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Prevalence of intestinal parasitic infections among people living with HIV/AIDS visiting a central hospital of Kathmandu Nepal

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ABSTRACT

Backgrounds: Intestinal Parasitic Infection (IPI) plays a vital role in the prognosis of People Living with HIV/AIDS (PLHA). **Aims and Objectives:** In this study, we aimed to measure the prevalence and associated factors of IPI among PLHA and non-HIV patients attending Sukraraj Tropical and Infectious Disease Hospital, Teku, Kathmandu. **Materials and Methods:** A cross-sectional study was conducted among 193 PLHA and 111 non-HIV patients having either of gastrointestinal disorders. Direct smear, Formalin ethyl acetate sedimentation and Kinyoun's modified acid fast staining methods were applied to detect intestinal parasites from stool samples and CD_4 T-cell counts of PLHA was recorded from ART centre of hospital. **Results:** The overall prevalence of IPI was found to be 16.12% (19.17% in PLHA and 10.81% in non-HIV subjects). Prevalence was higher in PLHA ($p<0.06$) in which poly parasitic infection was common (24%) with the protozoa predominating over helminths. CD_4 T-cell counts $<200/\mu l$ ($p<0.06$) and diarrhoea ($p<0.06$) were associated with increased IPI in PLHA. *Cryptosporidium parvum* was found in 19.05% cases of PLHA having CD_4 T-cell counts $<200/\mu l$. **Conclusions:** The higher prevalence of opportunistic protozoa among PLHA indicates the need of routine parasitic investigation using sensitive methods so that it will be helpful for the proper therapeutic management.

Key words: PLHA, ART, IPI, CD_4 T-cell counts

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INTRODUCTION

HIV/AIDS has become one of the most devastating infectious diseases to have been emerged in recent history.[1] Similarly Intestinal Parasitic Infections (IPI) is endemic worldwide and has been described as contributing the greatest single worldwide cause of illness and disease.[2] IPI plays a vital role in the prognosis of People Living with HIV/AIDS (PLHA).[3] It's endemic in many developing countries of Asia and Africa due to poor sanitation, poor hygiene and unavailability of safe drinking water.[4] Diarrhoea is a common gastrointestinal symptom in HIV positive patients occurring in more than 90% of patients in developing countries and 30-60% of patients in developed countries. It is an independent indicator of mortality and morbidity in PLHA.[5] Almost 80% of AIDS patients die from AIDS-related infections including intestinal parasites rather than HIV infection itself.[6] Several intestinal parasites previously considered non-pathogenic or with transient pathogenic potential in immune competent individuals opportunistically become aggressive and cause debilitating illness in HIV/AIDS patients.[7] If such co-infections is not diagnosed and prognoses properly, dramatically enhance the more rapid progression to AIDS, as a result of more rapid decline of the CD_4 T –lymphocytes count which characteristically falls below $200/\mu l$.[8]

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In Nepal, cumulative reported HIV positive cases were found 28865[9] and PLHIV currently on ART was 12,446 in July 2016.[10] It has been estimated that there are more than 60,000 PLHA in Nepal.[4] Similarly, IPI is highly prevalent among the general population of Nepal.[11] It is alone one of the most common public health problem in all over Nepal.[12] In 2011, in hospital visiting patients, it was 21.4%[13] and in the people included in a survey was 15.17%.[11] Previous studies among PLHA in Nepal have reported high prevalence of intestinal parasitic infections. It was reported 26.7% in 2006[14] and 22.4% in 2008.[4] Lower CD_4 T-cell counts; diarrhea; sex and some other factors were associated with increased IPI among PLHA.[4]

IPI creates a huge concern in the management and care of PLHA. Detecting these parasite and understanding the status and significance of the infection they cause will greatly help in proper therapeutic management of these people and will prevent further infection.[3] This study was to determine prevalence of IPI in PLHA and to compare with those infections in non-HIV patients in the regard of age, gender and diarrheal status. It was also to compare the IPI among PLHA currently under Antiretroviral therapy- ART (On ART PLHA) and not currently under ART (ART naïve PLHA) as well as among PLHA having CD_4 T-cell count <200/μl and ≥200/μl.

MATERIALS AND METHODS

Study was conducted from September, 2016 to February, 2017 in Sukraraj Tropical and Infectious Disease Hospital, Teku, Kathmandu, from which majority (about 1500) of PLHA and general patients of Kathmandu and from almost all parts of Nepal take regular health services. Being one of the largest governmental hospitals, it is accessible and affordable for all people in need.

Combined (Both qualitative and quantitative) research method was applied for cross-sectional study that was performed in PLHA and non-HIV subjects, both subjects having either diarrhoea or any other gastrointestinal (GI) disorders like flatulence, abdominal cramp, constipation, vomiting and tenesmus. But both subjects would have been visited the hospital for their respective GI problems.

Purposive random sampling was applied with recruiting eligible patients. By assuring confidentiality and anonymity of their information, informed consent was taken from patients. Tight, leak proof, waterproof and wide mouthed container was provided to participants after informing about proper technique of stool sample collection. Then sample was taken from participant, questionnaire was filled up also with the help of record found in ART Centre of hospital, container was sealed by paraffin wax; kept in protective transport box and was duly transported to and processed in Microbiology lab of Shi-Gan International College of Science and Technology, Maharajgunj. In case of anticipation of delay in transport by more than 2 hours, 10% formalin was added for preservation of parasites in stools. Unpreserved samples were processed by saline wet mount for detecting any motile form. Iodine wet mount was applied for colour contrast of cell organelles of parasites. Samples were further processed with formalin-ethyl acetate sedimentation. Concentrated deposits were observed by microscopy for different form of parasites that might be missed in direct wet mount. Centrifuged deposits were also processed by Kinyoun's Modified (cold) acid fast staining for oocyst of coccidian.[15]

Data management, processing and analysis

Data maintained in the computer sheets was organized and analysed by using SPSS software for Windows IBM 20. A value of $\alpha \leq 0.06$ was assumed and 94% confidence interval (CI) along with the exact p-value was presented.

RESULTS

A total of 304 stool specimens (193 from PLHA and 111 from non-HIV patients) were processed for the detection of intestinal parasites. Out of total PLHA, 97 (50.26%), 92 (47.67%) and 4 (2.07%) were male, female and transgender (TG) respectively while of the total non-HIV patients, 54 (48.65%) and 57 (51.35%) were male and female respectively. Age of PLHA ranged from 7-59 years with mean age 30.29 years and of the non-HIV patients age ranged from 6-65 years with mean age 28.45 years. 116 (60.1%) and 73 (65.77%) were diarrheal patients among PLHA and non-HIV patients respectively while remaining were having other GI disorder. Among PLHA, 125 (64.77%) were On ART PLHA and 68 (35.23%) were ART naive PLHA. Out of 193 PLHA, 21 (10.88%) and 172 (89.12%) were belonged to having CD_4-T cell count<200/μl and ≥200/μl respectively.

The overall prevalence of IPI was found to be 16.12% (19.17% (37/193) in PLHA and 10.81% (12/111) in non-HIV patients, p<0.06). Prevalence was found highest in age groups ≤15, followed by age group ≥36 among study population. Prevalence was found higher in female than male and it was highest in transgender (TG) among PLHA. But it was higher in male than in female among non-HIV patients. Prevalence of IPI among PLHA having diarrhoea was significantly higher than that in subjects having other GI disorders but difference was insignificant among non-HIV patients (Table 1).

Entamoeba histolytica was predominant intestinal parasite found among study population followed by *Giardia lamblia*. Hookworm and *Enterobius vermicularis* were common among both PLHA and non-HIV patients. *Entamoeba coli, Hymenolepsis*

Table 1: Distribution of intestinal parasites among study population

Study population	PLHA					Non-HIV patients				
	Parasite positive n (%)	Total n	OR (94% CI)	p-value	Chi-square value	Parasite positive n (%)	Total n	OR (94% CI)	p-value	Chi-square value
Age groups										
≤15	2 (100)	2	-	0.02	9.801[a]	3 (100)	3	-	0.000	30.567[a]
16-25	7 (13.73)	51				3 (7.5)	40			
26-35	19 (18.8)	101				2 (3.9)	51			
≥36	9 (23.07)	39				4 (23.53)	17			
Sex										
Male	18 (18.56)	97	-	0.942	0.121[a]	6 (11.11)	54	-	0.921	0.010[a]
Female	18 (19.57)	92				6 (10.53)	57			
Trans gender	1 (25)	4				0 (0)	0			
Diarrheal status										
Diarrhea	29 (25)	116	0.33 (0.223-0.449)	0.012	6.375[a]	9 (12.33)	73	-	0.475	0.510[a]
Other GI disorder	8 (10.38)	77				3 (7.89)	38			

OR, odds ratio; CI, confidence interval; PLHA, people living with HIV/AIDS; GI, gastrointestinal

nana, Cryptosporidium parvum, Taenia sp., *Trichuris trichiura* and *Ascaris lumbricoides* were found only among PLHA (Table 2).

Protozoa was found predominant over helminth among study population. Of the 37 IPI positive cases of PLHA, 75.68% (28/37) subjects revealed single intestinal parasites (16 protozoa and 12 helminths) in their stool. Remaining 24.32% (9/37) cases revealed more than one (poly) intestinal parasitic infection in single stool sample which was found mostly in PLHA having lower CD_4 T-cell counts and associated with *Entamoeba histolytica* and *Giardia lamblia* infection (Table 2 and 3).

IPI was significantly higher in case of CD_4 T-cell counts <200/μl than in ≥200/μl (Table 4). *Cryptosporidium parvum*, only one type of opportunistic intestinal parasite was found in 19.05% (4/21) subjects having CD_4 T-cell counts<200/μl. Of the total 4 *Cryptosporidium parvum* positive cases, 3 were found singly and one was along with *Entamoeba histolytica* (Table 3). ART naive PLHA revealed higher prevalence than among On ART patients (Table 4).

DISCUSSIONS

The overall prevalence of IPI in this study was found to be 16.12% which is lower than that reported by previous studies which ranges from 15.17 to 31.5.[11-14,16,17] Prevalence of IPI has been in decreasing trend in Nepal over two decade.[13, 16, 18] It ranges from13.5% to 62.3% in the world.[2,3,5-7,19-21] Such a huge difference may be associated with endemicity of parasite and sample size.[4] Sanitary practice, level of education, economic status, social behaviour and differently selective cases are also attributed to the variation.[22] IPI was significantly higher among PLHA. Similar result was found in India.[22] Naturally PLHA have diminished level of immunity.[2] Poverty striken

Table 2: Frequencies of intestinal parasites among study population

Intestinal parasites	Intestinal parasite posiive n (%)	
	In PLHA	In non-HIV patients
Entamoeba histolytica	11 (23.91)	6 (50)
Giardia lamblia	9 (19.57)	3 (25)
Entamoeba coli	6 (13.04)	-
Hymenolepsis nana	5 (10.87)	-
Cryptosporidium parvum	4 (8.7)	-
Hookworm	3 (6.52)	2 (16.67)
Taenia sp.	3 (6.52)	-
Trichuris trichiura	2 (4.35)	-
Ascaris lumbricoides	2 (4.35)	-
Enterobius vermicularis	1 (2.17)	1 (8.33)
Total	46 (100)	12 (100)

*Cases associated with poly parasitic infection. Here, total positive cases exceed 37 due to poly parasitic infection among some PLHA

Nepalese PLHA are highly predisposed to any AIDS related infections.[23] The prevalence of IPI among PLHA found in our study is lower than that reported earlier from elsewhere in Nepal[4,14, 24-26] and from developing countries of Africa and Asia.[3,27-29] But, it is higher than that of the developed countries[30] and of some developing countries.[31]

Predomination of protozoa over helminth was in consent with previous studies.[17,24] Time to time drug mass administration with albendazole could explain the low rate of helminthic infection.[5] *Entamoeba histolytica* was found as the predominant intestinal parasite ranking *Giardia lamblia* in second position. It was in contrast to previous study in Nepal.[16,32] and somewhere in the world[33] but similar result was reported in Nigeria[31] and Ethiopia.[28] IPI was highest in age group ≤15, followed by ≥36 among PLHA and non-PLHA. Generally IPI abounds in developing countries with school children carrying the heaviest burden of the associated morbidity.[34] Higher (p>0.06) IPI was found in

Table 3: Frequency of intestinal parasites found as per CD_4 T-cell count range of PLHA

Intestinal parasites	Frequency in CD_4 T-cell count <200/µl (%) n=21	Frequency in CD_4 T-cell count ≥200/µl (%) n=172
Entamoeba histolytica	2 (9.5)	3 (1.74)
Giardia lamblia	2 (9.5)	3 (1.74)
Hookworm	-	2 (1.16)
Hymenolepsis nana	1 (4.76)	1 (0.58)
Trichuris trichiura	1 (4.76)	1 (0.58)
Taenia sp.	1 (4.76)	2 (1.16)
Cryptosporidium parvum	3 (14.29)	-
Enterobius vermicularis	-	1 (0.58)
Ascaris lumbricoides	1 (4.76)	1 (0.58)
Entamoeba coli	-	3 (1.740
Entamoeba histolytica and *Entamoeba coli*	3 (14.29)	-
Entamoeba histolytica and *Giardia lamblia*	1 (4.76)	-
Entamoeba histolytica and *Hymenolepsis nana*	1 (4.76)	-
Entamoeba histolytica and *Cryptosporidium parvum*	1 (4.76)	-
Giardia lamblia and hookworm	-	1 (0.58)
Giardia lamblia and *Hymenolepsis nana*	-	2 (1.16)

Table 4: Distribution of intestinal parasites among PLHA in relation to CD_4 T-cell counts and ART status

CD_4 T-cell counts	<200/µl n=21	≥200/µl n=172	Chi-square value	OR (94% CI)	p-value
Intestinal parasite positive n (%)	17 (80.95)	20 (11.63)	58.043[a]	32.3 (10.36-100.68)	0.000
ART status	ART naive n=68	On-ART n=125	Chi-square value	OR (94% CI)	p-value
Intestinal parasite positive n (%)	15 (22.1)	22 (17.6)	0.565[a]	0.283 (0.163-0.491)	0.452

ART, Antiretroviral therapy

female than male among PLHA and more in male among non-HIV patients. Previous studies from Nepal indicate that sex is not the factor for acquisition of intestinal parasites.[4,11] Much higher prevalence was observed in transgender PLHA. Oral-anal and oral genital contact predisposes homosexuals to infection with enteric pathogens.[35]

IPI was higher among PLHA having CD_4-T cell counts <200/µl than having ≥200/µl, which shows agreements with reports from other countries regarding IPI in different CD_4 ranges[25,28,36,37] as well as from Nepal.[4,38] In studies conducted in Nepal, more than three-fifth to four-fifth of the HIV patients with CD_4 T-cell count <200 were found having parasitic infections.[25] The present study revealed the detection of opportunistic parasite, *Cryptosporidium parvum* only among PLHA. This finding has been supported by other findings reported from Nepal and elsewhere in the world.[4, 24, 26, 29, 33] Its 19.05% prevalence among PLHA having CD_4 T-cell counts <200 cells/µl was higher than previous study in Nepal[24, 25, 32] and other countries.[39] Most of those PLHA under our study had history of diarrhea and the prevalence rate of *cryptosporidiosis* among diarrheic HIV/AIDS patients is usually found significantly higher than 10%.[40] *Cyclospora cayetanensis*, *Isospora belli* and Microsporidia were also reported in previous study among immune-suppressed PLHA.[24] Administration of co-trimoxazole to most of the adult PLHA whose CD_4 T-cell counts decline to the threshold of 350/µl[41] might have helped to prevent those infection because co-trimoxazole is effective to isospora and cyclospora infections.[42,43] *Isospora belli* is more common in tropical and subtropical climate than in temperate climate.[5] Prevalence of Cyclospora infection may be influenced by study design, geographic area, age, immunologic status of population studied and seasonal variability of parasite.[44] Microsporidia was not reported in this study since Calcoflour White Staining which requires demanding laboratory set up,[15] was not applied.

However, use of ART is associated with parasite clearance and marked reduction in AIDS related morbidity and mortality[45] and it is supposed to provide psychological support to PLHA.[23] IPI was insignificantly higher in ART naive patients than in On ART patients. Similar result was found in Ethiopia.[33] Poor living condition, lower CD_4 T-cell counts, having contact with animal and using unprotected water for drinking are other factors accompanying IPI among HIV/AIDS patients.[7] Though the ART does not seem successful for significant reduction of intestinal parasitic infections, initiation of ART only after patients declining to threshold of CD_4 T-cell counts < 500/µl,[41] might have caused its less impact, since it was to apply just after confirmation of HIV cases as per WHO guidelines.[46]

Diarrhoea was found significantly associated with IPI among PLHA corresponding to previous study.[4,26] 24.3% prevalence of poly parasitic infection in PLHA was higher than that reported by previous study.[14, 25] It is associated

with severe immune status of PLHA which can't completely clear even single parasite from body.[38]

CONCLUSIONS

The higher prevalence of opportunistic protozoa among PLHA indicates the need of routine parasite investigation using sensitive methods so that it will be helpful for the proper therapeutic managements.

List of abbreviations used

IPI, Intestinal Parasitic Infection; PLHA, People Living with HIV/AIDS; GI, Gastrointestinal; OR, Odds ratio; CI, Confidence Interval.

DECLARATIONS

Ethical clearance and consent to participate

Study was approved by Ethical Review Board of Nepal Health Research Council (Reg. no. 191/2016). Informed consent was taken from participant. Patients diagnosed as being infected with intestinal parasites were referred appropriately for treatment.

Consent for publications

Not applicable

Availability of data and materials

The datasets (and or materials) used and or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

This article was the part of Master Thesis of Homa Nath Sharma, which was supervised by Anjana Singh, Bimal Sharma Chalise and Ganesh Rai and helped by Nabaraj Adhikari and Anup Bastola. All authors declare that they have no competing interests.

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HNS- Research design, sample collection, laboratory work, result analysis and manuscript preparation; BSC and GR- Research design, result analysis and manuscript preparation; NA and AB- Result analysis and manuscript preparation; AS- Overall supervision of the research project.

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