricoides* 4.20% each. The enteric parasites found with CD4 counts between 200-500 cells/µl included *Cryptosposidium spp.* 15.07% and *E.histolytica* 2.74%; and with CD4 counts >500 cells/µl included *E.histolytica* 10.71% and *Cryptosposidium spp.* 17.86%. Microsporidia were only present in those patients having CD4 counts <200 cells/µl. Continuous monitoring of CD4 counts in HIV seropositive patients is necessary to look for intestinal parasites in stool samples of these patients.

INTRODUCTION

The number of persons living with HIV infection exceeds 33.4 million, the majority of whom live in the developing countries of Sub Saharan Africa and South and South East Asia. According to estimates from the UNAIDS 2009 AIDS Epidemic Update, 31.3 million adults and 2.1 million children [1].

Opportunistic infections are common in HIV infection, which could be bacterial, fungal, parasitic or viral. The common parasitic infections are cryptosporidiosis, isosporiasis, cyclosporiasis, microsporidiosis, toxoplasmosis, generalized strongyloidiasis and giardiasis. The association between intestinal parasites and human immunodeficiency virus (HIV) infection is well documented. The intracellular intestinal protozoans *Cryptosporidium parvum, Cyclospora cayetanensis, Isospora belli* and the microsporidia are opportunistic in patients with acquired immunodeficiency syndrome (AIDS) and are often the major cause of uncontrollable, debilitating diarrhea [2]. These parasites cause morbidity and mortality in AIDS patients

worldwide, and are higher in developing countries due to higher prevalence of infections in the general population. These infections are often correlated with the CD4+T cell counts of the individuals. In AIDS patients with CD4+T cell count < 200 cells/μL with diarrhea, *Cryptosporidium* infection was at 56.5%, the highest and statistically significant compared with the other parasites. Microsporidium was detected in 30.4% of the AIDS patients. Diarrhea was common and most strongly associated in patients with low CD4+T cell counts [3].

Early diagnosis, antiretroviral therapy, chemoprophylaxis and treatment of opportunistic infections are important for the control of HIV replication and disease progression. So this study was carried out in a tertiary care hospital to find out the prevalent opportunistic intestinal parasites in HIV infected individuals and correlating them with the CD4 counts.

METHODS AND MATERIALS

Study comprised of all HIV seropositive patients, with diarrhea and without diarrhea during a period of one and a half years, i.e. from December 2008 to May 2010. Total 220 subjects were included in the study. The stool samples from these patients were collected in a wide mouthed container with a tight fitting leak proof lid and were examined for various enteric parasites. Their CD4+ T cell counts were determined and clinical profile was studied by reviewing their case papers and filling a pre-designed proforma. All HIV seropositive patients of all ages and both sexes who were tested for CD4+ T cell counts at Integrated Counselling and Testing Centre (ICTC) of Department of Microbiology were included in the study and all HIV seronegative patients detected at ICTC were excluded from the study. The protocol was reviewed and approved by the Institutional Ethics Committee.

Stool samples were examined in wet saline mounts and in iodine preparation for detection of protozoan oocysts, cysts, helminthic ova and larvae. Permanent stained smears were performed with Modified Ziehl-Neelsen staining (Cold Method) for detection of oocysts of Cryptosporidium, Isospora and Cyclospora [4] and Chromotrope 2R staining using Aniline Blue for detection of spores of microsporidia [5].

The CD4+ T-lymphocytes cell counts from the EDTA blood specimens of the HIV seropositive patients were determined by FACS Count System (Becton Dickinson). This system is an automated instrument designed specifically for enumerating the absolute cell counts of CD4+ and CD3 T-lymphocytes in unlysed whole blood. Guidelines for performance of the test, biosafety practices, trouble shooting and maintenance of equipment were strictly followed as recommended by the manufacturer for maintaining accuracy, reproducibility and comparability of the estimates.

The collected data was numerically coded and entered in Microsoft Excel 2007, and then transferred to the SPSS (version 15.0). Data was analyzed by applying appropriate statistical tests (Chi-square test and Fisher's exact test).

RESULTS

A total of 220 HIV seropositive patients were included in this study, of which 134 (60.91%) were males, 81 (36.82%) were females and 5 (2.27%) were transgender. Majority of patients, 105 (47.73%) were in the age group of 31-40 years and 49 (22.27%) were in the age group of 21-30 years.

Out of 220 seropositive patients, 119 (54.09%) had CD4+ T cell counts of < 200 cells/μl. In age group of 31-40 years, maximum number of cases 66/105 (62.86%) had CD4+ T cell counts of < 200 cells/μl, while majority of cases having CD4+ T cell counts of 200-500 cells/μl, 18/49 (36.73%) were in age group of 21-30 years.

Out of 220, a total of 182 (82.73%) had diarrhea and 38 (17.27%) did not have diarrhea. All patients (100%) in age group 11-20 years had diarrhea. Majority of patients 85/105 (80.95%), was in age group of 31-40 years and maximum number of patients also had diarrhea. No statistical association was found between age of the patients and diarrhea.

Table 1 shows the diarrheal cases in this study and their correlation with CD4+ T cell counts. Majority of the cases 104/182 (57.14%) having diarrhea had CD4+ T cell counts of < 200 cells/μl. The association between CD4+ T cell count and incidence of diarrhea was found to be statistically significant (P = 0.014).

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In the present study, the prevalence of intestinal parasites was 91/220 (41.36%), which included 34 by wet mount (Table 2), 51 by Z-N stain (Table 3) and 6 by Chromotrope 2R stain.

The various parasites detected in wet mount from HIV seropositive patients were correlated with the presence of diarrhea. It showed that in patients having diarrhea most common parasite detected in wet mount was *E.histolytica* (6.04%), followed by *Giardia lamblia* (2.19%), *S.stercoralis* (1.65%), *A.lumbricoides* (1.65%) and one in each of *A.duodenale* and *E.vermicularis,* while in patients without diarrhea, most common isolated parasite in wet mount was *A.lumbricoides* (5.26%). Majority of *E. histolytica/dispar/moschkovskii* 7/12 (58.33%) detected by wet mount in HIV seropositive patients had CD4+ T cell count of < 200 cells/ µl. *G. lamblia, A. lumbricoides, S. stercoralis, E. vermicularis, T. trichiura* and *A. duodenale* detected in these patients also had CD4+ T cell count of < 200 cells/ µl (Table 2).

Parasites were detected in 51/220 (23.18%) patients by Z-N staining, of which *Cryptosporidium species* was the predominant enteric coccidian parasite detected 40/51 (78.43%). Among the 182 patients with diarrhea, coccidian parasites were seen by Z-N stain in 50 patients as compared to patients without diarrhea, where in only one patient *Isospora* was detected. There was statistical significance seen between diarrhea and coccidian parasites (Chi-square = 11.533, DF = 3, P value = 0.009). *Cryptosporidium species* was detected in 24 HIV seropositive patients having CD4+ T cell count of < 200 cells/µl (Table 3). The isolation rates decreased with the increase in the CD4+ T cell counts.

Table 1: CD4+ T cell count Vs diarrhoea cases (n=220)

CD4+ T cell count	Diarrhoea >1 month		Total
	Present (%)	Absent (%)	
<200	104 (57.14)	15 (39.47)	119
200-500	60 (32.97)	13 (34.21)	73
>500	18 (9.89)	10 (26.32)	28
Total	182 (82.73)	38 (17.27)	220

Chi-square = 8.493, DF = 2, P value = 0.014.

Table 2: Parasites detected in wet mount from HIV seropositive patients in correlation with CD4+ T cell count (n=220)

Parasites detected	CD4+ T cell count (%)			Total
	<200 cells/µl	200-500 cells/µl	>500 cells/µl	
Cysts & trophozoites of *G. lamblia*	1 (0.84)	1 (1.37)	0 (0.0)	2
Cysts of *E. histolytica/dispar/moschkovskii*	7 (5.88)	2 (2.74)	3 (10.71)	12
Cysts of *G. lamblia*	5 (4.20)	0 (0.0)	0 (0.0)	5
Ova of *A. duodenale*	2 (1.68)	0 (0.0)	0 (0.0)	2
Ova of *E. vermicularis*	2 (1.68)	0 (0.0)	0 (0.0)	2
Ova of *T. trichura*	2 (1.68)	0 (0.0)	0 (0.0)	2
Larvae of *S. stercoralis*	4 (3.36)	0 (0.0)	0 (0.0)	4
Ova of *A. lumbricoides*	5 (4.20)	0 (0.0)	0 (0.0)	5
No pathogen found in wet mount	91 (76.47)	70 (95.89)	25 (89.29)	186
Total	**119 (54.09)**	**73 (33.18)**	**28 (12.73)**	**220**

Table 3: Parasites detected in Z-N staining from HIV seropositive patients and its co-relation with CD4+ T cell count (n=220)

Parasites detected	CD4+ T cell count (%)			Total
	<200 cells/µl	200-500 cells/µl	>500 cells/µl	
Oocysts of *Cryptosporidium* and *Isospora*	1 (0.84)	1 (1.37)	0 (0.0)	2
Oocysts of *Cryptosporidium*	24 (20.17)	11 (15.07)	5 (17.86)	40
Oocysts of *Isospora*	8 (6.72)	0 (0.0)	1 (3.57)	9
No pathogen found in Z-N stain	86 (72.27)	61 (83.56)	22 (78.57)	169
Total	119 (54.09)	73 (33.18)	28 (12.73)	220

Microsporidial spores were detected by Chromotrope 2R staining, only in cases with diarrhea 6/182 (3.3%) and were absent in cases without diarrhea, which was statistically insignificant. Microsporidia were only present in those patients having CD4+ T cell counts of < 200 cells/µl. There was no statistical significance seen between diarrhea and microsporidia (By Fisher's exact test, P value = 0.593).

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DISCUSSION

Diarrhea has been considered as an AIDS defining condition [3]. Many studies have shown that the CD4+ T cell count is the best indicator of the immediate state of immunologic competence of the patient with HIV infection. It is one of the most widely used surrogate markers for monitoring disease progression and initiating therapy in HIV seropositive persons. In untreated HIV infection, the CD4+ T cell count declines by approximately 4% per year [6]. Decline in CD4+ T cell counts and CD4 function is associated with development of clinical manifestations and opportunistic infections [7]. The prevalence of the enteric parasites in the HIV seropositive individuals is different in different studies as carried by various authors. In the present study, the prevalence of intestinal parasites was 91/220 (41.36%). The prevalence in the studies by Dwivedi et al, De et al, Vighnesh et al and Gupta et al was 62.7%, 39%, 37.1% and 55.8 % respectively [8,9,10,11].

In this study, in patients having diarrhea, most common parasite detected in wet mount was *E. histolytica* (6.04%), followed by *Giardia lamblia* (2.19%), *Strongyloides stercoralis* (1.65%) and *A. lumbricoides* (1.65%), while in patients without diarrhea, most common parasite detected in wet mount was *A. lumbricoides* (5.26%) (Table 2). In a study by De et al from Mumbai in 2009, study showed that commomly isolated parasite in wet mount was *G. lamblia* (7.7%) and *S. stercoralis* (5.1%) [9]. Another study by Dwivedi et al from New Delhi also showed *G. lamblia* as the commonest (13.3%) [8]. While in a study from Chennai by Vignesh et al [10], the most common parasite in wet mount was *Entamoeba histolytica/dispar/moschkovskii* (3.3%), *G. lamblia* (1.6%) and *S. stercoralis* (1.2%). This is in accordance with this study.

By Z-N staining, the most common prevalent coccidian parasite from diarrheal patients in this study was *Cryptosporidium species* 40/182 (21.98%), followed by *Isospora belli* 8/182 (4.39%). Also in 2 patients, there were mixed infection with oocysts of *Cryptosporidium* and *Isospora*. In patients without diarrhea, the most common prevalent coccidian parasite was *Isospora belli* 1/38 (2.63%) (Table 3)

The prevalence of *Cryptosporidium* infection at 56.5% was found by Sadraei et al [3] and was statistically significant as compared with the other parasites in HIV seropositive patients, while lowest of 2.9% was found by Vignesh et al [10]. In the latter study, they also found a statistically significant increase in the number of cases of *Isospora belli* ($p = 0.00037$) and a decline in the number of cases of *Cryptosporidium spp.* ($p = 0.00001$). Prevalence of *Cryptosporidium parvum* was around 33%, 33.3%, 20.6%, 10.8% and 39.8% by Dwivedi et al, De et al, Gupta et al, Mohandas et al and Tuli et al, respectively [8,9,11,12,13].

Study by De et al showed *Cryptosporidium species* (33.3%), *Isospora belli* (10.3%), *Cyclospora species* (2.6%), *Giardia lamblia* (7.7%) and *Strongyloides stercoralis* (5.1%) [9]. Enteric parasites were identified among 62.7% individuals, of which *Cryptosporidium* emerged as the single largest pathogen, predominant among 33% of the individuals ($P < 0.025$). In a study by Basak et al from Wardha, the coccidian parasites were found in HIV seropositive patients only and *Cryptosporidium species* (28.4%) was the most common, followed by *Isospora belli* (4.7%), which is comparable with this study [14]. In a study conducted by Gupta et al [11], enteric parasites were recovered in 30% of HIV infected individuals, with coccidian parasites comprising 76.3%. Their study showed that *Isospora belli* was the most common parasite in HIV positive patients, followed by *Cryptosporidium species*. Earlier studies from North India by Mohandas et al [12] and Sadraei et al [3] had found *Cryptosporidium species* to be the most common parasite while the *Isospora belli* was found to be much lower. This is in accordance with the present study.

In the study by Mohandas et al [12], *Isospora belli* were detected in 2.5% of the HIV seropositive patients while 2.7% were detected by Dwivedi et al [8]. According to De et al, Vignesh et al and Gupta et al, *Isospora belli* was found around 10.3%, 26.1% and 41.1% respectively [9,10,11]. Thus according to most of the studies, most predominant parasite found in HIV seropositive patients was *Cryptosporidium species,* followed by *Isospora belli* and this is significantly comparable with the present study. *Cyclospora cayetanensis* were detected in 3.3% of the HIV seropositive patients by Mohandas et al [12], 2.6% by De et al [9] and 2.9% by Gupta et al [11]. However, *Cyclospora cayetanensis* was not detected in this study.

In this study, Chromotrope 2R staining of the stool samples showed microsporidial spores in cases with diarrhea (3.3%) and were absent in cases without diarrhea. In a study by De et al [9], majority of the patients (73.3%) were in the age group of 21-40 years. Out of 16 microsporidia reported, liquid stool was seen in 37.5% cases, whereas abdominal pain was present in all 16 patients, but in this study diarrhea was seen in all patients with microsporidia (100%). According to Sadraei et al [3], microsporidia was

detected in 30.4% of the AIDS patients. By Dwivedi et al [8], the same was detected in 6.7% cases. Study conducted in Varanasi by Tulli et al [13] showed the infection rate of Microsporidia in HIV patients as 26.7%. On the contrary, this study detected 3.3% of Microsporidia. This decrease in isolation rates could be due to effective highly active antiretroviral therapy (HAART). In response to successful ART, the CD4+ T cell count typically increases by > 50 cells/µl within weeks of viral suppression [6].

In Lusaka, Zambia, Lindo et al [2] found a significantly higher prevalence of *Entamoeba coli, E. histolytica, Necator americanus,* and *Chilomastix mesniili* in HIV-negative individuals as compared with patients with AIDS. In contrast, *I. belli* and *C. parvum* were found exclusively in AIDS patients. Similarly, in Tanzania [15], significantly higher prevalence rates of *E. histolytica* and *A. lumbricoides* were reported in HIV-negative patients, while *C. parvum, S. stercoralis,* and *I. belli* were reported in HIV-positive patients.

In the present study, 119 HIV seropositive patients were with CD4 counts < 200 cells/µl, 73 HIV seropositive patients were with CD4 counts between 200- 500 cells/µl and 28 were with CD4 counts >500 cells/µl. Enteric parasitic infections were found with CD4 counts < 200 cells/µl, which included *Entamoeba histolytica/dispar/moschkovskii* 5.88%, *Cryptosposidium species* 20.17%, *Isopsora belli* 6.72%, *Giardia lamblia* and *Ascaris lumbricoides* 4.20% each. The enteric parasites were found with CD4 counts between 200- 500 cells/µl included *Entamoeba histolytica/dispar/moschkovskii* 2.74% and *Cryptosposidium species* 15.07%. Enteric parasitic infections were found with CD4 counts > 500 cells/µl included *Entamoeba histolytica/dispar/moschkovskii* 10.71% and *Cryptosposidium sppecies* 17.86% (Tables 1 & 2). Microsporidium was only present in those patients having CD4 counts < 200 cells/µl.

A study by Sharma et al in New Delhi between 2000 and 2003 showed that the majority (82.6%) of symptomatic HIV seropositive cases had CD4+ T cell count < 200 cells/µl and about 46% had CD4+ T cell count < 500 cells/µl [16]. About 99.6% of symptomatic cases had CD4+ T cell count < 500 cells/µl and about 83.4% of the symptomatic HIV seropositive cases had CD4+ T cell count < 200 cells/µl [17]. Although the findings are higher in proportion, they suggest that at lower CD4+ T cell counts, HIV infected persons are more likely to be symptomatic. However, in the study by Ajayi et al, majority of patients had CD4+ T cell counts of < 200 cells/µl [18].

Table 1 shows the diarrheal cases and their correlation with CD4+ T cell counts. Diarrhea in the HIV-infected group with CD4+ T cell counts > 500 cells/µl was 18/82 (9.89%) and in group with CD4+ T cell counts between 200 cells/ µl - 500 cells/µl, it was 60/182 (32.97%) In the patients with CD4+ T cell count < 200 cells/ µl, this rate was 57.14% (104/182), which is significant compared with the former two CD4+ groups and statistically significant.

In one study by Sadraei et al in North India [3], diarrhea in the HIV-infected group with (CD4+ T cell counts > 500 cells/ µl) was 14.7%, in group with (200 cells/ µl < CD4+ T cell counts < 500 cells/ µl) it was 29.8% and in patients with (CD4+ T cell counts < 200 cells/ µl) this rate was 56.1%, which is significant when compared with the former two CD4+ groups (P < 0.0005). Diarrhea was common and most strongly associated in patients with low CD4+ T cell counts. The maximum parasitic isolation was in the group of patients who had CD4+ T cell counts below 200cells/µl and *Cryptosporidium species* was found to be the most commonly acquired protozoa causing chronic diarrhea [3]. The isolation rates decreased with the increase in the CD4+ T cell counts. When compared to this study (Table 3), it also had *Cryptosporidium* as major predominant parasite and also had CD4+ T cell counts of < 200 cells/µl. This stress the importance of opportunistic protozoa in the HIV infected patients and that opportunistic protozoon should be expected in HIV-infected patients with diarrhea and with low CD4+ counts. Microsporidium were only present in those patients having CD4+ T cell counts of < 200 cells/µl in this study. Gamboa et al [19] showed that diarrhea due to microsporidium is frequently seen in advanced stages of AIDS with CD4+ T cell count below 200 cells/µl, which is also consistent with this study.

An inverse correlation between CD4+ T cell counts and isolation rates of parasites from diarrhoeal patients was observed. Enteric coccidian parasites were identified as significant agents associated with diarrhea, especially among those with improper hygiene, multiple infections and a lower CD4+ T cell count [8]. It has been postulated that colonization of the intestinal tract by parasites may be influenced by enteropathy induced by infection with HIV. Virus-induced structural and functional impairment leading to common gastrointestinal symptoms has been shown to occur independently of infection with enteric pathogens. This may selectively deter the establishment and or survival of extracellular and luminal parasites. In contrast, whereas the gut of HIV-infected individuals may not be a favorable environment for the establishment and or survival of extracellular parasites, intracellular and mucosal dwelling organisms

may not be adversely affected by the pathologic changes. The frequency of infection with *C. parvum, S. stercoralis,* and *I.belli* has been associated with increasing duodenal mucosa damage.

CONCLUSIONS

Early diagnosis, antiretroviral therapy, chemoprophylaxis and treatment of opportunistic infections are important for the control of HIV replication and disease progression. Therefore, all the HIV seropositive individuals should be enquired for symptoms like diarrhea. They should be referred for stool examination. The stool specimens should be subjected for special staining techniques like Modified Z-N stain and Chromotrope 2R staining for detection of the coccidian parasites. Continuous monitoring of CD4 counts in HIV seropositive patients is necessary to look for intestinal parasites in stool samples of these patients, so that prompt treatment for these enteric parasites can be initiated to avoid complications in these immunocompromised patients.

REFERENCES

1. Worldwide HIV and AIDS Statistics. www.avert.org/statindx.htm.
2. Lindo JF, Dubon JM, Ager AL, De Gourville EM et al. Intestinal parasitic infections in HIV positive individuals in San Pedro Sula, Honduras. Amer J Trop Med Hyg. 1998; 58: 431–5.
3. Sadraei J, Rizvi MA, Baveja UK. Diarrhea, CD4+ cell counts and opportunistic protozoa in Indian HIV-infected patients. Parasitol Res .2005; 97: 270–3.
4. Baron EJ, Peterson LR, Finegold SM. Diagnostic microbiology. 9th Ed. St. Louis: Mosby yearbook Inc. 1994; pp 776-86.
5. Ryan NJ, Sutherland G, Coughlan K, Globan M, Doultree J, Marshall J, et al . A new trichrome blue stain for detection of Microsporidial species in urine, stool and nasopharyngeal specimens. J Clin Microbiol. 1993; 31: 3264-9.
6. Betts MR, Ambrozak DR, Douek DC, Bonhoefer S. Analysis of total Human Immunodeficiency Virus (HIV) – specific CD4+ and CD8+ T-cell responses: Relationship to viral load in untreated HIV infection. J Virol. 2001;75:11983-91.
7. Clinical Overview of HIV Disease. HIV Insite Knowledge Base Chapter. January 2006. HIV Insite.com
8. Dwivedi KK, Prasad G, Saini S, Mahajan S, Lal S, Baveja UK. Enteric opportunistic parasites among HIV infected individuals: associated risk factors and immune status. Jpn J Infect Dis. 2007; 60: 76-81.
9. De A, Patil K, Mathur M. Detection of enteric parasites in HIV positive patients with diarrhea. Indian J Sex Transm Dis. 2009; 30: 55-6.
10. Vignesh R, et al. High Proportion of Isosporiasis among HIV-Infected Patients with Diarrhea in Southern India. Am J Trop Med Hyg. 2007; 77: 823-824.
11. S Gupta, S Narang, V Nunavath, S Singh. Chronic diarrhea in HIV patients: Prevalence of coccidian parasites. Indian J Med Microbiol. 2008; 26: 172-5.
12. Mohandas K, 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.
13. Tuli L, Gulati AK, Sundar S, and Mohapatra TM. 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. 2008; 8: 36.
14. Basak S, Bose S, Mallick SK, Ghosh AK. Intestinal Parasitic Infections In HIV Seropositive Patients – A Study. J Clin Diagn Res. 2010; 4: 2433-7.
15. Cegielski JP, Ortega YR, Mekee S, Madden JF, Gaido L, Schwartz DA, et al. Cryptosporidium, Enterocytozoon and Cyclospora infections in pediatric and adult patients with diarrhea in Tanzania. Clin Infect Dis. 1999; 28: 314-21.
16. Sharma SK, Kadhiravan T, Banga A, Goyal T, Bhatia I, Saha PK. Spectrum of clinical disease in a series of 135 hospitalized HIV-infected patients from North India. BMC Infect Dis. 2004; 4: 52.
17. Singh HR, Singh GB, Singh TB. Estimation of $CD4^+$ and $CD8^+$ T-lymphocytes in human immunodeficiency virus infection and acquired immunodeficiency syndrome patients in Manipur. Indian J Med Microbiol. 2007; 25: 126-32.
18. Ajayi AO, Ajayi EA, Fasakin KA. CD4+ T-cell Lymphocyte counts in adult with human immunodeficiency virus infection at the medical department of a tertiary health institute at Nigeria. Ann African Med. 2009; 8: 257-60.
19. Gamboa DA, Bencosme VC, Kato MM. Microsporidiasis in AIDS patients with chronic diarrhea. Experiences at the National Institute of Nutrition "Salvador Zubrirán". Rev Gastroenterol Mex 1999; 64: 70-4.