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Research Article

PREVALENCE OF INTESTINAL PARASITIC INFECTIONS IN HIV INFECTED PATIENTS PRESENTING WITH DIARRHEA AND THEIR ASSOCIATION WITH CD4+ COUNTS

Desh D Singh[1], Vinod Singh[2]

[1]Department of Microbiology, King George Medical University, Chowk, Lucknow U.P. INDIA-226003,
[2]Department of Microbiology, Barkatullah University, Bhopal, M.P.India-462026

Corresponding author's email: vinodsingh63@yahoo.co.in / drvinodsingh@hotmail.com

Abstract

Introduction: Intestinal parasitic infection has been an important problem in HIV patients, worldwide. Hence, this study was undertaken to establish the prevalence of intestinal parasitic infection among people with and without HIV infection and its association with diarrhea and CD4 T-cell count. we aimed to measure the prevalence and identify the factors associated with intestinal parasitic infection in people infected with HIV. **Methodology:** An analytical cross-sectional study in 1490 HIV-infected people attending for CD4 T-cell count was conducted. **Results:** The incidence of intestinal parasitic infection was 22.4% (95% CI 29.25 to 38.25). In univariate investigation, age, sex, longer time because diagnosis of HIV, CD4 T-cell count of <200/µL, diarrhoea, wedded status, and individual under tuberculosis (TB) treatment were drastically related with increased chances of intestinal parasite infection. Nevertheless, in the logistic malfunction representation, only the CD4 T-cell count of <200/µL (accustomed OR=6.3, 95% CI 3.75 to 10.5), diarrhoea (accustomed OR=4.2, 95% CI 2.7 to 6.45) and individual under TB cure (adjusted OR=4.35, 95% CI 2.7 to 6.45) remain 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:** HIV infection increased the risk of having intestinal parasites and diarrhoea. Therefore, raising HIV positive's immune status and screening for intestinal parasites is important. This study showed that Immunodeficiency increased the risk of having opportunistic parasites and diarrhea. Therefore; raising patient immune status and screening at least for those treatable parasites is important.

Key words: Intestinal parasite; HIV; CD4+ T cell counts; diarrhea

Introduction

Intestinal parasites cause major morbidity and mortality throughout the world, particularly in developing countries and in persons with comorbidities (Wiwanitkit, 2006). The intestinal mucosa becomes a site of significant HIV replication and destruction of CD4+ cells (Assefa *et al.*, 2009). Infections of the gastro-intestinal tract play a critical role in HIV pathogenesis, attainment a rate of up to 50 % in developed countries and 95 % in developing countries (Akinbo *et al.*, 2010). The progressive decline in immunological and mucosal defensive mechanisms predisposes HIV-positive individuals to gastro-intestinal infections thus increasing susceptibility to a number of opportunistic intestinal pathogens (Stensvold *et al.*, 2011). Intestinal parasites are endemic in many regions of the world where HIV is widespread such as sub-Saharan Africa (Kassu *et al.*, 2007). Some factors including scarcity and starvation can endorse the extend of both infections, and attempts to improve these fundamental circumstances may progress the situation (Tuli *et al.*, 2010). Ascaris lumbricoides, Trichuris trichiura and hookworms, Hymenolepis nana, Giardia duodenalis/Giardia intestinalis have been identified as common opportunistic pathogens affecting HIV-infected patients (Kurniawan *et al.*, 2007). Intestinal parasites remain a main cause of diarrhoea and other GI symptoms with subsequent weight loss. However their prevalence in HIV-infected patients has dramatically decreased in countries where antiretroviral treatment is widely available (National Center for AIDS and STD Control., 2010). Few studies have addressed the issue of intestinal parasites among HIV-infected persons in India (Alfonso and Monzote, 2011). We studied the prevalence of intestinal parasites in HIV-infected patients, taking into account their CD4+ count status and treatment course (Adamu, and Petros, 2009).

Methodology

Patients who were confirmed as HIV positive cases and whose CD4 count was being evaluated were taken as study subjects. The people intestinal parasitic infected with HIV were enrolled for this study, the national policy for eligibility to start HAART on the basis of CD4 T-cell count was a count of < 200/ µL. The subjects were selected from different hospital of India, while the study wascarried out at the Department of Microbiology, Barkatullah University Bhopal (MP) India. Irrespective of their signs and symptoms of gastrointestinal tract infection, each participant was provided with three standard stool collection containers labeled with the participant's code. Instructions were given for the collection of stool sample. Short questionnaire was maintained which included participant's present medical history: any complaints of diarrhoea, sociodemographic data: age, sex and types of drinking water whether or not on antiretroviral therapy. Stool from adults who were HIV negative were taken as controls.

Study design and data collection

We studied to determine the prevalence of intestinal parasitic infection in HIV infected patients. A total of 1490 participants were included in this study which took place from June 2010 to June 2013. 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 case 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 established by patients self history of enrollment 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 Lab. Every fecal sample was examined by three methods. First, a direct wet mount in normal saline Blood samples were analyzed for CD4+ T-lymphocyte cell counts, using a flow cytometer . Briefly, 20 µL of phycoerythrine-conjugated monoclonal antibody to human CD4 were gently mixed with 20 µL of whole blood into a test tube and incubated for 15 minutes at room temperature, protected from light. Next 800 µL of no-lyse buffer were added to the mixture. After homogenizing its content, the tube was plugged into the CyFlow Counter for automatic counting (Ibrahim et *al.,* 2007).

Statistical analyses

CD4+ counts were compared based on the former treatment threshold fix at CD4+ ≤ 200 cells/µL and the current treatment threshold fixed at ≤ 350 cells/µL (Evering *et al.,* 2006) . All statistical analyses were conducted using XLSTAT 2012 (Addinsoft SARL, Paris, France, 2012). Chi-2 test or Fisher exact test was used to investigate the association among prevalence of intestinal parasites, CD4+ counts, antiretroviral treatment, use of Co-trimoxazole, and symptoms of diarrhoea. Odds ratio was calculated to estimate the risk attributable to different factors with confidence intervals calculated using the Woolf's method. The level of significance was set at p-value = 0.05.

Results

A total of 1490 subjects were observed for intestinal parasites. About 42% of the participants were included in the study during the rainy season (July-September). regarding 31 % of the case patients had previously been positive tested for Tuberculosis (TB) and were under treatment. More than 80% of the participants were married, 11.7% of the case patients were under first-line HAART (highly active antiretroviral therapy). of the total participants had a CD4 T-cell count of < 200/µL in 43.89%, 25.77% had a CD4 T-cell count of 200-300/µL, and 30.33% had a CD4 T-cell count of >300/µL. The distinctiveness of case patients with intestinal parasitic infection was compared with those not infected and is shown in Table 2. Out of the total of 1490 stool samples analyzed from the same number of subjects, intestinal parasites were detected in 22.4% (95% CI 19.5 to 25.5) (334/1490). Among the total 334 volunteers harboring intestinal parasites, 83.9% (280/334) of the participants had a CD4 T-cell count of < 200/µL, whereas only 5.9% (20/334) of the participants had a CD4 T-cell count of > 300/µL (Table 1). The probability of being infected with an intestinal parasite was extensively higher in participants with a CD4 T-cell count of < 200/µL contrast to case patients with a CD4 T-cell count of > 300 (reference level) (unadjusted OR = 24.32, 95% CI 12.75 to 46.9). likewise, the prevalence of diarrhoea was 33.3% (95% CI, 44.85 to 55.05). The probability of having diarrhoea was considerably higher in case patients with a CD4 T-cell count of < 200/µL compared to case patients with a CD4 count of > 300 (OR = 34.35, 95% CI 19.2 to 61.8). (Table 1). CD4 T-cell count of < 200/µL, age, sex, marital status , diarrhoea, being under TB treatment, and a longer time in weeks in view of the fact that the first diagnosis of HIV status were significantly linked with more risk of intestinal parasitic infection (Table 2). All of these variables were integrated in concluding backward stepwise logistic regression model to adjust for confounders. nevertheless, in the backward stepwise logistic regression model, only the CD4 T-cell count of <

200/µL (adjusted OR = 8.4, 95% CI 3.75 to 14.0), diarrhoea (adjusted OR = 4.2, 95% CI 2.7 to 8.6) and being under TB treatment (adjusted OR = 4.35, 95% CI 2.7 to 6.9) remained independently associated with intestinal parasitic infection. The variables CD4 T-cell count of < 200/µL, diarrhoea, and being under TB treatment silent hang about statistically significant in the multiple logistic regression when the intestinal parasitic infection was stratified into protozoal infection and helminthic infection (Table 4). There was no evidence of statistical interface separately 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 3.

Table 1: Intestinal parasitic infection in different range 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	654 (43.9)	394 (79.4)	34.35(19.2-41.2)	< 0.001	280 (83.9)	24.3 (12.45-47.55)	< 0.001
200-300	384 (25.8)	74 (14.9)	3.6 (1.9-61.8)	< 0.001	34 (10.2)	3.15 (1.35-6.9)	0.07
> 300	452 (30.3)	14 (5.7)	1.5	-	10(5.9)	2	-
Total	1490 (100)	496 (33.3)			334 (22.4)		

Table 2: Intestinal parasitic infection with major associated factor (univariate analysis)

Variable	Cases	Controls	Unadjusted odds ratio (95% CI)	p-value
Number of participants	334	1156		
Male sex (%)	240 (71.8)	730 (63.1)	2.1 (1.5-3.1)	0.035
Median age (A1-A3)	32 (28-36)	30 (26-35)	-	0.009
Marital status	322 (96.4)	1060 (91.7)	7.2 (2.0-14.2)	0.04
Under TB treatment (%)	248 (74.2)	306 (26.4	12.0 (7.95-18.55)	< 0.001
Rainy season (July-September)	154 (46.1)	472 (40.8)	2.4 (1.8-2.7)	0.23
Median number of weeks since HIV diagnosis (A1-A3)	24 (12-36)	12 (5-24)	-	< 0.001
Diarrhoea	230 (68.8)	266 (23.1)	11.1 (7.35-16.65)	< 0.001
Mode of transmission				
Mother to child	6 (1.8)	62 (5.4)	0.6 (0.2-2.2)	0.052
Injecting drug use	164 (49.1)	576 (49.8)	1.35 (1.4-2.8)	0.891
Commercial sex	138 (41.3)	394 (34.1)	2.1 (1.8-2.85)	0.091
Sex with partner	26 (7.8)	122 (10.6)	1.4 (0.8-2.6)	0.342
Blood transfusion	0	1 (0.2)	-	-

Table 3: Intestinal parasites and CD4 T-cell counts

Intestinal parasites	CD4 count category			
	< 200/µL	**200-300/ µL**	**> 300/ µL**	**Total (%)**
Ascaris lumbricoides	30	6	4	40 (12.0)
Blastocystis hominis	2	0	2	4 (1.2)
Cryptosporidium parvum	46	0	0	46 (13.8)
Cyclospora cayetanensis	28	0	0	28 (8.4)
Entamoeaba histolytica	32	4	2	38 (11.9)
Giardia lamblia	38	10	4	52 (15.6)
Ancyclostoma duodenale	32	2	4	38 (11.4)
Hymenolepsis nana	10	2	2	14 (4.2)
Strongiloides stercoralis	4	0	0	24(1.2)
Trichuris trichuria	58	10	2	70 (21.0)
Total	**280**	**34**	**20**	**334**

Table 4: 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	6.3 (3.75-10.5)	8.1 (3.9-16.65)	4.5 (2.25-8.7)
Diarrhoea	4.2 (2.7-6.45)	3.75-7.50 (2.1-6.6)	2.8 (1.6-5.0)
TB Treatment	4.35 (2.7-6.9)	3.75 (2.1-6.9)	3.4 (1.8-6.3)

Discussion

The prevalence of intestinal parasitic infection and diarrhoea is most prevalent in HIV-infected people presence for CD4 T-cell count. 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 (Evering *et al.,* 2006). Likely, the less CD4 T-cell count was concerned with increased threat of diarrhoea. Cryptosporidium parvum and Cyclospora cayetanensis were the most recurrent opportunistic parasites observed only in case patients with lower CD4 T-cell counts (Faye *et al.*, 2010). We observed a high prevalence of intestinal parasitic infection rather Slightly higher than prevalence of intestinal parasitic infection (30.0%- 35.7%) has been reported from HIV-infected individuals from other study (Akinbo *et al.,* 2010). conversely, these studies were of lesser sample size. The prevalence of parasitic infections among HIV subjects ranged from 18.4% to 81.8% in different parts of the world (Stensvold *et al.*, 2011). 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 frequent among all participants and it was more frequent (80%) in participants with lower CD4 T-cell counts (Prasad *et al.,* 2000). Higher prevalence of diarrhoea in association with lower CD4 T-cell counts has been reported by several studies (Mukhopadhya et al.,2005). The inter-relationship between diarrhoea, lower CD4 T-cell count, and presence of intestinal parasites is complex and yet to be fully understood.

We studied that 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 (Mohandas., 2002). 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 (Ramakrishnan *et al.*, 2007). HIV-infected people with lower CD4 T-cell counts are not only at increased risk for protozoal infection but also for helminthes infection (Anand et al.,). 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 (Institutes National de la Statistique 2012)). 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 (Cello J P and Day L W 2009).

Trichuris trichuria was the most common parasite followed by Giarida lamblia and Cryptosporidium parvum (Nazeema ., 2012) . The occurrence of Cryptosporidium parvum and Cyclospora cayet anensis only below the CD4 T-cell count of < 200/µl indicates the typical opportunistic nature of these parasites. Other studies have also reported similar findings (Nitya *et al.*, 2012). This is an surveillance study in which HIV-infected people diagnosed with intestinal parasitic infection were evaluated with HIV-infected people diagnosed not to have intestinal parasitic infection. Some HIV-infected people did not submit the stool specimen for analysis; therefore they were not included in the observational study, and we do not know if these people differ systematically from the participants or not. We have not included data on any participant's personal cleanliness, hygiene, drinking water, dietary situation, and employ of antiparasitic medicines, which can as well influence the results. In adding up, we did not included data on period of diarrhoea and were not capable to classify the status of diarrhoea as acute or chronic, even if the patients generally indicated in the direction of having diarrhoea several weeks.

Conclusion

Intestinal parasitic infection and diarrhoea are common in HIV-infected people in india. The prevalence of intestinal parasites was higher among those HIV infected individuals with diarrhea, low CD4 count, and ART-naive group groups. Case patient's consequences conceive the need for allowing for early on detection and treatment of intestinal parasites in HIV infected case patients in sort to diminish their morbidity. These look for immense awareness by those scientific service providers who are working in the ART unit. Adherence counseling of ART, health information distribution on ecological and individual hygiene should also be given to HIV/AIDS patients. In addition auxiliary huge level revision by using dissimilar investigative techniques, HIV negative control and assess predispose concerns of intestinal parasites is recommended.

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