Enteric Parasitic Infection Among Antiretroviral Therapy Naïve HIV-Seropositive People: Infection Begets Infection-Experience from Eastern India

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ABSTRACT

Context: Parasitic opportunistic infections (POIs) frequently occur in HIV/AIDS patients and affect the quality of life. Aims: This study assessing the standard organisms in the stool of HIV-positive patients, their comparison with HIV-negative controls, their relation with various factors, is the first of its kind in the eastern part of India. Settings and Design: hospital-based case–control study. Materials and Methods: A total of 194 antiretroviral therapy naïve HIV-positive patients (18-60 years) were taken as cases and 98 age- and sex-matched HIV-negative family members as controls. Demographical, clinical, biochemical, and microbiological parameters were studied. Statistical Analysis Used: Odds ratio, 95% confidence interval, and P (< 0.05 is to be significant) were calculated using Epi Info 7 software. Results: POI was significantly higher among HIV-seropositive cases (61.86%) (P < 0.001). Cryptosporidium was the most common POI in HIV-seropositive patients overall and without diarrhea; Entameba was the most common POI in patients with acute diarrhea, and Isospora was the most common POI in the patients having chronic diarrhea. Entameba was the most common POI in CD4 count <350 cells/μl while for CD4 count >350 cells/μl Cryptosporidium was the most common POI. Mean CD4 count was significantly (P < 0.001) lower among people having multiple infections. Male sex, hemoglobin <10 g/dl, WHO Clinical Stage 3 or 4, tuberculosis, absolute eosinophil count of more than 540/dl, CD4 count <350 cells/μl, and seroconcordance of spouses were significantly associated with HIV-seropositive cases having POI (P < 0.05). Conclusions: Physicians should advise HIV-infected patients to undergo routine evaluation for POI, and provision of chemoprophylaxis should be made in appropriate settings.

Key words: Case–control, HIV, parasitic opportunistic infections

INTRODUCTION

Gastrointestinal infections are among the most common and debilitating infections associated with HIV, affecting almost 50% of AIDS patients in developed countries and 95% in developing countries.[1-3] In developed countries where most HIV infections occur among heterosexual persons, whereas in developing countries such as India, water- and food-borne transmissions are the principal modes of transmission of enteric organisms.[4-11] The severity and duration of symptoms associated with enteric pathogens are determined by level of immunosuppression, distribution of enteric parasitic pathogens, and clinical profiles of the HIV patients.[4-6] These parasitic opportunistic infections (POIs) are not only associated with symptomatic HIV-infected patients but also are more evident with decreasing immune state.

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(CD4+ cell count <200 cells/μl).[28,11] Antiretroviral therapy (ART) increases the immunity of HIV-positive persons and decreases the incidence of opportunistic infections.[6,7] Utilization of chemoprophylaxis can also act on intestinal parasites, reducing their prevalence.[8] The objective of this study is to explore epidemiology and clinical characteristics of HIV patients with POI.

**MATERIALS AND METHODS**

In this tertiary care hospital-based case-control study performed in Kolkata, West Bengal, India, following ethical clearance, sample of 194 ART naïve HIV-positive patients (both from outdoor clinic and wards) aged between 18 and 60 years were taken up as cases and 98 age- and sex-matched HIV-negative family members, friends, or close acquaintances were chosen as controls who shared the same socioeconomic-geographical milieu and the same access to health. HIV-positive patients on ART, patients on any form of antibiotics or antihelminthics during the last 3 months, and severely ill patients were excluded from the study.

Following informed consent, their demographical, clinical, and routine pathological and biochemical profiles were studied over 18 months. Single stool specimen was examined as a wet saline mount and iodine preparation for the detection of ova, larva, and cysts. The samples were then subjected to modified Ziehl–Neelsen staining for the isolation and identification of intestinal coccidian parasites. Modified trichrome staining was done for the identification of *Microsporidia*.

HIV infection was diagnosed by rapid or laboratory-based enzyme immunoassay: Pareekshak triline. This was confirmed by the second HIV-antibody test (rapid or laboratory-based enzyme immunoassay: Pareekshak trispol) relying on different antigens or of different operating characteristics. CD4 count testing in HIV-positive patients was done using Multiset V2.2 FACScalibur (#E97300192) system (Manufacturer BD Biosciences) and expressed in counts/dl.

Data were keyed into Microsoft Excel and had been double-checked to avoid any error in data entry. The qualitative data were described in proportions and the quantitative data were described in mean and standard deviation (SD). Odds ratio (OR), 95% confidence interval (CI), and P value were calculated using Epi Info 7 software (Manufacturer Centers for Disease Control and Prevention (CDC)). For establishing P value, Chi-square tests were applied for significance and P < 0.05 has been considered to be statistically significant and confidence interval is taken as 95% (95% CI).

**RESULTS**

Mean age (± SD) of the HIV-positive cases was 34.36 (±9.68) years. Control data were represented and compared with cases. Low education (75.51%), unemployment (58.76%), lower level of body mass index (BMI) (mean ± SD = 18.36 ± 4.23), concurrent or past history of tuberculosis (TB) (53.61%), lower level of hemoglobin (mean ± SD = 11.79 ± 1.46), *Cryptosporidium* sp. (29.38%), and *Isospora* sp. (14.43%) in stool were found to be significantly (*P < 0.01) associated with HIV-positive cases [Table 1]. On the contrary, dysentery (8.25%) and low absolute eosinophil count (<540 cells/dl) (44.33%) were significantly lower (*P < 0.05) among HIV-positive cases than the HIV-seronegative controls in our study [Table 1]. No significant difference in residence, and thus health access between cases and controls, were observed in our study [Table 1]. POI was significantly higher among HIV-seropositive cases (61.86%) than the seronegative controls (36%) (OR 0.35, 95% CI 0.21-0.59, *P < 0.001*).

*Cryptosporidium* sp. (31) was the most common POI in HIV-seropositive patients without diarrhea; *Entameba* sp. was the most common POI in patients with acute diarrhea; and *Isospora* sp. was the most common POI in the

| Points of comparison | HIV seropositive patients (%) | HIV seronegative controls (%) | COR‡ (95% CI) | P |
|----------------------|------------------------------|-----------------------------|--------------|---|
| Demography           |                              |                             |              |   |
| Low education        | 98 (50.53)                   | 74 (75.53)                  | 3.56 (2.11-6.01) | <0.001 |
| Unemployment         | 114 (58.76)                  | 28 (28.57)                  | 1.38 (0.79-2.42) | 0.27  |
| Rural residence      | 134 (69.07)                  | 74 (75.53)                  | 1.48 (0.83-2.62) | 0.18  |
| Clinical features    |                              |                             |              |   |
| Nausea + vomiting    | 54 (27.84)                   | 34 (34.69)                  | 1.37 (0.81-2.21) | 0.27  |
| Dysentery            | 16 (8.25)                    | 16 (16.33)                  | 2.17 (1.03-4.55) | <0.05 |
| Diarrhea             | 74 (38.14)                   | 28 (28.57)                  | 0.64 (0.38-1.09) | 0.11  |
| Risk factors         |                              |                             |              |   |
| Concurrent tuberculosis* | 104 (53.61)               | 8 (8.16)                    | 0.07 (0.03-0.16) | <0.001 |
| AEC<550 cells/dl POI | 86 (43.31)                   | 68 (69.39)                  | 2.84 (1.70-4.76) | <0.001 |
| Number of organisms  |                              |                             |              |   |
| *Cryptosporidium* sp. | 57 (29.38)                  | 2 (2.04)                    | 0.05 (0.01-0.21) | <0.001 |
| *Cyclospora* sp.     | 2 (1.03)                     | 0                           | 0             | 0.55  |
| *Entameba* sp.       | 38 (19.59)                   | 26 (26.53)                  | 1.48 (0.83-2.62) | 0.18  |
| *Giardia* sp.        | 20 (10.31)                   | 10 (10.20)                  | 0.9 (0.44-2.20) | 1     |
| *Isospora* sp.       | 28 (14.43)                   | 4 (4.08)                    | 0.25 (0.08-0.74) | <0.01 |
| *Microsporidia* sp.  | 4 (2.06)                     | 0                           | 0             | 0.30  |

*‡*Tuberculosis (includes pulmonary and extrapulmonary, past and concurrent) at the time of diagnosis of parasitic opportunistic infections, †AEC: Absolute eosinophil count, ‡COR: Crude odd ratio, §CI: Confidence interval, ||POI: Parasitic opportunistic infections (significant parameters have been made bold).
patients having chronic diarrhea [Table 2]. *Cryptosporidium* sp. (57) was the most common POI in HIV-seropositive patients and the other common POIs are found to be *Cyclospora* sp. (2), *Entamoeba* sp. (38), *Giardia* sp. (20), *Isospora* sp. (28), and *Microsporidia* sp. (4) *Entamoeba* sp. was the most common POI in CD4 count <350/μl while above CD4 count of 350/μl *Cryptosporidium* sp. was the most common POI [Table 3]. Almost 76.67% of cases having POI had single infection while 23.33% had multiple infections. In our study, mean CD4 count (± SD) was significantly (P < 0.001) lower among people having multiple infections (274.28 ± 124.44) compared to those with single infection (404.30 ± 170.36) and no infection (580 ± 227.88). Unemployment, male sex, rural residence, hemoglobin <10 g/dl, WHO Clinical Stage 3 or 4, TB, absolute eosinophil count of more than 540/dl, CD4 cell count <350 cells/μl, and seroconcordance of spouses were significantly associated with HIV-seropositive cases having POI [Table 4].

**DISCUSSION**

In our single-centered cross-sectional study from Eastern India, the high prevalence of POI among (61.86%) HIV-seropositive cases was corroborative with other parts of India such as 62.7% in the study by Dwivedi *et al.* from Delhi, 44.7% in the study by Ramana and Mohanty from Nalgonda, 25% in the study by Gupta from Gujarat, 35% in the study by Kulkarni *et al.* from Pune; and also with the results from other developing countries such as 33.7% in Ethiopia, 30.13% in Nepal, and 12.38% in Brazil[2,3,6,8,9,11,12]. There has been no such study till date from the eastern part of India and most of the studies have not included HIV-seronegative controls. In our study, taking HIV-seronegative relatives of HIV-seropositive patients as controls is a novel concept as they share the same food, roof, and sanitations. POI was present in 36% of control groups which corroborates with the prevalence of POI among the general population (24.78%).[3]

Among HIV-seropositive cases, *Cryptosporidium* sp. was found to be the most common POI corroborating with literature.[2,14-16] *Strongyloides* sp., *Ascaris* sp., *Ankylostoma* sp., *Trichuris* sp., and *Hymenolepis* sp. were not found in our study unlike the study from Brazil by Tatiana Paschoalette Rodrigues Bachur *et al.*[6] *Cyclospora* sp. was only found in people with CD4 count <350/μl. In our study, *Cryptosporidium* sp. was the most common POI in HIV-seropositive patients without diarrhea; *Entamoeba* sp. was the most common POI in patients with acute diarrhea, and *Isospora* sp. was the most common POI in the patients having chronic diarrhea. This finding is similar to the study by Gupta *et al.*, except in their study *Cryptosporidium* sp. and *Isospora* sp. were more common in patients with acute diarrhea.[9]

### Table 2: Distribution of parasitic opportunistic infection among HIV-seropositive cases with respect to nature of diarrhea

| POI                  | No diarrhea (%) | Acute diarrhea (%) | Chronic diarrhea (%) | Total (%) |
|----------------------|-----------------|--------------------|----------------------|-----------|
| *Cryptosporidium* sp. | 32 (25.83)      | 12 (26.19)         | 15 (46.88)           | 57 (29.38) |
| Cyclospora sp.        | 0               | 2 (4.76)           | 0                    | 2 (1.03)  |
| Entamoeba sp.         | 12 (10.00)      | 22 (52.38)         | 4 (12.50)            | 38 (19.59) |
| Giardia sp.           | 14 (11.67)      | 6 (14.29)          | 0                    | 20 (10.33) |
| Isospora sp.          | 4 (3.33)        | 4 (9.52)           | 20 (62.50)           | 28 (14.43) |
| Microsporidia sp.     | 0               | 2 (4.76)           | 2 (6.25)             | 4 (2.06)  |

POI: Parasitic opportunistic infection

### Table 3: Distribution of parasitic opportunistic infections among HIV-seropositive cases with respect to CD4 counts

| POI                  | CD4 <350 cells/μl | CD4 350-500 cells/μl | CD4 >500 cells/μl |
|----------------------|-------------------|----------------------|-------------------|
| *Cryptosporidium* sp. | 17 (28.33)       | 19 (28.79)           | 21 (30.88)        |
| Cyclospora sp.        | 2 (3.33)          | 0                    |                   |
| Entamoeba sp.         | 18 (30.00)        | 14 (21.22)           | 6 (8.82)          |
| Giardia sp.           | 14 (23.33)        | 6 (9.09)             |                   |
| Isospora sp.          | 10 (16.67)        | 10 (15.15)           | 8 (12.76)         |
| Microsporidia sp.     | 4 (6.67)          | 0                    |                   |

POI: Parasitic opportunistic infection

### Table 4: Demographical, clinical, and biochemical comparison among HIV-seropositive patients with parasitic opportunistic infections and HIV-seropositive patients without parasitic opportunistic infections

| Parameters                  | POI present (n = 120) (%) | POI absent (n = 74) (%) | COR \(95\% CI)\) | \(P\) |
|-----------------------------|---------------------------|-------------------------|-------------------|------|
| Age >40 years               | 34 (28.33)                | 14 (18.92)              | 0.59 (0.29-1.19)  | 0.37 |
| Sex                         |                           |                         |                   |      |
| Male                        | 78 (65.00)                | 30 (40.54)              | 2.72 (1.49-4.94)  | 0.001|
| Rural residence             | 88 (73.33)                | 46 (62.16)              | 1.67 (0.90-3.11)  | 0.11 |
| Seroconcordance of spouse   | 66 (55.00)                | 54 (72.97)              | 0.45 (0.24-0.84)  | 0.01 |
| BMI* <18 kg/m²              | 68 (56.67)                | 32 (43.74)              | 1.71 (0.95-3.07)  | 0.07 |
| Unemployment                | 70 (58.33)                | 43 (59.46)              | 1.04 (0.58-1.88)  | 1.00 |
| WHO† Stage 3, 4             | 86 (71.67)                | 20 (27.03)              | 0.14 (0.07-0.28)  | <0.002|
| Good functional capacity    | 102 (85.00)               | 72 (97.30)              | 0.15 (0.03-0.69)  | <0.006|
| Concurrent tuberculosis§    | 86 (71.67)                | 10 (14.29)              | 7.86 (4.05-15.27) | <0.001|
| Hemoglobin <10 g/dl         | 20 (16.67)                | 2 (2.70)                | 7.20 (1.63-31.78) | <0.002|
| AEC <540 cells/dl           | 20 (16.67)                | 66 (89.19)              | 0.02 (0.01-0.05)  | <0.002|
| CD4 <350 cells/μl           | 52 (43.33)                | 8 (10.81)               | 6.30 (2.78-14.29) | <0.002|

*BM*: Body mass index, *WHO*: World Health Organization, *Tuberculosis* (includes pulmonary and extrapulmonary; past and concurrent) at the time of diagnosis of parasitic opportunistic infections, *AEC*: Absolute eosinophil count, *COR*: Crude odd ratio, *CI*: Confidence interval, POI: Parasitic opportunistic infection, (significant parameters have been made bold)
POI was more in patients with low CD4 count (<350/μl) which corroborates with other studies except the study by Amâncio where no association was found between CD4 count and POI.\cite{2,3,6-8,10,11} In our study, Entameba was the most common POI in CD4 count <350/μl while in CD4 count of 350/dl-500/μl and >500/μl Cryptosporidium was the most common POI, in contrast to the study by Kulkarni et al., where Cryptosporidium was the most common pathogen in CD4 count <200/μl, Entameba was the most common parasite in 200-499/μl, and Hymenolepis was most common POI in CD4 count >500 cells/μl.\cite{6} In our study, multiple POIs among HIV-seropositive cases were associated with significantly lower CD4 count similar to the study by Dwivedi et al.\cite{12} This can be explained by progressive immunosuppression with decreasing CD4 count.

Lower level of BMI (mean 18.36 ± SD 4.23) was found to be significantly associated with HIV infection in our study. In the study by Swaminathan et al., BMI was found to be <18.5 among HIV-seropositive patients.\cite{17} Prevalence of TB was found to be significantly higher (54%) among HIV-seropositive cases in our study. A study carried out by Padyana et al. at a tertiary care hospital in South India, among 200 HIV-positive patients, showed that 54 (27%) patients had HIV/TB co-infection.\cite{18} The lower level of hemoglobin (mean 11.79 ± SD 1.46) was observed among HIV seropositive cases. A study from Nepal revealed a 55.8% prevalence of anemia in people living with HIV/AIDS.\cite{19} In our study, all the HIV patients were heterosexual which is unlike the study by Bachur et al. from Brazil.\cite{6}

POI was higher in unemployed people and in those coming from rural areas probably due to low socioeconomic status, poor hygiene, unavailability of safe drinking water, and frequent contact with livestock as was also found in the study by Ramana and Mohanty.\cite{8} In our study, cases having POI belonged to worse WHO clinical stages. This aspect has never been studied before. We attribute this to poorer immune and hygiene status and increased morbidity in patients with higher stages. In spite of these, patients were working or ambulatory probably due to the low economic background. Seroconcordance of spouses was significantly associated with HIV-seropositive cases having POI. This has not been studied before and is probably because they were immunocompromised and shared the same household and sanitation.

TB came out to be significantly associated with POI in HIV-seropositive patients. This is a novel finding from our study. This may be attributable to significant association of tumor necrosis factor-α and interleukin-5 and other cytokines both with TB and POI and also poor socioeconomic and sanitary status.\cite{10,20,21} In our study, among the HIV-seropositive patients, lower level of hemoglobin level (<10 g/dl) was found to be significantly associated with POI probably due to gastrointestinal blood loss. Higher absolute eosinophil count (>540/dl) was a significant association with POI unlike the study by Amâncio et al.\cite{10}

From this hospital-based cross-sectional study, we could conclude that proportion of POI cases were high among the HIV-infected population. HIV seropositivity, especially with low CD4 count, co-infection with TB, and worse WHO clinical staging should prompt the use of chemoprophylaxis. Although administration of routine chemophylaxis has been a normal practice in this group, it does not appear to make sense for parasitic infections in particular. First, mass administration of antibiotics empirically could trigger the development of resistant strains. Second, chemophylaxis might be under dosage to efficiently kill the parasites. Third, routine administration of antibiotics to reduce the prevalence of intestinal infections could shift the clinician’s attention from parasitological diagnosis to empirical treatment. Physicians should order stool examination with special staining for early detection of POI inappropriate setting. Prevention, by improvement of the basic sanitary and health systems, and proper management in the form of frequent fecal examinations, especially in high-risk individuals (i.e., CD4 counts <200 cells/μl), and early treatment can halt the progression of disease and decrease morbidity of HIV/AIDS patients and thus improving the quality of life. Larger population-based and multicentric cohort study should be conducted to look into the role of chemophylaxis and ART in the management of POI among HIV/AIDS patients.

Our study concerning POI in HIV is the first of its kind from the eastern part of India. Furthermore, case-control studies on HIV and POI are lacking in our country and taking controls from the same socioeconomic-demographic background is a novel attempt. To the best of our knowledge, no other contemporary studies have shown association of POI with TB, seroconcordance of spouse, WHO clinical staging. On the other hand, our study was a single center-based cross-sectional study and assessment of viral load and polymerase chain reaction-based detections of POI were not done.

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Conflicts of interest

There are no conflicts of interest.

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