High Prevalence of Giardiasis and Strongyloidiasis Among HIV-Infected Patients in Bahia, Brazil

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Diarrhea due to intestinal microbial infections is a frequent manifestation among HIV-infected patients. It has been postulated that HIV-infected patients may have special types of intestinal infections, and that immune activation from such parasites may affect the progression of HIV disease. To evaluate these associations, the frequency of infections was examined in HIV-infected patients in Bahia, Brazil. To determine the potential impact of the presence of intestinal parasitic infections on HIV disease progression, a retrospective study approach was used. The medical charts of 365 HIV-infected patients who had been treated at the AIDS Clinic of the Federal University of Bahia Hospital were reviewed, and the prevalence of parasites was compared with 5,243 HIV-negative patients who had attended the hospital during the same period of time. Among HIV-infected subjects, CD_4 count, RNA plasma viral load (VL), and number of eosinophils were compared according to their stool examination results. The overall prevalence of each parasite was similar for HIV-positive and HIV-negative patients. However, the prevalence of *S. stercoralis* ($p<10^{-7}$) and *G. lamblia* ($p=0.005$) was greater for HIV-infected subjects. The mean CD_4 count and viral load of HIV patients in our clinic who had stool examinations was 350 cells ± 340 and 4.4 ± 1.4 log RNA viral load, respectively. In this patient group there was no clear association between the level of the absolute CD_4 count or the viral load and a specific parasitic infection. The presence of an intestinal parasitic infection was not associated with faster progression of the HIV disease among HIV-infected patients. We conclude that strongyloidiasis and giardiasis are more frequent in HIV-infected patients in Bahia, Brazil. If this association is due to immune dysregulation, as has been proposed elsewhere, it must occur in patients after only minor shifts in CD_4 count from normal levels, or as a result of immune dysfunction not represented by CD_4 count. These infections do not appear to alter the progression of HIV disease.

Key Words: HIV-infected patients, strongyloidiasis, giardiasis.

Diarrhea is one of the most prevalent manifestations of disease among AIDS patients [1]. Such a high frequency of diarrhea is basically associated with the presence of intestinal parasites, and the identification of infections caused by *Isospora belli* and *Cryptosporidium sp*. are considered AIDS diagnostic events [2,3]. On the other hand, common intestinal pathogens are also diagnosed frequently among HIV-infected subjects, mostly in the developing world [3-7]. In Brazil, there have only seen a few reports about the prevalence of intestinal parasites and worms among HIV-infected patients.

There is some controversy about the potential effects of co-infection by intestinal worms on HIV disease. Some authors suggest that it could be an important factor of immune activation in this population, leading to a faster disease progression as a result of such interaction [8]. Immunological evaluation of a group of

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HIV-negative Ethiopians, who migrated to Israel, showed strong evidence in favor of immune dysregulation; if this were to occur in HIV-positive patients, one could predict the faster progression of HIV disease in these patients [8-10]. Since intestinal parasites can cause immune activation, the high prevalence of intestinal parasites could be a factor involved in AIDS progression among African patients [8-12].

We conducted this study to evaluate the frequency of common intestinal parasites among HIV-infected patients in Bahia, Brazil. In addition, we evaluated the association between the presence of parasites in their stools, the level of CD_4^+ cells, and plasma RNA viremia among these subjects.

Materials and Methods

Subjects: The medical charts of 690 HIV infected patients attended at the AIDS Clinic of Professor Edgard Santos University Hospital (HUPES) at the Federal University of Bahia, Brazil, from January, 1997, to June, 1999, were reviewed. All patients had HIV infection confirmed by Western blot. Demographic data, CD_4^+ cell count (CD_4), and HIV-1 RNA plasma viral load (VL) were recorded, as well as the frequency and results of stool examinations performed during that time interval. In addition, presence of gastrointestinal symptoms and white blood cell (WBC) results were recorded. For purpose of analysis, we considered the values of CD_4 and VL performed at the time closest to stools examination. We also randomly selected approximately 7,000 (each third) stool examinations from a total of 21,000 tests performed at the HUPES, corresponding to 5,243 HIV negative patients, during the same period of time.

Laboratory methods: RNA VL was assessed in all patients by Nuclisens (Organon Teknika, Boxtel, Holland), with a lower detection limit of 40 HIV-RNA copies/ml. Determination of CD_4 count was performed by flow cytometry. Analysis of fecal specimens is routinely done according to the methods of Hoffmann, Faust, and Baermann (when indicated by physicians). Detection of opportunistic pathogens was attempted by modified acid-fast staining.

Statistical analysis: Two different analyses were performed. First, we compared only the prevalence rate for HIV-positive and HIV-negative subjects. Patients were not paired according to age and/or sex, since this information was not available for HIV-negative controls. Second, we compared the overall prevalence and the specific prevalence for each parasite among HIV-infected patients.

A comparison of CD_4, VL, absolute WBC, and eosinophils was done for HIV-infected individuals according to the presence of any intestinal parasite and for each detected worm, specifically. Means were compared using analysis of variance. Proportion was evaluated by chi-square test. Logistic regression analysis was performed to assess the relationship between CD_4, VL, and the presence of intestinal parasites.

Viral load measures were transformed to logarithmic values and used in analytical procedures. Initially, absolute CD_4 cell counts were compared; the counts were stratified in two levels ($\geq$200 CD_4^+ cells or $\leq$200 CD_4^+ cells) and treated as a dummy variable. Fisher's exact test was used when testing differences in qualitative variable distributions. Student's T test was used when testing differences in quantitative variables among groups. An alfa level of 0.05 was arbitrarily chosen. Statistical 4.0 for Windows and StatXact-3 were used for computational and statistical procedures.

Results

A total of 1,043 medical charts of HIV-positive patients were reviewed, corresponding to the total number of subjects attended at the HUPES AIDS Clinics from January, 1997, though June, 1999. From this group, a total of 690 patients were followed for at least 6 months, and 365 subjects had at least one stool examination recorded.

For HIV-negative controls, we randomly selected a sample of 5,243 stool examinations performed on HIV-negative individuals, from a total of 20,972 tests performed during that time period.

Table 1 shows the prevalence of non-opportunistic intestinal parasites diagnosed, according to the HIV serological status. The overall prevalence of any parasites was similar for both HIV-positive and HIV-negative patients, but *Giardia lamblia* (p=0.005) and *Strongyloides stercoralis* ($p<10^{-7}$) were significantly associated with HIV infection. *Entamoeba coli* (p=0.0002) and *Endolimax nana* (p=0.004) were less frequently diagnosed among HIV-positive patients. Stool test results revealed that 105 (64%) individuals had only 1 diagnosed parasite, while 40 (25%) had 2 parasites, 13 (8%) had 3 parasites, 4 (2%) had 4 parasites, and 1 patient had 6 different parasites detected.

As expected, patients with at least 1 diagnosed parasite had a higher number of stool samples tested. Mean age, frequency of gender, absolute WBC, absolute eosinophils count, and presence of gastrointestinal symptoms were similar for HIV-positive and HIV-negative groups (Table 2). However, we did observe a trend for lower mean age among patients harboring *G. lamblia* (25.7 years vs. 33.2 years; p=0.057). We did not detect any difference when comparing means of absolute CD_4 cell counts and presence of a diagnosed parasite, in general, or any parasite in particular (Table 3). However, when stratified into 2 groups, we found an association between higher CD_4 counts (>200) and presence of at least 1 parasite (prevalence of 66% and 51% for higher and lower CD_4 counts, respectively, p<0.01). In addition, a univariate analysis showed a lower viral load for patients presenting any diagnosed parasites (p=0.02) but, after controlling for CD_4 levels, the difference disappeared. However, patients harboring *Entamoeba histolytica* maintained a significantly lower viral load (p=0.01) and a trend to a higher CD_4 >200 count (p=0.06).

Discussion

Diarrhea is a common manifestation of disease among AIDS patients in Brazil: a previous study showed that diarrhea was diagnosed in 70% of symptomatic individuals attended at HUPES [3]. However, there is scarce information about the prevalence of common intestinal worms in this population. This study evaluated the prevalence of intestinal parasites in AIDS patients, comparing this group with other of HIV-negative subjects tested during the same time period at the same laboratory. Our findings showed similar prevalence of parasites for both groups (HIV-infected/non-infected), but *S. stercoralis* and *G. lamblia* were significantly more prevalent among HIV-infected individuals than among negative controls. On the other hand, non-pathogenic agents such *E. nana* and *E. coli* were associated with absence of HIV infection. Of note, the prevalence we found was similar to that found in previous work in Bahia, by Moreira, et al., but it was lower than that observed by Moura in Rio de Janeiro, Brazil [3,14].

Several reports on the interaction between HIV and enteric parasites showed a significant association between *S. stercoralis* and HIV infection [5,15-17]. However, this association is not observed consistently for other parasites: in a study conducted among sugar-estate residents in Ethiopia, Fontanet, et al. found a higher prevalence of amoebic parasites in HIV patients than in negative patients [13]. Also, Mendez, et al. detected a positive association between HIV infection and *E. histolytica,* and *I. bütschlii*, in Buenos Aires [6]; while Lindo, et al. found an association between *S. stercoralis* and HIV infection, but a lower risk for infestation by *G. lamblia, A. lumbricoides* and *T. trichiurus,* in Honduras [5].

It seems clear that there is no standard pattern of occurrence of parasites among AIDS patients. The prevalence rate of a specific parasite may vary from country to country, and even from different regions in the same country [3-5,12,14,21,22]. Variation in the designs of such studies make a direct comparison between the results impossible. Nevertheless, the association between *S. stercoralis* and HIV infection

Table 1. Prevalence of intestinal parasites according to HIV infection status

Intestinal parasites	HIV+ (%) n= 365	HIV- (%) n= 5,243	OR (95% CI)	p value
Any	161 (44.1%)	2,478 (47.3%)	0.88 (0.71-1.1)	NS
E. nana	69 (18.9%)	1,349 (25.7%)	0.67 (0.51-0.89)	0,004
E. coli	29 (7.9%)	801 (15.3%)	0.48 (0.32-0.71)	0,0002
A. lumbricoides	43 (11.8%)	495 (94%)	1.28 (0.98-1.80)	NS
T. trichiurus	19 (5.2%)	291 (5.5%)	0.93 (0.56-1.54)	NS
E. hystolytica	20 (5.5%)	215 (4.1%)	1.36 (0.82-2.21)	NS
A. duodenalis	16 (4.4%)	198 (3.8%)	1.17 (0.87-2.01)	NS
G. lamblia	18 (4.9%)	127 (2.4%)	2.2 (1.23-3.59)	0,005
I. butschlii	4 (1.1%)	93 (1.8%)	0.61 (0.19-1.79)	NS
S. mansoni	9 (2.5%)	78 (1.5%)	1.67 (0.78-3.48)	NS
S. stercoralis	20 (5.5%)	39 (0.74%)	7.74 (4.3-13.8)	$< 10^{-7}$
E. vermicularis	2 (0.5%)	56 (1.1%)	0.51 (0.09-2.14)	NS
H. nana	1 (0.3%)	16 (0.3%)	0.9 (0.12-6.78)	NS

NS = p value > 0.05.

Table 2. Characteristics of HIV-infected patients according to the presence or absence of diagnosed intestinal parasites

	At least 1 parasite (n)	No diagnosed parasite (n)	p value
Age	32.7 ± 12	33.35 ± 12	0.15
Gender			
male	100 (43%)	130 (57%)	0.6
female	52 (46%)	60 (54%)	
Absolute WBC	5.647 ± 2.387	5.657 ± 2511	0.9
Eosinophilis (%)	7.9 ± 7.7	7.2 ± 6.8	0.54
GI symptoms			
Yes	74 (44%)	96 (56%)	0.8
No	86 (45%)	107 (55%)	
Number of stools samples examined (mean ± sd)	1.8 ± 1.1	1.6 ± 1.0	0.01

GI = gastrointestinal.

Table 3. CD_4 level and HIV RNA plasma viral load of patients according to the presence of intestinal parasites (means ± SD)

Parasites identified	CD_4 levels (means ± SD)			Viral load(log_{10} RNA copies/ml)		
	Yes	No	p value	Yes	No	p value
ANY	396 ± 340	336 ± 330	0.1	4.3 ± 1.0	4.5 ± 1.4	0.02*
A. duodenalis	277 ± 306	367 ± 337	0.3	4.8 ± 1.2	4.4 ± 1.1	0.58
A. lumbricoides	370 ± 289	362 ± 342	0.9	4.2 ±1.0	4.5 ± 1.2	0.07
E. coli	407 ± 260	360 ± 341	0.8	4.3 ± 0.9	4.5 ± 1.2	0.14
E. vermicolaris	262 ± 305	364 ± 336	0.7	4.0 ± 1.4	4.5 ± 1.1	0.41
G. lamblia	482 ± 516	357 ± 323	0.3	4.5 ± 1.3	4.4 ± 1.1	0.92
E. hystolytica	427 ± 302	359 ± 337	0.4	4.0 ± 1.0	4.5 ± 1.1	0.012
E. nana	399 ± 351	355 ± 332	0.5	4.4 ± 1.1	4.5 ± 1.2	0.25
I. butschlii	329 ± 135	364 ± 337	0.8	3.5 ± 0.6	4.5 ±1.1	0.04
S. mansoni	234 ± 225	367 ± 337	0.2	4.3 ± 1.3	4.5 ± 1.1	0.91
S. stercoralis	356 ± 326	480 ± 452	0.3	4.9 ± 1.3	4.4 ± 1.1	0.19
T. trichiurus	407 ± 226	361 ± 340	0.6	4.3 ± 1.0	4.5 ± 1.2	0.26
H. nana	390 ± 0	363 ± 336	0.94	3.0 ± 0	4.5 ± 1.1	0.23

* p value > 0.05 after controlling for CD_4 count.

seems consistent and is the only common conclusion of the majority of the studies [5,12,15-17]. Of note, both Strongiloidiasis and giardiasis have been found in patients with immunologic dysfunction other than HIV infection.

Another question concerns the effects of parasitic infection on HIV disease. Although some *in vitro* evidence suggests that a immune activation may be a consequence of parasitic infection, the eventual impact on HIV progression remains an unanswered question. Shapira-Nahor, et al. showed an increased susceptibility of PBMC from chronically immune activated individuals to HIV-infection, and hypothesized it could be a result of chronic parasitic infection [11]. For example, the effects of nematode infection on immune systems of HIV-negative individuals induces an impaired Th1 response and seems to be dependent on cytokine balance (23,25). However, we cannot rule out other potential causes of such activation, like other infectious diseases. Bentwich agrees with that point of view, but also recognizes that other factors could play a role in immune activation in such population [8-11].

The laboratory results we found in this study show a similar immunological pattern for HIV-infected patients, regardless of the stool examination results. We observed no difference between age, gender, absolute WBC, eosinophils and CD_4 counts for both groups. The mean eosinophil count was above the normal value for both groups, but this finding is probably a result of the immune dysregulation caused by HIV, and not a consequence of parasitic infection. However, mean viral load was significantly lower for patients with at least 1 parasite infection and, specifically, for patients harboring *E. histolityca*, even after controlling for CD_4 counts. Thus, in our study, we did not detect any association between intestinal parasites and evidence of HIV disease progression.

The significance of a higher prevalence of non-pathogenic protozoa like *E. coli* and *E. nana* among HIV-negative individuals, compared to infected patients is not clear. We can only hypothesize that HIV

may cause changes in the bowel environment, making it unfavorable to such organisms. However, the limited information provided by this study does not provide support for this conclusion.

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