INTESTINAL PARASITIC PREVALENCE IN HUMAN IMMUNO-DEFICIENT VIRUS (HIV) INFECTED PATIENTS WITH AND WITHOUT DIARRHOEA AND ITS ASSOCIATION WITH CD4 T CELLS COUNTS

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Abstract

Background & objectives: Intestinal parasitic infections are major cause of diarrhoea in HIV infected individuals. The present study was undertaken to detect intestinal parasites in HIV infected patients with and without diarrhoea and to determine association between enteric parasites and CD4 T cell count.

Methods: The study was carried out at Department of Microbiology, Rural Medical College, Loni, India, between September 2010 and August 2012 among consecutively enrolled 127 HIV infected patients presenting with and without diarrhoea. Stool samples were collected and examined for enteric parasites by microscopy and special staining methods. CD4 cell counts records of patients were taken from Antiretroviral Treatment Centre (ARTC) of the hospital.

Results: Out of total 127 cases intestinal parasites were detected in 27 cases. The incidence of intestinal parasitic infection was 21.25%. Of 27 cases where parasites detected in total, *Entamoeba histolytica* 13 (48.14 %) was found to be most prevalent parasite followed by *Cryptosporidium parvum* 9 (33.33%) followed by *Giardia lamblia* 3 (11.11 %) followed by *Taenia spp* 2 (7.40%). In HIV infected patients with CD4 count < 200 cells/µl, *C. parvum* was the most commonly observed (88.88%) parasite. Whereas the proportion of intestinal parasites in patients with CD4 count 200 – 499 cells/µl was significantly higher as compared with other two groups of patients with CD4 count < 200 and ≥ 500 cells/µl

Interpretation & conclusions: Parasitic infections were detected in 21.25% HIV infected patients and low CD4 count was significantly associated with opportunistic infection. Detection of aetiologic pathogens might help clinicians to decide appropriate management strategies thereby to reduce morbidity and mortality due to intestinal parasitic infections.

Keywords: Diarrhoea, Intestinal parasites, CD4 T cell count, HIV

1. Introduction:

India is estimated to have the third largest HIV positive population in the world, with 2.5 million people living with HIV/AIDS and prevalence rate approximately 0.30%. Diarrhoea is the most common gastrointestinal infection occurs in 30-60 % of AIDS patients in developed countries and in about 90 % of AIDS patients in developing countries. The aetiologic spectrum of enteric pathogens causing diarrhoea includes bacteria, parasites, fungi and viruses. Diarrhoea, which is caused by opportunistic protozoa, is one of the commonest complications seen in the course of the HIV disease and it is a cause of considerable morbidity and mortality. In HIV infected patient, continuous impairment of immune system makes them extremely vulnerable to variety of common and opportunistic infections. Recently, many studies reported the presence of protozoa like *Cryptosporidium parvum*, *Isospora belli*, *Cyclospora cayetanensis*, *Microsporidia*, *Entamoeba histolytica* and *Giardia lamblia*, which account for a significant number of cases of diarrhoea in this population. Number of studies has been conducted to detect the presence of enteric parasites associated with diarrhoea in HIV/AIDS patient from India and different parts of the world. But the incidence and prevalence of infection with a particular enteric parasite in HIV/AIDS patients is likely to depend upon the endemicity of that particular parasite in the community. As well as the prevalence rate of parasitic agent differ significantly from region to region. Presently, CD4+ T cell count has been shown to be an excellent indicator which defines the degree of immune suppression. A decrease in CD4+ T-lymphocyte counts is responsible for the profound immune-deficiencies that lead to various opportunistic infections in HIV-infected patients. There have been reports on frequency of various pathogens causing diarrhoea from different parts of India. However, there appears to be a scarcity...
of data on correlation of CD4+ T-cell counts and the aetiology of diarrhoea among the HIV patients in this part of India. Thus, this study was conducted in rural tertiary care hospital with the aim to determine the prevalence of enteric parasites affecting the HIV patients with and without diarrhoea. An attempt was also made to elucidate the association between CD4 and presence of enteric parasites.

2. Materials and Methods:
A total of 120 HIV seropositive patients were enrolled in the study. Out of which 54 patients present with diarrhoea whereas 73 non diarrhoeal patients were also included in the study to analyse the prevalence of asymptomatic infection by enteric parasites. The HIV seropositive patients were defined as those who had been tested positive for HIV antibodies by any of the two tests i.e. ELISA/Rapid/Simple as per the recommendation given by WHO13.

2.1 Stool examination: Three consecutive freshly-voided stool specimens from each patient were collected in a sterile wide mouth container from the patients enrolled in the study. Diarrhoea is defined as the passage of abnormal liquid or unformed stool at an increased frequency14. The consistency of stool samples was noted. A direct wet mount of stool in normal saline (0.85%) was prepared and examined for the presence of motile intestinal parasites and trophozoites under light microscope. Lugol's iodine staining was used to detect cysts of intestinal parasites. Formalin ether concentration was the method of choice when stool did not reveal any parasites by direct microscopy. The modified Ziehl Neelsen staining technique was used for coccidian parasites.15

2.2 CD4+ T cell estimation: The CD4+ T-cell count estimation of the patients was taken from the ART centre. Patients were categorized into 3 categories by their immune status according to the 1993 – revised classification system for the HIV infection by CD4 T-cell categories.16

2.3 Statistical methods: Statistical analysis was done using Chi square test to evaluate any association between HIV, diarrhoea and parasitic infections. Observed differences in data were considered significant and noted in the text if P < 0.05 was obtained.

3. Result:
Stool samples of total 127 HIV seropositive patients were screened for intestinal parasites during the period of Sept.2010 to August 2012. Out of total 127 HIV seropositive patients, 71 (55.90 %) were males and 56 (44.09%) were females. The mean age of the male and female was 35.01yrs and 33.23yrs respectively. Table 1 show that the incidence of HIV infection is more in the age group 31-40 in both male and female. The maximum incidence of diarrhoea associated with HIV infection was seen in the age group of 31-40 years, followed by the age group 21-30 years in both male and female [Fig. 1]. Out of the 127 patient, 54 (42.51%) patients were presenting diarrhoea whereas 73 (57.48%) were non diarrhoeal. In the present study, most of the patients were males but interestingly the percentage of HIV infected females presenting diarrhoea (68.51%) was more than that of males (31.48%) [Table 2]. Out of 127 HIV seropositive patients, parasitic infections were detected in 27 (21.25%) cases. Of the 54 diarrhoeal cases, intestinal parasites were found in 23 (42.59%) cases and in 4 (5.47%) cases of HIV-positive patients without diarrhoea (controls) [Table 3]. Statistical analysis was done using Chi square test about diarrhoeal Vs. non-diarrhoeal cases, p value was 0.001. Out of 127 patients studied, 13 (10.23%) were infected with Entamoeba histolytica. Out of 54 diarrhoeal cases studied, 12 (22.22%) were of Entamoeba histolytica and of 73 non-diarrhoeal cases studied; only 1 (1.36%) case were found to be infected with Entamoeba histolytica. Statistical analysis was done using Chi square test about Entamoeba histolytica in diarrhoeal Vs non-diarrhoeal cases, p value was 0.003. Of 27 cases where parasites detected in total, Entamoeba histolytica 13 (48.14 %) was found to be most prevalent parasite followed by Cryptosporidium parvum 9 (33.33%) followed by Giardia lamblia 3 (11.11 %) followed by Taenia spp. 2 (7.40%). Out of 54 diarrhoeal cases Cryptosporidium parvum was found in 7 (12.96%) and 2 (2.73%) in non-diarrhoeal cases. Whereas Giardia lamblia was found only in diarrhoeal patient 3 (5.55 %) and Taenia spp. was identified in 1 (1.85%) diarrhoeal & 1 (1.36%) non diarrhoeal case [Fig. 2]. The p value for Giardia lamblia, Cryptosporidium parvum and Taenia spp in diarrhoeal Vs non diarroeal cases was 0.12, 0.09 and 0.86 respectively. E.histolytica and C.parvum shows significant association with diarrhoeal and non- diarrhoeal cases as compared to Giardia lamblia and Taenia spp.

The study population consisted of 27 patients with CD4 count > 500 cells/µl, 39 patients with
CD4 count 200-499 cells/µl and 61 patients with CD4 count < 200 cells/µl. Among 61 patients with CD4 count less than 200 cells/µl, parasites could be identified in 9 (14.75%) cases. Cryptosporidium parvum was the most common parasites identified in 8 (88.88%) in this group. Of the 39 patients with CD4 count 200-499 cells/µl, parasites were identified in 12 (30.76%) cases in which Entamoeba histolytica was found to be most prevalent parasite, identified in 7 (17.94%) cases. Other parasites such as Giardia lamblia (5.12%), Cryptosporidium parvum (1.56%) also found in this study population. Parasites were detected in 6 (22.22%) cases out of 27 patients with CD4 count > 500 cells/µl, of which E.histolytica was detected in 5 (18.51%) and G. lamblia was found in only 1 (3.70%) case.

The proportion of intestinal parasites in patients with CD4 count 200-499 cells/µl was higher (12(9.44%)) than that in the other two groups of patients with CD4 count < 200 cells/µl (9(7.08%)) and CD4 count >500 cells/µl (6(4.72%)). As shown in Table 4 and by applying Chi-square test there is a significant association between diarrhoal and non-diarrhoal cases and intestinal parasites and CD4 counts (χ² = 29.56, p< 0.01).

Table 1: Age and sex wise distribution of HIV infected patient.

| Age group | Male | Female | Total |
|-----------|------|--------|-------|
| 0-10      | 9    | 4      | 13    |
| 11-20     | 6    | 4      | 10    |
| 21-30     | 8    | 14     | 22    |
| 31-40     | 19   | 20     | 39    |
| 41-50     | 14   | 7      | 21    |
| 50-60     | 15   | 7      | 22    |
| Total     | 71   | 56     | 127   |

Table 2: Sex wise distribution of HIV patient with and without diarrhoea.

| Sex       | With Diarrhoea | Without Diarrhoea | Total  |
|-----------|----------------|-------------------|--------|
| Male      | 17(31.48%)     | 54 (73.97%)       | 71(55.90%) |
| Female    | 37(68.52%)     | 19(26.02%)        | 56(44.09%) |
| Total     | 54(42.51%)     | 73 (57.48%)       | 127    |

χ² = 22.7, d.f.=1, p<0.05, significant

Table 3: Prevalence of the enteric parasites among HIV patients with diarrhoeal and non-diarrhoeal complaints

| Enteric parasites | HIV patient | Total (n = 127) |
|-------------------|-------------|----------------|
|                   | Diarrhoal   | Non Diarrhoal  |
| Positive          | 23(42.59%)  | 4 (5.47%)      | 27(21.25%) |
| Negative          | 31(57.40%)  | 69(94.52%)     | 100(78.74%) |

χ²= 25.51, d.f.=1, p<0.05, significant

Table 4: The association between diarrhoal and non-diarrhoal complaints, parasites isolated and CD4 counts of 127 AIDS patients.

| Enteric parasites | CD4 cells< 200 cells/µl N = 61 | CD4 cells 200–499 cells/µl N = 39 | CD4cells >500 cells/µl N = 27 | Total N = 127 |
|-------------------|---------------------------------|----------------------------------|--------------------------------|---------------|
|                   | Diarrhoal N= 29 | Non Diarrhoal N= 32 | Diarrhoal N=17 | Non Diarrhoal N=22 | Diarrhoal N= 8 | Non Diarrhoal N=19 |               |
| Entamoeba histolytica | 1(3.44%) | - | 7(41.17 %) | - | 4(50%) | 1(5.26%) | 13(10.23%) |
| Giardia lamblia | - | - | 2(11.76%) | - | 1(12.5%) | - | 3(2.36%) |
| Cryptosporidium parvum | 7(24.13%) | 1(3.12%) | - | 1(4.54%) | - | - | 9(7.08%) |
| Taenia spp. | - | - | 1(5.88%) | 1(4.54%) | - | - | 2(1.57%) |

χ² = 29.56, d.f.=5, p< 0.01, highly significant
4. Discussion
Diarrhoea is an important manifestation of the intestinal parasitic infestation in HIV-infected patients\(^1\). Diarrhoea commonly occurs in HIV infected patient at some point of their illness. It is reported that diarrhoea occurs in 30-60 % of AIDS patients in developed countries and in about 90 % of AIDS patients in developing countries\(^2\). In India, prevalence of intestinal parasitic infection in AIDS ranges from 20 % to 30%\(^3\). Slightly higher prevalence of intestinal parasite in HIV infected patients is reported from other countries (33-50%)\(^4\). These parasites can cause self-limiting diarrhoea of short duration in healthy individuals, but in the immunocompromised host, including AIDS patients, the diarrhoea is usually chronic and sometimes, life-threatening\(^5\).

The present study was done on 127 HIV positive patient attending Pravara Rural Hospital. The maximum incidence of HIV seropositive patient and HIV associated diarrhoea was seen in the age group 31-40 in both male & female whereas the proportion of diarrhoea was higher in female as compared to male, which is similar to the results of Deorukhkar, et al.\(^6\). The etiological agents have been identified in various studies from India and other parts of world includes, *E. histolytica*, *G. lamblia*, *Cryptosporidium parvum*, *Isospora belli*, *Microsporidia*, *H. nana*, *Ascaris lumbricoidea*, *Ancylostoma duodenale* etc.\(^7\). Various studies from India and other countries have reported a high prevalence of intestinal parasite, ranging from 25 to 60 percent\(^8\). Gupta et al from northern India has detected enteric parasites in 55.8% HIV patients with diarrhoea and *Isospora belli* was found in 41.1% of chronic diarrhoea and 6.3% in non-diarrhoeal cases\(^9\). Recently Malaji et al from Baglkot and Venkatesh Naik R et al from Karnataka and Vyas et al from Jaipur have documented parasitic prevalence in diarrhoeal cases i.e. 44%, 21.8% and 60.9% respectively\(^10,11\). In all these studies *C. parvum* was found to be most common pathogen\(^9,12,13\). In Maharashtra, Kulkarni et al\(^3\) from Pune reported parasitic infection in 35% patient with diarrhoea and *Cryptosporidium parvum* (12%) as prevalent parasite. In our study enteric parasites were detected in 27 (21.25%) cases in total out of 127 patients and *E. histolytica* (10.23%) was the most frequently encountered pathogen in the study population followed by *Cryptosporidium parvum* (7.08%). *Giardia lamblia* were found in 3 (2.36%) patients and *Taenia* in 1 (1.57%) patient. Whereas Kotigire from Wardha, reported *C. parvum* as most prevalent pathogen followed by *E. histolytica*\(^14\). Mixed infection with *E. histolytica* and *E. coli* was seen only in 2 cases of diarrhoea in present study. But due to uncertainty of pathogenesis of *E. coli* in diarrhoea, mixed infection was not considered. The variation in the prevalence of intestinal parasitic infection demonstrated in different studies can be attributed to the difference in geographical distribution of parasites, awareness of hygiene, medical facilities and different selection of cases. In our study high prevalence of *E. histolytica* is due to poor water supply and lack of personal hygiene, which is a common situation of rural area.

Further the study tried to determine the association between intestinal parasites in diarrhoeal and non-diarrhoeal patients infected with HIV at different levels of immunosuppression by means of CD4 T cell count. CD4 counts play an incredibly important role in the presentation of diarrhoea as well as in the control of protozoa in HIV-infected individuals\(^15\). The impairment of the immune system in HIV patients makes them extremely vulnerable to the specific opportunistic infections which are unable to establish in immunocompetent hosts\(^16\). In our study, intestinal parasites were more commonly found in patients with CD4 count 200-499 cells/µl and the correlation between CD4 cell count and intestinal parasitic infection was found to be highly significant (p<0.01). Several other studies on coccidian parasites reported increased parasitic infection in CD4 count < 200 cells/ µl. Similarly, in our study the *C. parvum* was mainly associated with CD4 count < 200 cells/ µl. This is in concordance with the findings in the study by Kulkarni et al.\(^3\). Whereas Sulcithangam G et al also found prevalence of Coccidian parasites in CD4 count < 200 cells/ µl but reported no association between parasites, diarrhoea and CD4 count in HIV patients\(^4\). Various other studies have reported the correlation between CD4 count and diarrhoea and presence of parasites in HIV positive patients\(^5,6,17,23\). The differences in results may be due to difference in opportunistic and non-opportunistic parasites occurrence at particular territory. This variation may also be pertaining to the infectivity of parasites at different level of immunosuppression. For example, infections by coccidian parasites such as *C. parvum*, *Microspora* mainly seen in the lower immunity, whereas other protozoa like *E. histolytica/E.dispar* most commonly seen in the
patient with higher CD4 T-cell level in diarrhoeal as well as non-diarrhoeal patient. Only a few studies regarding the prevalence of intestinal parasite and their association with diarrhoea of HIV infected patients and levels of immunosuppression are available from rural part of Maharashtra, India at present. So our study is helpful to decide proper clinical management strategies in this area in order to reduce the mortality and morbidity due to parasitic infections.

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