Original Article

Intestinal parasitic infection among the HIV-infected patients in Nepal

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Abstract

Introduction: Intestinal parasitic infection has been a significant problem in HIV patients, worldwide. In this study, we aimed to measure the prevalence and identify the factors associated with intestinal parasitic infection in people infected with HIV and attending National Public Health Laboratory in Kathmandu, Nepal, for CD4 T-cell count.

Methodology: An analytical cross-sectional study in 745 HIV-infected people attending for CD4 T-cell count was conducted.

Results: The prevalence of intestinal parasitic infection was 22.4% (95% CI 19.5 to 25.5). In univariate analysis, age, sex, longer time since diagnosis of HIV, CD4 T-cell count of <200/µL, diarrhoea, marital status, and being under tuberculosis (TB) treatment were significantly associated with increased odds of intestinal parasite infection. However, in the logistic regression model, only the CD4 T-cell count of <200/µL (adjusted OR=4.2, 95% CI 2.5 to 7.0), diarrhoea (adjusted OR=2.8, 95% CI 1.8 to 4.3) and being under TB treatment (adjusted OR=2.9, 95% CI 1.8 to 4.6) remained as significant predictors. On stratification, CD4 T-cell count of <200/ µL was independently associated with higher odds of protozoal as well as helminthes infection. The parasites *Cryptosporidium* and *Cyclospora* were observed only in participants with CD4 T-cell counts <200/µL.

Conclusions: Both protozoal and helminthic intestinal parasitic infections are common in HIV-infected people seeking care in healthcare facilities. The poor immune status as indicated by low CD4 T-cell count and TB may account for such a high risk of parasitic infection.

Key words: HIV; intestinal parasite; CD4; diarrhoea

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Introduction

Human immunodeficiency virus (HIV) infection is a significant health problem with most of the cases in Asia and Africa. In Nepal, about 60,000 people are living with HIV and many in need are facing problems to access life-saving highly active antiretroviral therapy (HAART) [1]. Similarly, intestinal parasitic infections are endemic in many developing countries of Asia and Africa due to poor sanitation, poor hygiene, and unavailability of safe drinking water. In Nepal, intestinal parasitic infections are highly prevalent in all age groups and geographical regions of the country [2,3].

Previous studies among people infected with HIV in many tropical and sub-tropical countries including Nepal have reported high prevalence of intestinal parasitic infection [4-8]. The inter-relationship of HIV and intestinal parasite infection is complex and yet to be fully understood. The higher prevalence of intestinal parasitic infection in HIV-infected individuals has been reported to be associated with many factors including lower CD4 T-cell count, diarrhoea, living in a rural area, and poor nutrition [7, 9-11]. Some studies have reported that, in HIV-infected people, intestinal parasite prevalence is higher only in cases of opportunistic protozoal parasites, but not in the case of helminthes infestation [8,12]. Similarly, a high prevalence of intestinal parasites has been observed in HIV and TB co-infected patients [13].

HIV treatment programs can benefit from the knowledge of the magnitude and predictors of important HIV co-infections. In Nepal, data is not available on the magnitude of the relationship between intestinal parasitic infection and important factors associated with it. To date, this is the largest study in Nepal aimed at measuring the prevalence and identifying the factors associated with intestinal

parasitic infection in people infected with HIV and attending the National Public Health Laboratory in Kathmandu, Nepal, for CD4 T-cell count.

Methodology

Setting

This study was conducted among people infected with HIV attending the National Public Health Laboratory (NPHL) for CD4 T-cell count in Kathmandu, Nepal. The majority of the participants were visiting the facility for the first time to measure their CD4 T-cell count. During the period of this study, the national policy for eligibility to start HAART on the basis of CD4 T-cell count was a count of < 200/ μL. NPHL is the largest referral center in the country equipped with an automated CD4 T-cell count facility. Although the facility was located in the capital city, the participants came from various part of the country as CD4 T-cell count service was not available in other parts of the country until the middle of 2008.

Study design and data collection

We conducted an analytical cross-sectional study to measure the prevalence of intestinal parasitic infection and to identify the associated risk factors. A total of 745 participants were included in the study which took place from March 2005 to December 2008. The study was briefly explained to the participants and they were assured of the confidentiality as well as anonymity of the collected information. An informed verbal consent was obtained from all the volunteers. Participants were requested to collect and submit a stool specimen by themselves.

A participant was defined as intestinal parasite positive if the stool specimen was positive for at least one of either a pathogenic protozoal or a helminth in microscopic examination. Similarly, a participant was categorized as intestinal parasite negative if the stool specimen on microscopic examination was not positive for pathogenic intestinal parasites. The status of diarrhoea was ascertained by self-report of participants having loose stools three or more times a day. Information about other medical conditions and demographic details was collected from a patient register maintained at NPHL.

Laboratory investigation

Microscopic examination of the stool samples was performed by wet mount, formal-ether sedimentation technique and modified acid-fast staining [14]. Three milliliters of blood from each participant was collected for CD4 T-cell count. The CD4 T-cell count was performed by a FACS counting system (Becton Dickinson, San Jose, CA, USA) [15].

Statistical analysis

Data was entered in to an Excel spreadsheet (Microsoft, Redmond, WA, USA) and statistical analysis was performed by STATA (version 10; Stata Corp, College Station, Texas). The measure of association between different independent variables and intestinal parasitic infection (outcome variable) was expressed in terms of odds ratio (OR) with corresponding 95% confidence interval. Binomial logistic regression was performed to identify the possible factors associated with the presence of intestinal parasitic infection in univariate analysis. Factors significantly associated with the intestinal parasitic infection at $p < 0.05$ in the univariate analysis were adjusted for confounders in a final backward stepwise multiple logistic regression model. The independently associated predictor variables in the final model are reported with their corresponding adjusted odds ratio. The independently associated variables were tested for statistical interaction using a test of homogeneity. Chi-square test was used for testing statistical significance in categorical variables and the Wilcoxon rank-sum test was used for continuous variables. A p-value of < 0.05 was considered statistically significant.

Ethics

The study was approved by the ethics committee of Tribhuvan University, Kathmandu, Nepal. Participation was fully voluntary. Informed verbal consent was taken from each participant. Participants diagnosed as being infected with intestinal parasites were referred appropriately for treatment.

Results

A total of 745 volunteers were screened for intestinal parasites. The median age of the participants was 30 years (Q1 and Q3, 26 and 35 years, where Q1 and Q3 denote first and third quartile respectively). About 65% were male. The median time in weeks since the first diagnosis of HIV to the day of participation in the study was 12 weeks (Q1 and Q3, 6 and 26 weeks). About 42% of the participants were enrolled during the rainy season (June-September). More than 90% of the participants were married. About 37% of the participants had already been diagnosed with TB and were under treatment. Only 8.7% of the participants were receiving first-line highly active antiretroviral therapy (HAART). About

44% of the participants had a CD4 T-cell count of < 200/µL, 26% had a CD4 T-cell count of 200-349/µL, and 30% had a CD4 T-cell count of >349/µL. The characteristics of participants with intestinal parasitic infection was compared with those not infected and is shown in Table 1.

Out of the total of 745 stool samples analyzed from the same number of participants, intestinal parasites were detected in 22.4% (95% CI 19.5 to 25.5) (167/745). Among the total 167 volunteers harboring intestinal parasites, 83.9% (140/167) of the participants had a CD4 T-cell count of < 200/µL, whereas only 5.9% (10/167) of the participants had a CD4 T-cell count of > 350/µL (Table 2). The odds of being infected with an intestinal parasite was significantly higher in participants with a CD4 T-cell count of < 200/µL compared to participants with a CD4 T-cell count of > 350 (reference level) (unadjusted OR = 16.2, 95% CI 8.3 to 31.7) (Table 2). Similarly, the prevalence of diarrhoea was 33.3% (95% CI, 29.9 to 36.7). The odds of having diarrhoea was significantly higher in participants with a CD4 T-cell count of < 200/µL compared to participants with a CD4 count of > 350 (OR = 22.9, 95% CI 12.8 to 41.2) (Table 2).

In the univariate analysis, a CD4 T-cell count of < 200/µL, diarrhoea, age, sex, marital status, being under TB treatment, and a longer time in weeks since the first diagnosis of HIV status were significantly associated with higher risk of intestinal parasitic infection (Table 1). All of these variables were included in final backward stepwise logistic regression model to adjust for confounders. However, in the backward stepwise logistic regression model, only the CD4 T-cell count of < 200/µL (adjusted OR = 4.2, 95% CI 2.5 to 7.0), diarrhoea (adjusted OR = 2.8, 95%

Table 1. Factors associated with intestinal parasitic infection (univariate analysis)

Variable	Cases	Controls	Unadjusted odds ratio (95% CI)	p-value
Number of participants	167	578	-	
Median age (Q1-Q3)	32 (28-36)	30 (26-35)	-	0.008
Male sex (%)	120 (71.8)	365 (63.1)	1.4 (1.0-2.2)	0.03
Median number of weeks since HIV diagnosis (Q1-Q3)	24 (12-36)	12 (5-24)	-	< 0.001
Diarrhoea	115 (68.8)	133 (23.1)	7.4 (4.9-11.1)	< 0.001
Rainy season (June-September)	77 (46.1)	236 (40.8)	1.2 (0.9-1.8)	0.22
Marital status	161 (96.4)	530 (91.7)	2.4 (1.0-7.1)	0.03
Under TB treatment (%)	124 (74.2)	153 (26.4)	8.0 (5.3-12.1)	< 0.001
Under HAART (%)	15 (8.98)	50 (8.65)	1.0 (0.5-1.9)	0.89
Mode of transmission				
Mother to child	3 (1.8)	31 (5.4)	0.3 (0.1-1.1)	0.05
Injecting drug use	82 (49.1)	288 (49.8)	0.9 (0.7-1.4)	0.89
Commercial sex	69 (41.3)	197 (34.1)	1.4 (0.9-1.9)	0.09
Sex with partner	13 (7.8)	61 (10.6)	0.7 (0.4-1.3)	0.34
Blood transfusion	0	1 (0.2)	-	-

Note: HAART = Highly Active Anti-retroviral Therapy; TB = tuberculosis

Table 2. Risk of diarrhoea and intestinal parasitic infection in different categories of CD4 T-cell count (univariate analysis)

CD4 T-cell/ µL	Total (%)	Diarrhoea (%)	Crude odds ratio (95% CI)	p-value	Parasites (%)	Crude odds ratio (95% CI)	p-value
< 200	327 (43.9)	197 (79.4)	22.9 (12.8-41.2)	< 0.001	140 (83.9)	16.2 (8.3-31.7)	< 0.001
200-349	192 (25.8)	37 (14.9)	3.6 (1.9-6.9)	< 0.001	17 (10.2)	2.1 (0.9-4.6)	0.07
> 350	226 (30.3)	14 (5.7)	1	-	10 (5.9)	1	-
	745 (100)	248 (33.3)			167 (22.4)		

CI 1.8 to 4.3) and being under TB treatment (adjusted OR = 2.9, 95% CI 1.8 to 4.6) remained independently associated with intestinal parasitic infection. The variables CD4 T-cell count of < 200/μL, diarrhoea, and being under TB treatment still remained statistically significant in the multiple logistic regression when the intestinal parasitic infection was stratified into protozoal infection and helminthic infection and analyzed separately (Table 3). There was no evidence of "statistical interaction" between independently associated variables as indicated by the "test of homogeneity".

Altogether 10 different species of intestinal parasites were detected. Among the intestinal parasites, *Trichuris trichuria* (21%) was the most frequently detected, followed by *Giardia lablia*, and *Cryptosporidium parvum*, respectively. The opportunistic parasites *Cryptosporidium parvum* and *Cyclospora cayetanensis* were observed only when the participants had CD4 T-cell counts of < 200/μL. The distribution of different parasites in different categories of CD4 T-cell counts is shown in Table 4.

Discussion

The prevalence of intestinal parasitic infection and diarrhoea was common in HIV-infected people attending for CD4 T-cell count in Kathmandu, Nepal. CD4 T-cell count of < 200/μL, diarrhoea, and being under treatment for TB were the independent predictors of intestinal parasitic infection. Lower CD4 T-cell count was associated with increased risk of both protozoal as well as helminthes infection. Similarly, the lower CD4 T-cell count was associated with increased risk of diarrhoea. *Cryptosporidium parvum* and *Cyclospora cayetanensis* were the most frequent opportunistic parasites detected only in participants with lower CD4 T-cell counts.

Our study showed a high prevalence of intestinal parasitic infection. Slightly higher prevalence of intestinal parasitic infection (30.0%- 35.7%) has been reported from HIV-infected individuals from Kathmandu Valley [4,5]. However, these studies were of smaller sample size. The prevalence of parasitic infections among HIV subjects ranged from 18.4% to 81.8% in different parts of the world [8,9,16-18]. Such a huge difference in the prevalence of intestinal parasitic infection may be associated with the different levels of endemicity of such parasites.

Diarrhoea (33.3%) was common among all participants and it was more frequent (80%) in

Table 3. Factors associated with intestinal parasitic infections (multiple-regression analysis)

	All intestinal parasitic infections	**Protozoal infection**	**Helminthes infection**
Variable	Adjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)
CD4 T-cell count < 200/ μL	4.2 (2.5-7.0)	5.4 (2.6-11.1)	3.0 (1.5-5.8)
Diarrhoea	2.8 (1.8-4.3)	2.5 (1.4-4.4)	2.8 (1.6-5.0)
TB Treatment	2.9 (1.8-4.6)	2.5 (1.4-4.6)	3.4 (1.8-6.3)

Table 4. Intestinal parasites and CD4 T-cell counts

Intestinal parasites	**CD4 count category**			
	< 200/μL	**200-349/ μL**	**> 350/ μL**	**Total (%)**
Ascaris lumbricoides	15	3	2	20 (12.0)
Blastocystis hominis	1	0	1	2 (1.2)
Cryptosporidium parvum	23	0	0	23 (13.8)
Cyclospora cayetanensis	14	0	0	14 (8.4)
Entamoeaba histolytica	16	2	1	19 (11.9)
Giardia lamblia	19	5	2	26 (15.6)
Ancyclostoma duodenale	16	1	2	19 (11.4)
Hymenolepsis nana	5	1	1	7 (4.2)
Strongiloides stercoralis	2	0	0	2 (1.2)
Trichuris trichuria	29	5	1	35 (21.0)
Total	**140**	**17**	**10**	**167 (100.0)**

participants with lower CD4 T-cell counts. Higher prevalence of diarrhoea in association with lower CD4 T-cell counts has been reported by several studies [10,19-22]. A recent study from Denmark has also reported diarrhoea as a common symptom in HIV-infected individuals; however, it emphasized that the diarrhoea is not associated with lower CD4 T-cell count or the presence of intestinal parasites [23]. The inter-relationship between diarrhoea, lower CD4 T-cell count, and presence of intestinal parasites is complex and yet to be fully understood.

Our study showed that lower CD4 T-cell count, presence of diarrhoea, and being under TB treatment as independent predictors of intestinal parasitic infection, with lower CD4 T-cell count being the strongest predictor. There was a large difference in the unadjusted and adjusted values of odds ratios, indicating the confounding effect of variables included in the logistic regression model; however, there was no "interaction" among the three independently associated variables. This finding has important implications for improvement in HIV treatment programs. Screening, treatment, and measures for prevention of parasitic infection should be a part of HIV treatment programs for better outcomes in patients.

This study showed that HIV-infected people with lower CD4 T-cell counts are not only at increased risk for protozoal infection but also for helminthes infection. This finding contrasts with those of some other studies which have reported an increased risk of being infected with protozoal parasite but not with helminthes parasites [24]. In addition, our study did not show any association between the rainy season and risk of parasitic infection, unlike a study from India which showed a higher prevalence in the rainy season [19].

In our study, *Trichuris trichuria* was the most common parasite followed by *Giarida lamblia* and *Cryptosporidium parvum*. The occurrence of *Cryptosporidium parvum* and *Cyclospora cayetanensis* only below the CD4 T-cell count of < 200/ul indicates the typical opportunistic nature of these parasites. Other studies have also reported similar findings [10,11,20,21]. However, we did not detect any *Microsporidium* and *Isospora*, which are also reported to be important opportunistic parasites from other parts of the world.

Our study has some limitations. This is an observational study in which HIV-infected people diagnosed with intestinal parasitic infection were compared with HIV-infected people diagnosed not to have intestinal parasitic infection. We did not study the prevalence of intestinal parasite infection and the risk factors in HIV-negative people comparable to our study population. Although the size of our study is moderately large, we did not perform an *a priori* sample size calculation. Some HIV-infected people did not submit the stool specimen for analysis; hence they were not included in the study, and we do not know if these people differ systematically from the participants or not. We did not collect data on any participant's personal hygiene, sanitation, drinking water, nutritional condition, and use of antiparasitic medicines, which can also affect the outcome. In addition, we did not collected data on duration of diarrhoea and were not able to categorize the status of diarrhoea as acute or chronic, although the patients mostly indicated toward having diarrhoea some weeks.

Conclusion

Intestinal parasitic infection and diarrhoea are common in HIV-infected people in Nepal. HIV-infected people presenting with lower CD4 T-cell count, diarrhoea, and undergoing TB treatment are significantly more likely to be infected with these infections. Management of HIV treatment programs should consider these facts for more effective treatment outcomes.

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