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Original Article

Opportunistic intestinal parasites and CD4 count in HIV infected people

Amatya R[1], Shrestha R[2], Poudyal N[1], Bhandari S[2]

[1]*Department of Microbiology, BP Koirala institute of Health sciences*
[2]*Department of Internal medicine, BP Koirala institute of Health sciences*

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ABSTRACT

Background: Opportunistic intestinal infections cause a significant morbidity and mortality among the HIV infected people. The present study was undertaken to find the prevalence of intestinal opportunistic parasitic infections among the HIV infected populace in eastern Nepal and to correlate the occurrence with the CD4 T cell counts.

Materials and Methods: Stool from 122 HIV infected people were examined microscopically for the presence of parasitic ova/cyst. CD4 T cell enumeration was done using FACS Count (Becton Dickinson). Stool from 100 age matched HIV negative controls were also examined.

Results: A male preponderance in the parasite positivity was seen. Twenty five of symptomatic and 2.8% of asymptomatic harboured one or more intestinal parasites.12.3% of the study population had intestinal parasitoses with 7.3% being infected with opportunistic parasites. The mean CD4 count of the subjects was 307 while those with parasitoses were 204. A statistically significant difference was seen between the CD4 counts of symptomatic and asymptomatic patients.

Conclusion: Coccidian parasites are frequent opportunistic intestinal parasites infecting HIV infected patients. A lowered CD4 count predisposes to acquisition of these agents. Regular monitoring of CD4 counts and screening for these opportunistic agents in the HIV infected will help reduce the mortality and morbidity associated with infections by these agents.

INTRODUCTION

Since 1998, when the first case of HIV was detected in Nepal, there has been a steady rise in the HIV infected population in Nepal. Currently, Nepal is in the concentrated epidemic phase with 12,933 HIV positive and 2,151 people living with AIDS by the end of 2009.[1] For a poverty stricken country like Nepal, the scourge of HIV/AIDS is most unfortunate. It is the concomitantly occurring opportunistic infections in the HIV infected that take a major toll in terms of economic loss, morbidity and mortality.

Diarrhea is one of the AIDS defining conditions and is an important clinical problem associated with significant impairments in health related quality of life.[2] Besides the usual intestinal pathogens causing diarrhea, opportunistic agents like coccidian parasites and Blastocystis hominis are frequently encountered in people living with AIDS.[3-5]

Correspondence:
Dr. Ritu Amatya, MD
Department of Microbiology, KIST Medical College,Lalitpur, Nepal
Email:ritu484@yahoo.co.in

Table 1: Parasitic ova/cyst observed in microscopic examination of stool from HIV infected patients

Parasite	No. of positive samples
Giardia lamblia	7
Cryptosporidium spp	5
Cyclospora spp	4
Blastocystis hominis	2
Hookworm	2
Isospora	1
Hookworm	1

Table 2: Coccidian infection and mixed parasitic infection.

Coccidian parasite detected (no. of patients)	No of patients with mixed infection	Coinfected with (n)
Cryptosporidium (5)	5	Giardia lamblia (3) Hookworm (1) B hominis + cyclospora (1)
Cyclospora (4)	1	Cryptosporidium + B hominis (1)
Isospora (1)	1	Giardia lamblia (1)

There are at present only two reports from central and one from western Nepal regarding frequency of various pathogens causing diarrhea in HIV/AIDS patients in Nepal.[6-8] However, there is none yet from the Eastern region of Nepal. Moreover, the published reports are lacking on data on correlations of CD4 levels on gastrointestinal opportunistic agents.

With this background, this study was conducted to find the spectrum of gastrointestinal parasites in HIV infected at various CD4 levels so as to guide the treatment and prophylaxis of diarrhea in HIV/AIDS patients in Nepal. An attempt was also made to elucidate the association between CD4 and diarrhea; CD4 and parasitic infection and significant correlation if any between parasitic infection and source of drinking water.

MATERIALS AND METHODS

This study was a hospital based cross-sectional study conducted from January 2007 to December 2008 in the Department of Microbiology, BP Koirala institute of health science (BPKIHS), Nepal.

Study cases

Patients who were confirmed as HIV positive cases and whose CD4 count was being evaluated were taken as study subjects. Irrespective of their signs and symptoms of gastrointestinal tract infection, each participant was provided with three standard stool collection containers labeled with the participant's code. Instructions were given for the collection of stool sample.

Short questionnaire was maintained which included

a. sociodemographic data: age, sex
b. behavioral data: types of drinking water
c. participant's present medical history: any complaints of diarrhea, whether or not on antiretroviral therapy and whether or not on Trimethoprim-Sulphamethoxazole (TMP-SMX) prophylaxis. Stool from hundred age matched adults who were HIV negative were taken as controls.

For CD4 cell count, 5ml blood in K3EDTA was taken between 10am and 11am from all participants and was analyzed by FACS Count (Becton Dickinson).

Parasitological examination was done in collected stools samples immediately for the presence of parasites. Microscopic examination was carried out by direct wet mount using normal saline (0.9%), Lugol's iodine (0.5%) and 10% formol ether concentrated mount. The parasite count was recorded as per high power field for protozoal cysts and as number of ova per coverslip for helminths. Duplicate smears were made from each specimen and stained with modified Zeihl-Nelsen staining technique. All the stained smears were screened by two independent observers for oocyts of cryptosporidium, isospora and cyclospora. Modified acid fast stain positive oval to round structures with size varying from 2-6µm, with or without the presence of retracted cytoplasm were identified as cryptosporidium oocyst. Cyclospora oocysts were identified as acid-fast round structures with crumpled celophane appearance and approximate size of 8-10µm. Similarly, pink oval structures 20-30µm by 10-19µm were identified as isospora oocysts.

RESULTS

A total of 122 HIV positive individuals (369 stool sample) were screened for intestinal parasites during this study. CD4 enumerations for each were also performed. There was a male preponderance in the number of participants (86 male and 36 female). Similar majority of males was seen amongst the intestinal parasite positive cases. The mean age of the patients with intestinal parasitosis was 33.6 years as compared to the mean age of the participants (30.7 years). The age range of participants was 4 years to 50 years.

Twenty five percent (13/52) of the symptomatic patients and 2.8% of asymptomatic patients (2/70) had intestinal parasitosis. Overall, 12.3% of the participants were excreting parasitic ova/cyst in their stool (Table 1) Opportunistic agents were found in 9 (7.3%) individuals.

Five had mixed infections as shown in Table 2. Statistically significant association between the presence of coccidian parasites and diarrhea however could not be established. The two asymptomatic patients had Blastocystis hominis and hookworm infestation each.

Ten participants although on TMP-SMX prophylaxis, were positive for intestinal parasites. Similarly, both the anti retroviral therapy (ART) naive and those on ART were found infected. The mean CD4 T cell count for parasite positive cases was lower than the mean for the entire participant group (204 versus 307). A statistically significant difference (p value < 0.001) in the mean T cell count was seen between the symptomatic diarrhoeal HIV patients (mean: 164.3) and the asymptomatic HIV patients (mean: 414).

Although a statistically significant association between the source of drinking water and parasite positivity was not seen, 20% of those taking direct tap water for drinking purposes and 12.5% of those using bore well water had intestinal parasitosis. Majority of the participants (61%) relied on municipality supplied tap water.

Out of the 100 stools from the HIV negative controls, none had coccidia infection.

DISCUSSION

In the HIV/AIDS era, the infections by opportunistic agents are on the rise. Opportunistic infections of the gastrointestinal tract are one of the major causes of morbidity and mortality in HIV positive individuals worldwide.[9] Amongst these, the coccidian parasites are often implicated for protracted diarrhea which may even prove fatal.[10]

This study tried to determine the prevalence and profile of intestinal parasites among the HIV positive patients. The overall parasite prevalence among the study subjects was 13.2% with the coccidian parasites comprising 52.8%. Similar observation was made a study done amongst North Indian HIV positives.[11] However, the overall prevalence of parasites and percentage of coccidia in their study exceeded ours (30% and 76.3%) respectively. The parasite prevalence in symptomatic diarrhoea cases were more than that in the asymptomatic cases [25% vs. 2.8%]. Similar observation was seen in a study conducted in Chennai.[12]

Our study showed cryptosporidium as the most frequent coccidian parasite. Similar preponderance was seen in other studies from central and western Nepal and other parts of the world.[6,8,13,14] The lifetime risk of infection by cryptosporidium in HIV patient is 10%.[15] Cyclospora cayetenensis was another frequently occurring coccidian. It is endemic in Nepal and has been described as an important among HIV/AIDS patients in previous reports. Other studies however have found isospora and microsporidium as the commonest coccidia amongst the HIV/AIDS patients.[16-18]

In agreement with previous studies those parasites associated with HIV were encountered more often as the CD4+ T cell count fell below 200/µl.[17,18] Immunodeficient state makes them more susceptible to these infections and once established they are not able to prevent the proliferation or clear the infecting agent.[19] HAART is the best way to improve immune status in HIV patients and thus avoid potentially fatal opportunistic infections. Multiple infections were more common in HIV infected than in the HIV negative patients. This finding corresponds well with other studies and is a common observation in areas where various types of parasites are prevalent.[20]

Since TMP-SMX does not cover cryptosporidium, these coccidian were recovered from 2 patients out of the 59 receiving prophylactic TMP-SMX. Three on prophylaxis had cyclospora and one had isopora infection. The recovery of these parasites despite prophylactic treatment with TMP-SMX [one double strength 160mg/800mg daily] for PCP could be because of the inadequate dosage or poor patient compliance favoring drug resistance. Further studies may be required to look into this. Some investigators have proposed higher doses for treatment and prophylaxis of isospora.[21,22]

The use of direct tap water in this locale has been shown to have an association with acquisition of intestinal parasites.[23] Boiled or appropriately treated water should be used for drinking purposes especially by those who are immunocompromised.

The absence of cryptosporidium among the adult controls is conspicuous as another study done in our hospital had shown a high prevalence of cryptosporidium among the pediatric diarrhea cases.[24] This can be explained by the development of immune tolerance to the parasite among the immunocompetent adults in the endemic areas due to frequent exposure.[25]

There were some limitations to our study. Diarrhoeal etiologies were not known in 75% of the symptomatic cases. Search for other etiologic causes like bacteria, virus, and fungi would have made it more comprehensive. Calcofluor white staining technique for screening for microsporidium was not available. Since this was a hospital based study, the inference drawn from this may not always be representative of the general people living with HIV/AIDS (PLWHA) in the community.

CONCLUSION

The coccidian parasites are significantly more frequently seen in the stool of both symptomatic and asymptomatic HIV patients than the HIV negative patients. Clinical microbiology laboratories receiving stool samples from HIV patients should make it a routine practice to screen for coccidian parasites as well. Timely detection and treatment would avoid the serious consequences of infection and also

prevent transmission.

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