

High carriage rate of intestinal parasites among asymptomatic HIV-seropositive individuals on antiretroviral therapy attending the tertiary care hospital in Varanasi, India

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Abstract

Introduction: Infections by intestinal parasites (IPs) are prevalent among human immunodeficiency virus (HIV)-seropositive individuals. Although diarrhea is the usual manifestation, asymptomatic presentations are common. In such situations, the problem of parasitosis is often underestimated. This study was performed to assess the presence of IPs among asymptomatic HIV-seropositive patients.

Materials and Methods: A case-control study was designed, including stool samples from 75 asymptomatic HIV-seropositive patients and 75 seronegative patients with gastrointestinal discomfort. IPs were identified by microscopy and polymerase chain reaction (PCR) for *Entamoeba* spp, *Cryptosporidium* and *Microsporidium* spp. The data were statistically analyzed using the Chi-square test. **Results:** The prevalence of IPs in HIV-seropositive cases (25.33%) was statistically significantly more ($P < 0.05$) than that of controls (4%). Helminths predominated in both the groups. PCR detected IPs in 5 (6.6%) additional microscopy-negative cases. The presence of IPs was associated with CD4 count <200 cells/ μ l. **Conclusion:** The asymptomatic carriage of IPs in HIV patients poses an important reservoir in the community. To prevent transmission, frequent screening is needed.

Key words: Asymptomatic, helminths, parasites, reservoir, stool

INTRODUCTION

Human immunodeficiency virus (HIV) infection and its complications remain a major global health problem. According to a recent survey, it was estimated that the prevalence of HIV in India among adults (15–49 years) was 0.26%.^[1] In 2016, India had 80,000 new HIV infections and 62,000 HIV infection-related deaths.^[2] It has been reported that 80% of the mortality in HIV-seropositive patients is

because of related opportunistic infections including intestinal parasitic infection.^[3] The spectrum of intestinal parasites (IPs) in HIV-seropositive individuals varies from one geographical region to another.^[4] The most commonly reported IPs

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in HIV-positive patients include *Cryptosporidium parvum*, *Cystoisospora belli*, *Cyclospora* species, *Microsporidium* species, *Giardia lamblia*, and *Entamoeba histolytica*. Diarrhea is the most common clinical symptom of the HIV-seropositive patients as reported in 30%–60% of the cases in the developed countries and 90% of the cases in developing countries.^[5] Because of this reason, diarrheal cases dominate in the studies related to the presence of IPs in HIV-seropositive patients. Consequently, the prevalence of pathogenic parasites in the HIV-infected nondiarrheal patients remains undiagnosed, and they act as potential cyst shredders and reservoirs in the population. HIV shows definite interaction with the CD4 molecules of T-lymphocytes. As the disease progresses, CD4 count goes down, leading to the weakening of the immune system of the individual. Thus, because of debilitated immune response, the patient becomes susceptible to various bacterial, fungal, and parasitic infections.

In this context, this study was performed to screen the presence of IPs in the asymptomatic HIV-seropositive nondiarrheal cases presenting with differential immune status.

MATERIALS AND METHODS

Study design

A case-control study was conducted from May 2016 to April 2017 in the Department of Microbiology and Integrated Counseling and Testing Center of a tertiary care hospital in Varanasi, North India.

Study cases and controls

A total of 150 participants of both sexes were included in this study, of which 75 HIV-seropositive patients without diarrhea or any intestinal symptoms and who were on antiretroviral therapy (ART) were included as the study participants. While the same number of HIV-seronegative patients attending various outpatients department due to gastrointestinal discomfort during the same study period were included as the controls. Briefly, the same number of controls from the samples received for routine stool microscopy from the symptomatic patients was included on a particular day as the number of cases based on age or sex matching. Only those patients were considered controls that had HIV-seronegative report within the past 3 months. The demographic data, including age, sex, occupation, residence, and CD4 count (for HIV seropositive only), were collected from each patient. Cases with chronic diarrhea or diarrhea in the past 6 months were excluded from the study.

Sample collection

A single-stool specimen from each patient was collected in sterile screw-capped dry containers and immediately brought to the microbiology laboratory for processing. All samples were divided into two parts, one used for microscopic examination and the other was kept at -20°C for the molecular study.

Microscopic examination

Stool samples were concentrated based on formol-ether sedimentation method.^[6] All the stool samples were examined by direct microscopy through saline and Lugol's iodine wet mounts for the screening of ova, cysts, or larvae of parasites. Along with it, modified acid-fast (MAF) staining using 3% acid alcohol was done for the screening of oocysts of *Cryptosporidium*, *Cystoisospora*, *Cyclospora*, and *Microsporidium* spp.

DNA extraction

DNA extraction was carried out from all the stool samples using the QIAamp DNA stool mini kit (QIAGEN, Germany) following the manufacturer's instructions. The quantification of the extracted DNA was done using NanoDrop™ One (Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India). The DNA was stored at -20°C .

Polymerase chain reaction amplification

Conventional polymerase chain reaction (PCR) was carried out for the screening of *Microsporidium* spp.,^[7] and nested PCR for *Cryptosporidium* spp.^[8] and *Entamoeba* spp.^[9] using species-specific primers. Briefly, 20 μL reaction mixture was used containing 2.5 μL of 10X reaction buffer (GeNei, Bengaluru, India), 2.0 μL of 200 M concentrations of each of the deoxynucleoside triphosphates (GeNei, Bengaluru, India), 0.3 μL of 5U Taq DNA polymerase (GeNei, Bengaluru, India), and 1 μL of each oligonucleotide primers. Five microliters (50 ng) of the DNA template/amplicon from the first cycle and milli Q were added to maintain the final volume of 25 μL . The resulting amplicons were subjected to 1.5% agarose gel electrophoresis using ethidium bromide to visualize the desired bands.

Ethical statement

The study protocol, including the consent form, was approved by the Institutional Ethical Committee. Informed consents were obtained from the patients before participating in the study.

Statistical analysis

The occurrence of IPs and their association with CD4 count were statistically analyzed using the

Chi-square test between the cases and controls. The calculations were performed using the Medcalc Statistical software version 16.4.3 (Medcalc Software Ltd., Acaciaaan, Ostend, Belgium).

RESULTS

The mean age of the HIV-seropositive cases was 36.93 ± 2.68 years, including 45 males and 30 females, making a male-to-female ratio of 3:2, and in the control group, the mean age was 41.24 ± 1.43 years with 41 males and 34 females.

In the seropositive group, microscopy detected the presence of IPs in 14 cases comprising 13 helminths, including hookworm (7, [9.3%]), *Ascaris* (5, [6.6%]), *Strongyloides* (1, [1.3%]), and in 1 (1.3%) case of *Giardia* [Figure 1]. No acid-fast structures were seen in MAF staining. Through PCR, the presence of IPs in 5 (6.6%) more stool samples was detected [Figure 2]. The nested multiplex PCR for *Entamoeba* species showed the presence of *E. histolytica* in 3 (4%) cases and *Entamoeba dispar* in 1 (1.3%) case. Along with this, the presence of *Cryptosporidium* was seen in 2 (2.6%) patients. Thus, a total of 19 (25.3%) pathogenic parasites were found in the HIV-seropositive group and 1 (1.3%) nonpathogenic *E. dispar* was seen [Table 1].

In control group of patients with gastrointestinal discomfort, the presence of IPs was seen in 3 (4%) cases which included hookworm in 2 (2.6%) cases and *Giardia* in 1 (1.3%) case by microscopy. Specific parasite PCR was negative for all the samples in this group. On statistical analysis, the occurrence of the IPs in the HIV-infected individuals was statistically significantly higher in comparison to the control group ($P < 0.05$, $P = 0.0002$). No case of multiparasitism was observed.

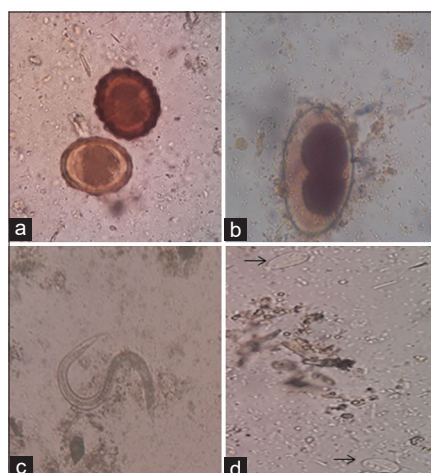


Figure 1: Microscopic images (×40) of (a) *Ascaris* ova (b) Hookworm ova (c) *Strongyloides* larvae (d) *Giardia* cyst (arrows)

Further, the mean CD4 count of the HIV-seropositive cases was 213.96 ± 55.3 . The prevalence of the IPs in the HIV-seropositive nondiarrheal patients was statistically significantly higher ($P < 0.05$) if the CD4 count was below 200 cells/ μ l [Table 2].

DISCUSSION

HIV is a major health problem in developing countries such as India. There have been studies on IPs in HIV-infected patients with diarrhea.^[10-13] However, the presence of IPs in nondiarrheal HIV-seropositive patients goes unnoticed. The present study revealed a significantly higher prevalence of IPs in asymptomatic seropositive patients who might be potential reservoirs for the transmission of these IPs.

Interestingly, all these cases were on ART. It has been reported that ART has markedly reduced the incidence of many opportunistic parasitic infections by decreasing the viral load and increasing the CD4 count.^[10] At the same time, it also has been commented that in spite of free ART facility, opportunistic IPs are “still a threat.”^[11] This study revealed a significantly higher prevalence of IPs in HIV-infected patients as compared to HIV-seronegative control group. This finding is in concordance with other studies conducted in resource limited, low socioeconomic countries like ours.^[5,12] However, contrary to our results, a study showed that there is no significant difference between the presence of IPs in nondiarrheal HIV patients when compared with controls.^[3]

The similar prevalence rate of IPs was reported in India,^[13] but the incidence rate as higher as 30%–70% has also been stated globally including India.^[14,15] The most commonly found parasite in our test group was hookworm (9.3%) as contrary to other studies, where the highest prevalence of *Cryptosporidium* and *Cystoisospora* has been reported.^[5,16,17] This is because helminths are dominantly found in the nondiarrheal formed stools as compared to diarrheal.

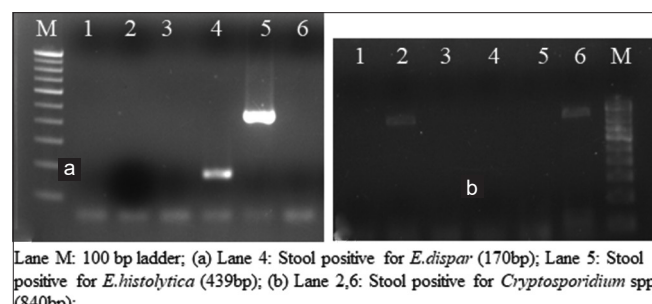


Figure 2: Polymerase chain reaction amplification of (a) *Entamoeba* spp. and (b) *Cryptosporidium* spp. in stool samples

Table 1: Occurrence of intestinal parasites among on antiretroviral therapy human immunodeficiency virus-seropositive and human immunodeficiency virus-seronegative individuals

IPs	On-ART seropositive individuals (cases; n=75), n (%)	Seronegative individuals (controls; n=75), n (%)
<i>Ascaris</i>	5 (6.6)	0
Hookworm	7 (9.3)	2 (2.6)
<i>Strongyloides</i>	1 (1.3)	0
<i>Entamoeba</i> spp.	3 (4)	0
Giardia	1 (1.3)	1 (1.3)
<i>Cryptosporidium</i>	2 (2.6)	0
Other coccidian parasites	0	0
Total	19 (25.3)*	3 (4)

* $P < 0.05$ ($P = 0.002$). ART=Antiretroviral therapy, IPs=Intestinal parasites

Table 2: Association of intestinal parasitosis with CD4 count

CD4 counts	Number of cases with IPs
<200	14*
201-500	2
>500	3

* $P < 0.05$ ($P = 0.0007$). IPs=Intestinal parasites

It was seen in this study that microscopy was less sensitive to detect the presence of opportunistic parasite, *Cryptosporidium* sp. as well as pathogenic *E. histolytica*. This may be because the sensitivity of acid-fast staining is low, and it requires a minimum concentration of more than 50,000 oocysts/ml of stool whereas PCR can detect the presence of a single oocyst in the clinical samples.^[18] In addition, as cases were already on ART which is known to affect parasitic infections, the load of parasites must have been low, and therefore, undetectable by microscopy.

This study also showed that the deterioration of immune system of an individual significantly increases its chances of intestinal parasitic infections as the majority (73.6%) of HIV patients suffering from parasitosis had CD4 count <200. Similar findings have been reported in various studies conducted in other developing countries including India.^[19]

CONCLUSION

To conclude, asymptomatic carriage of IPs is considerable (25.3%) in seropositive patients even on ART which pose an important reservoir in the community. Molecular methods are a better option for screening asymptomatic carriage in these patients with a low parasitic load. Accurate and timely detection of the IPs will reduce the sufferings of the infected individuals as well as check transmission in the community.

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Conflicts of interest

There are no conflicts of interest.

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Association of abnormal cervical cytology with a coinfection of human papillomavirus and *Chlamydia trachomatis*

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2020

Study of mucocutaneous manifestations of HIV and its relation to total lymphocyte count

PK Ashwini, Jayadev Betkerur, Veeranna Shastry