

## INCIDENCE OF VARIOUS INTESTINAL PARASITES IN STOOL SAMPLES OF HIV INFECTED/AIDS PATIENTS AND THEIR RELATIONSHIP TO BLOOD CD4 COUNTS

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### Abstract

**Background:** Intestinal parasites are endemic in many regions of the world where Human Immunodeficiency Virus/Acquired Immunodeficiency syndrome (HIV/AIDS) is also prevalent. The present study is, therefore, aimed to determine the prevalence and pattern of intestinal parasites in people with HIV infection and also their association with diarrhoea and immune status. **Materials and Methods:** This cross-sectional study was conducted at Department of Microbiology, JLNMC, Bhagalpur, Bihar from August, 2023 to January 2024. People who test HIV-positive at the hospital and referred from other health institutions are attached to the antiretroviral therapy (ART) clinic for clinical and laboratory investigations to monitor their disease status. Immunological assessment using CD4+ T-cell count at enrolment and every three months follow-up visits helps to identify those who are eligible for ART. On the same day of interview and CD4+ T-cell measurement, a single stool sample was collected from each participant and examined for intestinal parasites. Stool specimen were processed using direct technique (saline and iodine mounts) to identify trophozoite and cyst of protozoan parasites and using formal-ether concentration technique to detect eggs and larva of helminths. Modified acid fast stain was used to detect oocysts of *Cryptosporidium* species and *Isospora belli*. **Results:** A total of 200 individuals were screened for intestinal parasites during the study period. Majority of the participants were urban dwellers (87%) and females (54%). Twenty-two subjects (11%) were less than 20-year old, 288 (72%) were in the age range 20 - 40-years and 55 (10%) were above 40-year old. Of the total study subjects, participants with HIV infection were 57%. The mean age of HIV infected participants was 30.2 years (range 17-67 years; SD 8.8). The male to female ratio was 1:2 in HIV positive individuals. **Conclusions:** raising immune status of HIV infected patients with anti-retroviral therapy may help to reduce acquisition and/or proliferation of HIV associated parasitic infections and the likelihood of experiencing diarrhoea.

## INTRODUCTION

Intestinal parasites are endemic in many regions of the world where Human Immunodeficiency Virus/Acquired Immunodeficiency syndrome (HIV/AIDS) is also prevalent. Sub-Saharan Africa is among the regions where intestinal parasitic infections are entrenched,<sup>[1]</sup> and the largest burden of AIDS cases exist.<sup>[2]</sup> The same factors including poverty and malnutrition could promote transmission of both infections in the region, and attempt to improve the underlying conditions may revert the situations.

Studies investigated the existence of interaction between HIV and parasitic infections in co-infected individuals. Parasitic infections particularly helminths cause chronic immune activation,<sup>[3,4]</sup> in addition to skewing the immune response toward T helper-2 immune responses.<sup>[5]</sup> Though proving evidences are insufficient, such immune modulation was shown to increase host susceptibility; thereby, promoting HIV infection and disease progression.<sup>[6-8]</sup> Thus, chronic immune activation was suggested as one factor that adversely influences epidemics of HIV/AIDS in Africa.<sup>[9]</sup>

On the other hand, HIV infection has increased significance of parasitic infection. More importantly, with emergence of AIDS, the epidemiology as well as outcome of diseases caused by opportunistic parasites was significantly modified.<sup>[10,11]</sup> But, the effect of HIV on some other parasites is not clearly understood. Overall, either backed by HIV or independently, intestinal parasitic infections have continued to be major cause of morbidity and mortality in humans.<sup>[12]</sup>

Ethiopia is among the sub-Saharan countries with overlapping high rate of HIV and parasitic infections. However, there have been few studies to ascertain whether epidemiology of intestinal parasites take different picture as population with HIV/AIDS is growing. The present study is, therefore, aimed to determine the prevalence and pattern of intestinal parasites in people with HIV infection and also their association with diarrhoea and immune status.

## MATERIALS AND METHODS

This cross-sectional study was conducted at Department of Microbiology, JLNMC, Bhagalpur, Bihar from August, 2023 to January 2024. People who test HIV-positive at the hospital and referred from other health institutions are attached to the antiretroviral therapy (ART) clinic for clinical and laboratory investigations to monitor their disease status. Immunological assessment using CD4+ T-cell count at enrolment and every three months follow-up visits helps to identify those who are eligible for ART.

The study population consisted of HIV-positive individuals, who gave blood for CD4 T-cell count at their first enrolment to monitor disease status at the ART clinic during the study period and clients tested HIV-negative at the hospital in the same period. Patients receiving anti-parasitic treatment were excluded from the study. In total, 200 consecutive Patients were included in the present study.

All study subjects were interviewed on sociodemographic factors and asked about current symptom and duration of diarrhoea. On the same day of interview and CD4+ T-cell measurement, a single stool sample was collected from each participant and examined for intestinal parasites. Stool specimen were processed using direct technique (saline and iodine mounts) to identify trophozoite and cyst of protozoan parasites and using formal-ether concentration technique to detect eggs and larva of helminths. Modified acid fast stain was used to detect oocysts of *Cryptosporidium* species and *Isospora belli*.<sup>[13]</sup>

Data entry and analysis was performed using SPSS Version-15 software. Different characteristics of study participants were described using mean, range and percentage as appropriate. Statistical

significance of differences in proportions was evaluated by Pearson's Chi-square test. Fisher Exact test was used in place of Pearson's Chi-square when more than 20% of cells in contingency tables have expected count less than 5. Odds ratio was used to measure the strength of association between outcome and its correlates. A given statistical test was reported significant whenever it resulted in a  $p$ -value  $< 0.05$ . The study was approved by the Ethics Committees.

## RESULTS

A total of 200 individuals were screened for intestinal parasites during the study period. Majority of the participants were urban dwellers (87%) and females (54%). Twenty two subjects (11%) were less than 20-year old, 288 (72%) were in the age range 20 - 40-years and 55 (10%) were above 40-year old.

Of the total study subjects, participants with HIV infection were 57%. The mean age of HIV infected participants was 30.2 years (range 17-67 years; SD 8.8). The male to female ratio was 1:2 in HIV positive individuals.

During microscopic examination of stool samples the most frequently detected parasites were *E. histolytica/dispar* (25%) and *A. lumbricoides* (15%) (Table1). Infected individuals were found to harbour one and up to five types of parasites. Multiple infections (polyparasitism) were observed in 30% of the total examined patients. [Table 1]

*Cryptosporidium* species and *I. belli* occurred exclusively among HIV positive individuals. The association of hookworm infection with HIV status was marginally non-significant ( $p = 0.05$ ).

Although single infection was not associated with HIV status, higher rate of mixed infection occurred among HIV positives.

HIV positive patients with CD4 counts less than 200 cells/ $\mu$ L had reported an excess risk of having diarrhea independent of parasitic infection compared with those having 500 cells/ $\mu$ L and above (OR = 2.7; 95% CI 1.24 to 5.91).

The rate of parasitic infection was increased with decreasing CD4 T-cell count among HIV infected individuals (Table 2). The highest infection rate was at CD4 counts of less than 200 cells/ $\mu$ L and it was about six-fold higher compared with individuals having counts greater than 500 cells/ $\mu$ L (OR = 6.3; 95% CI 2.6 to 15.1). Similarly, an increased rate of mixed parasitic infection was observed at the same lower counts of CD4 T-cells (OR = 3.1; 95% CI 1.1 to 8.9). Higher rate of *S. stercoralis* (OR = 3.2; 95% CI 1.4 to 7.3), *I. belli* (OR = 7.5; 95% CI 3.1 to 18.7) and *Cryptosporidium* species (OR = 12.1; 95% CI 5.5 to 26.3) were detected among individuals with CD4 counts less than 200 cells/ $\mu$ L compared with those having 200 cells/ $\mu$ L and above. [Table 2]

**Table 1: Distribution of different intestinal parasites among HIV positive**

Type of parasite	HIV positive (N = 200) Number (%)	P-value
<b>Helminths</b>		
A. lumbricoides	28(14%)	0.75
T. trichiura	14(7%)	0.07
Hookworm species	12(6%)	0.04
E. vermicularis	2(1%)	1.00
S. stercoralis	18(9%)	0.00
Taenia species	4(2%)	0.20
H. nana	6(3%)	0.37
S. mansoni	6(3%)	0.30
<b>Protozoa</b>		
E. histolytica/dispar	54(27%)	0.10
G. lamblia	20(10%)	1.00
I. belli	14(7%)	0.00
Cryptosporidium species	26(13%)	0.00
E. coli	1(1%)	0.00
T. hominis	1(1%)	0.00

**Table 2: Distribution of HIV-associated parasites in different categories of CD4 T-cell counts**

CD4 T-cell count/ $\mu$ L	Total (%) examined	Positive (%) for any parasite	S. stercoralis	I. belli	Cryptosporidiumspecies
< 200	61(31%)			20(32.5%)	30(50%)
200-349	51(26%)			10(15.5%)	10(15.5%)
350-499	41(21%)			1(1.5%)	5(9.5%)
$\geq$ 500	47(28%)			0	1(1.5%)

## DISCUSSION

This study determined the prevalence and pattern of intestinal parasites among HIV positive and negative individuals to assess if trend of occurrence was evident. The study also attempted to investigate whether the distribution of parasites was affected by immune status, and finally to provide information regarding diarrhoea associated parasites. The overall prevalence of intestinal parasites was 58% among the study subjects. Compared to the current study, a lower rate of parasitic infection (39.8%) was shown among individuals with and without HIV/AIDS in south western Ethiopia (Jimma).<sup>[14]</sup> Higher rate of parasitic infection among farmers in the present study may be explained by increased occupational exposure to contaminated soil. The preponderance of parasitic infection among merchants may also be due to the common habit of placing fingers in their mouth in attempt to ease counting of money with moisten fingers.

In this study, HIV status was associated with infection of Cryptosporidium, I. belli, and S. stercoralis. However, HIV had non-significant effect to modify prevalence of other protozoan or helminthic infections. In agreement, several other studies reported higher rate of Cryptosporidium,<sup>[10, 11, 14-16]</sup> and I. belli infection,<sup>[10,11]</sup> among HIV positive individuals. Predominance of S. stercoralis infection in the same sero-group was also reported elsewhere.<sup>[14,17]</sup> In contrast to our study, however, predominance of S. mansoni, T. trichiura and hookworm infection was additionally detected among HIV positive individuals in Jimma.<sup>[14]</sup> On the other hand, higher rate of T. trichiura, A. lumbricoides, and G. lamblia in Honduras,<sup>[16]</sup> and A.

lumbricoides, I. butschlii and C. mesnili in Zambia,<sup>[11]</sup> were reported among HIV negative individuals. These reports did not confirm similar trend of parasite occurrence in relation to HIV status.

In agreement with previous studies,<sup>[10,18]</sup> those parasites associated with HIV were more likely encountered as the CD4 T-cell count fell below 200 cells/ $\mu$ L. This may be because immunodeficient patients were either more susceptible to acquire particular parasites and/or unable to clear once infection is established. However, the mechanism by which immunodeficiency facilitate selective establishment of certain parasites is not yet clear. Indeed, the magnitude of impairment on innate or acquired immunity alters the range of pathogens to which the host is susceptible.<sup>[19]</sup>

Mixed infection is a common phenomenon in areas where various types of intestinal parasites are encountered.<sup>[12]</sup> Although most participants in the present study harbour mixed infection, difference was observed by HIV status. Increased rate of mixed infections among HIV positive individuals, particularly in those with CD4 counts below 200 cells/ $\mu$ L may be because of higher prevalence of certain parasites among the risk group, which favours the frequent mixing up.

It has been reported that diarrhoea is an important clinical problem among HIV-infected patients and associated with significant impairments in health-related quality of life.<sup>[20]</sup> The present study shows that diarrhoea is a concern among the participants regardless of their HIV status though it more likely takes chronic course among HIV-infected participants than HIV uninfected group. HIV and various parasitic infections were reported to

associate with diarrhoea.<sup>[10,11]</sup> Similarly, in this study, the role of either HIV or parasitic infection independently or as a co-infection to cause any type of diarrhoea or chronic diarrhoea was significant. A more than twofold increase of diarrhoea among patients with CD4 counts less than 200 cells/ $\mu$ L in the present study may re-affirm the view that diarrhoea to be an AIDS defining condition.<sup>[21]</sup>

This hospital based cross-sectional study provided preliminary data for further detailed information in the particular area. A study using a longitudinal design with stronger power and representative sample reliably investigates the possible immunologic and epidemiologic interaction between HIV infection and intestinal parasites. Our reliance on participants report to assess symptom and duration of diarrhoea may introduce bias. Thus, results of this study should be interpreted in light of the study limitations.

## CONCLUSION

The high prevalence of intestinal parasitic infection in the study population warrants the urgent need of intervention so as to avoid its consequences. Infection of *Cryptosporidium*, *I. belli* and *S. stercoralis* was significantly higher among HIV positive subjects, particularly in those with lower CD4 T-cell counts. As no cure is available for cryptosporidiosis, people with HIV should be advised on how to avoid infection, including the potential benefits of drinking boiled water and avoiding contact with animals. Screening of HIV infected individuals is also essential for early treatment of *I. belli* and *S. stercoralis* infection. Moreover, raising immune status of HIV infected patients with anti-retroviral therapy may help to reduce acquisition and/or proliferation of HIV associated parasitic infections and the likelihood of experiencing diarrhoea.

## REFERENCES

1. World Health Organization (WHO): Prevention of schistosomiasis and soil transmitted helminthiasis: Report of WHO Expert Committee. WHO Technical Report Series 912. Geneva. 2002, 10.1038/nm0402-319.
2. UNAIDS/WHO, AIDS epidemic update 2006. [http://www.un-ngls.org/spip.php?page=article\_s&id\_article=186]
3. Grossman Z, Meier-Schellersheim M, Sousa AE, Victorino RM, Paul WE: CD4+ T-cell depletion in HIV infection: are we closer to understanding the cause. *Nature Medicine*. 2002, 8: 319-323. 10.1128/CMR.17.4.1012-1030.2004.
4. Borkow G, Bentwich Z: Chronic immune activation associated with chronic helminthic and human immunodeficiency virus infections: Role of hypo-responsiveness and anergy. *Clin*

- Microbiol Rev. 2004, 17 (4): 1012-1030. 10.1128/IAI.70.2.427-433.2002.
5. MacDonald AS, Araujo MI, Pearce EJ: Immunology of parasite helminth infections. *Infect Immun*. 2002, 70: 427-433. 10.1128/IAI.71.11.6668-6671.2003.
6. Secor WE, Shah A, Mwinzi PM, Ndenga BA, Watta CO, Karanja DM: Increased density of Human Immunodeficiency Virus type 1 co-receptors CCR5 and CXCR4 on the surfaces of CD4+ T cells and monocytes of patients with *Schistosoma mansoni* infection. *Infect Immun*. 2003, 71: 6668-6671. 10.1006/clim.2001.5040.
7. Kalinkovich A, Borkow G, Weisman Z, Tsimanis A, Stein M, Bentwich Z: Increased CCR5 and CXCR4 Expression in Ethiopians Living in Israel: Environmental and Constitutive Factors. *Clin Immunol*. 2001, 100 (1): 107-117. 10.1006/clim.2001.5040.
8. Shapira-Nahor O, Kalinkovich A, Weisman Z, Greenberg Z, Nahmias J, Shapiro M, Panet A, Bentwich Z: Increased Susceptibility to HIV-1 infection of peripheral blood mononuclear cells from chronically immune activated individuals. *AIDS*. 1998, 12: 1731-1733. 10.1016/S0001-706X(03)00063-9.
9. Fincham JE, Markus MB, Adams VJ: Could control of soil-transmitted helminthic infection influence the HIV/AIDS pandemic? *Acta Tropica*. 2003, 86 (2-3): 315-333. 10.4103/0255-0857.40536
10. Gupta S, Narang S, Nunavath V, Singh S: Chronic diarrhea in HIV patients: prevalence of coccidian parasites. *Indian J Med Microbiol*. 2008, 26: 172-175. 10.1186/1471-230X-9-7.
11. Kelly P, Todd J, Sianongo S, James M, Sinsungwe H, Max K, Farthing MJ, Feldman RA: Susceptibility to intestinal infection and diarrhoea in Zambian adults in relation to HIV status and CD4 count. *BMC Gastroenterology*. 2009, 9: 7-10.1186/1471-230X-9-7.
12. Habtamu B, Kloos H: Intestinal parasitism. *Epidemiology and Ecology of Health and Diseases in Ethiopia*. Edited by: Berhane Y, Hailemariam D, Kloos H. 2006, Addis Ababa: Shama books, 519-538. 1
13. Cheesbrough M: *District laboratory practice in tropical countries*, Part 1. 2000, Cambridge University Press, United Kingdom, 2
14. Awole M, Gebre-Selassie S, Kassa T, Kibru G: Prevalence of Intestinal Parasites in HIV-Infected Adult Patients in Southwestern Ethiopia. *Ethiop J Health Dev*. 2003, 17: 71-78.
15. Lindo JF, Dubon JM, Ager AL, De Gourville EM, Solo-Gabriele H, Klaskala WI, Baum MK, Palmer CJ: Intestinal parasitic infections in human immunodeficiency virus (HIV)-positive and HIV-negative individuals in San Pedro Sula, Honduras. *Am J Trop Med Hyg*. 1998, 58: 431-435.
16. Adesiji YO, Lawal RO, Taiwo SS, Fayemiwo SA, Adeyeba OA: Cryptosporidiosis in HIV infected patients with diarrhoea in Osun State southwestern Nigeria. *Eur J Gen Med*. 2007, 4: 119-122. 10.1590/S1413-86702001000600008.
17. Feitosa G, Bandeira AC, Sampaio DP, Badaro R, Brites C: High prevalence of Giardiasis and strongyloidiasis among HIV-infected patients in Bahia, Brazil. *Braz J Infect Dis*. 2001, 5: 339-344. 10.1186/1471-230X-1-3.
18. Wiwanitkit V: Intestinal parasitic infections in Thai HIV-infected patients with different immunity status. *BMC Gastroenterol*. 2020, 1: 3-10.1186/1471-230X-1-3.
19. Evering T, Weiss LM: The immunology of parasite infections in immunocompromised hosts. *Parasite Immunol*. 2021, 28: 549-565. 10.1097/01.mcg.0000225694.46874.fc.
20. Siddiqui U, Bini EJ, Chandarana K, Leong J, Ramsetty S, Schiliro D, Poles M: Prevalence and impact of diarrhea on health-related quality of life in HIV-infected patients in the era of highly active antiretroviral therapy. *J Clin Gastroenterol*. 2022, 41 (5): 484-490. 10.1097/01.mcg.0000225694.46874.fc.
21. Mith PD, Jana EN: Infectious diarrhea in human immunodeficiency virus infection. *Gastroenterol Clin North Am*. 2023, 17: 587-598.