Human immunodeficiency virus (HIV), the causative agent of AIDS, is fast becoming a major threat in the Indian subcontinent, with an estimated 5.2 million persons being infected with HIV (UNAIDS, 2006). Infections of the gastrointestinal tract play a critical role in HIV pathogenesis, reaching a rate of up to 50% in developed countries and 95% in developing countries (Smith, 1993). The progressive decline in immunological and mucosal defensive mechanisms predisposes HIV-positive individuals to gastro-intestinal infections thus increasing susceptibility to a number of opportunistic intestinal pathogens, among which protozoan and coccidian parasites such as Cryptosporidium sp., Cystoisospora sp. and Cyclospora sp., amongst others, are frequently detected (Tarimo et al., 1996; Mohandas et al., 2002). Intestinal parasitic infections in the HIV-infected population can result in faster disease progression, which can be attributed to their role in immune activation (Feitosa et al., 2001). TCD4+ cell counts are crucial in defining the immune status of HIV-infected individuals. Previous studies have suggested that lower counts of TCD4+ are related to increased incidences of opportunistic parasitic infections in HIV-seropositive patients (Certad et al., 2005). The present study aimed to screen opportunistic intestinal parasites in HIV-infected individuals who were freshly diagnosed and presented with diarrhoea or a previous history of diarrhoea. Stool specimens were initially screened routinely and later were screened after use of a concentration technique, and the results were compared. The absolute TCD4+ counts were calculated and correlated with the opportunistic intestinal parasitic infection.

The study included 452 HIV-infected individuals who were antiretroviral therapy naive and who visited Kamineni Institute of Medical Sciences, Narketpally, Nalgonda, from January 2004 to December 2007. The Ethical Committee of the institution approved the current study and the subjects gave their informed consent to the work. The patients’ HIV status was confirmed by three different types of commercially available ELISA kit, as recommended by the National AIDS Control Organization (NACO, 2006).

Routine stool examination was carried out on all faecal samples by simple wet/saline and iodine mounts. A concentration technique involving formol-ether sedimentation method was simultaneously carried out on all stool samples and the concentrated samples were then screened for the presence of parasites. Smears from all the stool samples after concentration were stained by modified acid-fast stain or Kinyoun’s stain for coccidian parasites (Bray, 1957; Markell et al., 1992). The absolute TCD4+ counts were determined by flow cytometry (Vajpayee et al., 2003).

Among the 452 individuals enrolled in our study, 223 (49.3%) were males and 229 (50.7%) were females. The majority, 333 (73.7%), of the subjects were in the age group of 21–40 years. Eleven per cent of all patients were below the age of 20 years. A history of low socio-economic status and poor personal hygiene was mentioned by 69 and 76.3% patients, respectively. Of the 229 females 17 (7.4%) referred to a previous history of sex outside marriage (multiple partners), whereas 135 (60.5%) of the 223 infected males described the same behaviour.

General examination of the subjects’ stool showed the presence of intestinal parasites in 202 (44.7%) of them. Concentration using the formol-ether sedimentation method increased the positivity rate to 269 (59.5%). Of the 269 samples positive for intestinal parasites 121 (45.0%) showed coccidian parasites and 117 (43.5%) revealed protozoan parasitic infections, with 31 of them (11.5%) involving helminths. Cryptosporidium sp., Cystoisospora sp. and Entamoeba histolytica were found at 17.8, 17.1 and 15.6%, respectively, constituting the majority of infections, followed by Giardia lamblia (12.6%), Entamoeba coli (10.4%) and Cyclospora sp. (10%). Blastocystis hominis (0.4%) was also observed. Among the helminthic parasites, eggs of Ascaris lumbricoides (4.8%) and Rodentolepis nana (5.2%) were observed. Larvae of Strongyloides stercoralis were cited in only one (0.4%) case. Mixed infections with more than one parasite and not more than three were observed in 41 (9%) stool samples. E. histolytica (35), Entamoeba coli (25), G. lamblia (5), B. hominis (11) and Cryptosporidium sp. (12) and R. nana (3) were present in infections involving more than one parasite. The absolute CD4+ cell counts in the case of coccidian infections was 365 cells mm$^{-3}$ ± 86.43, whereas in the case of other protozoan, helminthic and mixed parasitic infections it was $468 \pm 40.50, 429 \pm 190$ and $312 \pm 65.74$ cells mm$^{-3}$, respectively.

Although HIV is the causative agent of AIDS, most of the morbidity and mortality seen in AIDS patients results from opportunistic infections, which take advantage of deficient cell-mediated and humoral defence mechanisms (Smith et al., 1988). A wide variety of infections are encountered in HIV-positive individuals at different stages, including bacterial, fungal, viral, protozoan and helminthic infections (Escobedo & Núñez, 1999). The absolute TCD4+ counts were evaluated in all cases and were compared (Flanigan et al., 1992; Büyükbahar Boral et al., 2004). The study results demonstrated that using a concentration method improves the chances of finding the parasites in stool samples. This signifies the importance of technical skill and expertise in the parasitological laboratory. Our study revealed a comparatively low rate of infection with Cystoisospora sp., contradicting a study performed in South India that showed a higher rate of Cystoisospora sp. infections (Mukhopadhyay, 2007).
The TCD4+ immune mechanism in HIV-infected parasites make use of the diminished unsolved as most of the non-pathogenic though their role in pathogenesis remains studies suggest (Vignesh et al., 2007). The E. histolytica, G. lamblia and Cryptosporidium sp. infection rate was considerably higher in our study compared with a study done in North India (Mohandas et al., 2002). The incidence of helminthic infections by hook worm (1.4%) and Strongyloides (0.3%) was surprisingly third in the list of other protozoan infections, behind E. histolytica and Giardia. B. hominis was observed in 4.0% of cases (Brites et al., 2005). Helminthic infections were seen in 11.5% of cases in our study compared to 4.4% in a Korean study (Guk et al., 2005). We report both Entamoeba coli and B. hominis, though their role in pathogenesis remains unsolved as most of the non-pathogenic parasites make use of the diminished immune mechanism in HIV-infected patients, and may well contribute to the disease progression.

The TCD4+ counts, when correlated with the parasite, revealed that coccidian parasites (365 ± 86.43 cells mm^-3) and mixed parasitic infections (312 ± 65.74 cells mm^-3) had lower CD4+ counts than those observed in case of other protozoan (468 ± 40.50 cells mm^-3) and helminthic (429 ± 190 cells mm^-3) infections (Certad et al., 2005). This indicates that as the disease progresses and the TCD4+ counts decline the HIV-seropositive individuals are prone to intestinal parasitic infections of which protozoan, and in particular coccidian parasites, play a major role. In contrast to previous studies where coccidian parasitic infections were associated with TCD4+ counts less than 100 cells mm^-3, our study revealed higher TCD4+ counts (Certad et al., 2003). The higher counts in our study can be attributed to the early infection. The high incidence of opportunistic intestinal parasitic infection in our study may be due to low socioeconomic status, poor hygiene, unavailability of safe drinking water and frequent contact with livestock. Emphasis on specific measures to prevent opportunistic infections, and initiating appropriate antiparasitic therapy, are important because of the limitations of highly active antiretroviral therapy (HAART).

Since the treatment of an opportunistic infection can only get control of the present illness but not prevent reinfec­tion, physicians caring for HIV-infected patients can substantially reduce the morbidity and mortality caused by opportunistic infections by early diagnosis and prompt elimination of the parasite. In conclusion, our findings highlight and reassert the importance of screening the stool samples of HIV-seropositive individuals and evaluating the absolute TCD4+ counts regularly. Our study also suggests that the intestinal parasitic infections vary with the geographical areas and studies must be carried out in respective regions to evaluate the frequent causes of intestinal parasitic infections in HIV-seropositive individuals. Early diagnosis of opportunistic infections and prompt treatment definitely contribute to an increased life expectancy of infected individuals by delaying the progression to AIDS.

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