Article/Artigo

Enteric parasites in HIV-1/AIDS-infected patients from a Northwestern São Paulo reference unit in the highly active antiretroviral therapy era

Enteroparasitas em pacientes infectados pelo HIV-1/AIDS em uma unidade de referência do noroeste paulista na era da terapia antirretroviral de alto impacto

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ABSTRACT

Introduction: We describe the epidemiology of intestinal parasites in patients from an AIDS reference service in Northeastern São Paulo, Brazil. **Methods:** Retrospective evaluation was done for all HIV-1/AIDS-positive patients whose *Hospital de Base/São José do Rio Preto* laboratorial analysis was positive for enteroparasites after diagnosis of HIV-1 infection, from January 1998 to December 2008. Statistical analysis was performed using the R statistical software version 2.4.1. The level of significance adopted was 5%. **Results:** The most frequent protozoan was *Isospora belli* (4.2%), followed by *Giardia lamblia* (3.5%), *Entamoeba coli* (2.8%), and *Cryptosporidium parvum* (0.3%). *Ancylostoma duodenale* (1.4%) was the most frequently detected helminth, while *Taenia saginata* and *Strongiloides stercoralis* were found in 0.7% of the samples. The results showed that diarrhea was significantly associated with giardiasis and isosporiasis. However, no association was observed between CD4+ cell counts, viral load, and the characteristics of any particular parasite. **Conclusions:** Our data may be useful for further comparisons with other Brazilian regions and other developing countries. The data may also provide important clues toward improving the understanding, prevention, and control of enteric parasites around the world.

Keywords: Epidemiology. HIV-1. AIDS. Enteroparasites. HAART. Brazil.

RESUMO

Introdução: Descrevemos a epidemiologia de enteroparasitoses em pacientes de um serviço de referência de AIDS, no noroeste paulista, Brasil. **Métodos:** Durante o período de janeiro de 1998 a dezembro de 2008, foi realizado este estudo retrospectivo por meio da análise dos prontuários dos pacientes diagnosticados com HIV-1/AIDS atendidos no Ambulatório de Doenças Infecto-Parasitárias do Hospital de Base, São José do Rio Preto, São Paulo. As análises estatísticas foram realizadas usando a versão 2.4.1 do *software* estatístico R. O nível de significância adotado foi de 5%. **Resultados:** O protozoário mais frequente foi o *Isospora belli* (4,2%), seguido da *Giardia lamblia* (3,5%), *Entamoeba coli* (2,8%) e *Cryptosporidium parvum* (0,3%). O *Ancylostoma duodenalis* (1,4%) foi o helminto mais detectado, enquanto a *Taenia saginata* e o *Strongiloides stercoralis* foram observados em 0,7% das amostras. Os resultados mostraram que a diarreia foi significativamente associada com giardíase e isosporíase. Entretanto, nenhuma associação foi observada entre as contagens de células T CD4+, carga viral e a característica de qualquer parasita em particular. **Conclusões:** Nossos dados podem ser úteis para futuras comparações com outras regiões do Brasil e outros países em desenvolvimento. Os dados também podem fornecer pistas importantes para a melhoria da compreensão, prevenção e controle de parasitas entéricos em todo o mundo.

Palavras-chaves: Epidemiologia. HIV-1. AIDS. Enteroparasitas. HAART. Brasil.

INTRODUCTION

HIV-1 infection is a disease that goes with progressive depression of the immune system; its symptoms appearance may vary, in general, from 6 months to 10 years. Acquired immune deficiency syndrome (AIDS) is the expression of the advanced form of the disease, characterized by the appearance of opportunistic diseases that can have infectious or neoplastic characteristics[1]. The prevalence of people living with HIV-1 has increased to 33.2 million in the last 3 years, and an estimated 22.1 million have died from diseases related to HIV-1[2].

In Latin America, a third of HIV-1-seropositive individuals live in Brazil. In the Southeast of the country, 67.8% of the cases are found[1]. The South leads with the number of deaths (8.7/100,000 inhabitants) in cases that 90% of people that live five years after the diagnosis[2]. However, there is a trend of increasing cases in the North (16,103) and Northeast (53,089), and of stabilization in the South (89,250), Southeast (289,074), and Central West regions (26,757). According to the World Health Organization (WHO), the concentration of the epidemic is in the population between 15 and 49 years old, with a prevalence of HVI-1 tax of infection of 0.6%[2].

Diarrhea stands out as one of the possible manifestations of AIDS, especially among patients who are immune depressive. Although there is relevance in the association between AIDS and diarrhea, there is scant literature available that explains the participation of enteric pathogens in this population. Some reports indicate the occurrence of diarrhea in 30-60% of patients with AIDS in developed countries and in 90% of cases in developing countries[3]. The relevance of enteropathogens as colonizers of gastrointestinal mucosa in HIV-1 is unquestionable. Diarrhea may or may not be infectious, but the first form occurs

more frequently in developed countries, being more common than infections caused by viruses, bacteria, or protozoa. Independently of these reasons, intestinal parasite infections are the most studied in the group of HIV-1-seropositive/AIDS patients. Some opportunistic protozoan parasites, such as *Cryptosporidium parvum*, *Isospora belli*, and microsporídia contribute to chronic diarrhea, with resultant loss of weight in HIV/AIDS patients[3]. Besides, protozoa such as *Giardia lamblia* and *Entamoeba histolytica* and the helminths *Strongyloides stercoralis*, *Ascaris lumbricoides*, and *Ancylostoma duodenale*[4-6] are regarded as the main pathogens that affect these patients.

We observe that the prevalence of enteroparasites in HIV/AIDS patients varies in many regions of the world, mainly influenced by seasonality[7, 8]. The same holds true in Brazil. In the State of Ceará, in the Northeast, a greater prevalence of *G. lamblia*[9] in patients and an association between diarrhea and the presence of *C. parvum* and *Microsporidium*[10] are found. In the Southeast, contradictory results are observed, as studies done in the State of Rio de Janeiro showed no evidence of the presence of enteroparasites in the etiology of diarrhea. However, *Cryptosporidium* was the most commonly found, followed by *G. lamblia*, *E. histolytica*, and *I. belli*. On the other hand, two other studies in the States of Minas Gerais and São Paulo showed the presence of diarrhea in patients with *I. belli*, *G. lamblia*, and *Cryptosporidium*[11, 12]. Few effects were attributed to helminths in the natural history and epidemiology of AIDS; although Strongyloidiasis has been more frequently described in Brazilian medical history, its prevalence in AIDS cases was 4.5% compared to 1.4% in the population in general[12].

The definition of the cause of diarrhea in HIV patients is a challenge as this disease can be noninfectious; it is the most common collateral effect of highly active antiretroviral therapy (HAART)[13]. Since 1996, with the progressive introduction of HAART, which has helped control HIV-1 infection and reconstruct the immune system, changes have been observed in the profile of morbi-mortality among HIV-1/AIDS patients, reflected in the reduction in opportunistic infections, including those caused by enteroparasites. A study conducted in the State of Ceará showed that with the use of HAART, the chances of being infected by enteroparasites became 5.6 times lower[14].

Some studies about the hospitalization of HIV-1-seropositive patients have shown that gastrointestinal diseases constituted the main cause of hospitalization of patients about 41 years old (24 by 1,000 people per year). It was also shown that for each increase of 50 cells/mm^{3} in the TCD4+ lymphocyte (LTCD4+) counts, there was an 8% reduction in the risk of hospitalization. Even under the use of HAART, hospitalization rates remained the same while comorbidities did change[15]. Therefore, this study aims to describe the epidemiology of intestinal parasitosis in HVI-1-seropositive patients in a hospital located in the Northeast of the State of São Paulo.

METHODS

This retrospective, transversal, and descriptive study was conducted during the period January 1998 to December 2008, in the HAART era, to analyze the medical forms of HIV-1/AIDS patients at the ambulatory for infectious and parasitic diseases at Hospital de Base, located in the City of São Jose do Rio Preto in the State of São Paulo, Brazil. Hospital de Base is a hospital school associated with the courses of medicine and nursery of the Faculdade de Medicina de São José do Rio Preto (FAMERP). This hospital has 800 berths available to the Brazilian public health system (SUS), has level 4 specialized attendance, is controlled by the State of São Paulo, and

receives patients from 101 cities in the Northwest of São Paulo. The City of São Jose do Rio Preto is located 443km from the City of São Paulo[16], and its population is about 420,000[17].

During the period of 10 years, 1,100 patients were registered at the ambulatory of infectious and parasitic diseases, and 300 (27%) of the forms were inactive for a period over 3 years. Among the active forms, only 500 were used for the enteroparasite analysis. The data were collected using a questionnaire with the following items: age, sex, category of virus exposure, information about antiretroviral treatment, laboratory exams, clinical criteria for diarrhea, and parasitological fecal exam.

The exams were done at the Hospital de Base laboratory, and the fecal samples were analyzed using microscopic methods to detect eggs, larvae, and cysts. The methods of Hoffman, Pons and Janer, Faust, and Baermann-Moraes were used. The modified Ziehl-Neelsen stain was also used to microscopically identify *C. parvum* and *I. belli*. A stool sample was collected from each patient. Each participant was provided with a standard fecal collection vial containing 4mL of 5% formalin and a spatula. Approximately 10g of feces was obtained for the parasitological analysis. The lamina analysis was done using an optical microscope, with 10x and 40x objective lenses. The patients' anti-HIV antibodies as detected by *enzyme-linked immunosorbent assay* (ELISA) or *Western blot* (Wb), viral load (VERSANT HIV-1 RNA 3.0 bDNA Assay, Siemens, Bayswater, Victoria, Australia), and immunological profile (Tritest CD4 FITC/CD8 PE/CD3 PerCP, Becton Dickinson, CA) were obtained from the medical forms. Statistical analyses were made using the R statistical software version 2.4.1. (R Foundation, ISBN 3-900051-070, http://www.r-project.org, Vienna, Austria). To obtain interdependence among proportions, chi-square distribution and Fisher's exact tests were used. The statistical significance level was 5%.

Ethical considerations

The study was approved by the ethics and research council of FAMERP (protocol #3361/2008) according to resolution 196/96.

RESULTS

The average age of the HIV-1 patients was 42.3 years, and most of them were men (61.2%). Heterosexual relationship was the main cause of the disease in this period. Among the male patients, sexual relationships were the main source of HIV infection (32.7% had sex with women), while nonsexual behaviors were less causative. A significant association was observed among women infected through sexual relations (chi-square, p < 0.0001). The risk of being infected by parental transfusion was more significant among men (chi-square, p= 0.0004). The smallest risk of infection was in haemophilia (0.3%). There was no observed relationship between gender and haemophilia.

As shown in **Table 1**, there is an association between diarrhea and the use of HAART in the parasitological exam results for HIV-1/AIDS patients. Among those who presented a negative result on the exam, 416 (86.5%) patients had used HAART, and 125 (26%) patients had had diarrhea (p = 0.0001). Among the fecal samples tested, 19 (3.8%) were positive for enteroparasites, and 18 (94.7%) were classified as diarrhea, corresponding to 3.6% of all patients. A significant association was observed only between the presence of enteroparasites and the diarrheic fecal aspect (Fisher's exact test, p > 0.0001).

The association between the presence of parasites and the fecal aspect in the HAART era is shown in **Table 2**. Most of the individuals did not have diarrhea (71.2%); there was a concomitance

TABLE 1 - Clinical characteristics of the HIV-1/AIDS patients and the parasitological results in the HAART era, São José do Rio Preto, Brazil, 2011.

| | Fecal parasitologic evaluation | | | |
| | positive | | negative | |
Variable	n	%	n	%
Access to HAART				
patients using	18	94.7	416	86.5
patients not using	1	5.3	84	17.5
total	**19**	**100.0**	**481**	**100.0**
Diarrhea status				
yes	18*	94.7	125	26.0
no	1	5.3	356	74.0
total	**19**	**100.0**	**481**	**100.0**

*Fisher's exact test: p = 0.0001; **HAART:** highly active antiretroviral therapy.

TABLE 2 - Association between the presence of parasites and the fecal aspect, São José do Rio Preto, Brazil, 2011.

	Fecal aspect				
	non-diarrheic		diarrheic		
	(n = 357)		(n = 143)		
Enteroparasites	n	%	n	%	P value*
Giardia lamblia	-		5	3.5	0.0023
Cryptosporidium parvum	1	0.3	-		
Isospora belli	-		6	4.2	0.0007
Endolimax nana	-		3	2.1	
Entamoeba coli	-		4	2.8	
Ancylostoma duodenale	-		2	1.4	
Taenia saginata	-		1	0.7	
Strongyloides stercoralis	-		1	0.7	
None		99.7	125	7.4	

*Fisher's exact test.

between two parasites in four fecal samples. The positive results for enteroparasites were observed mainly in samples with diarrhea; the mean number of enteroparasites found was 1.21 for each positive fecal sample. In the diarrheic samples, the more frequent protozoan was *Isospora belli* (4.2%), followed by *Giardia lamblia* (3.5%), *Entamoeba coli* (2.8%), and *Endolimax nana* (2.1%). In only one non-diarrheic sample was *Cryptosporidium parvum* (0.3%) found. *Ancylostoma duodenale* (1.4%) was the most frequently detected helminth, while *Taenia saginata* was observed in 0.7% of samples. Only one sample presented *Strongyloides stercoralis,* and it was from a male patient. There was a significant association between diarrhea and the presence of *G. lamblia* and *I. belli* (Fisher's exact test, p = 0.0023 and p = 0.0007, respectively).

The TCD4+ lymphocyte count (obtained up to 3 months before or after fecal analysis) was available only for 14 of the 19 patients because of the absence of this information in the medical forms. The detection was verified in patients with lymphocyte levels of 200-350 LTCD4+ (33.3%) and < 200 LTCD4+ (50%). Among patients with a HIV-1 viral load > 100.000 copy/mL, *Isospora belli* was detected in three cases. Among individuals with a viral load of 10.000-100.000 copy/mL, the presence of *Entamoeba coli* and *Giardia lamblia* was observed. In patients with a superior viral load, only the nonpathogenic amoeba *Endolimax nana* was found. No statistically significant association was observed between the LTCD4+ level and viral load and the presence of enteroparasites.

DISCUSSION

There is no uniformity in the dissemination of HIV-1, which shows different behaviors in different regions of the world. Here we discussed the epidemiologic aspects of enteroparasites in a HIV-1-seropositive adult population at a hospital in the Northwest of the State of São Paulo.

The results presented in this study showed that the HIV-1 infection was mainly among male individuals in their 40s (42.3 years), which is in accordance with the reports of national media (40 and 59 years) and associated with the increase in life expectancy[18]. The data on the method of transmission of HIV-1 confirm those found in the other regions of the country, in which sexual relations was predominant[19, 20], especially among individuals with a low level of education. Intestinal parasite infection presents a problem among HIV-1 patients because of the decreased immunity and the infestation by pathogenic microorganisms, which may result in diarrhea[20, 21]. Data on the LTCD4+ count were found only in some forms; therefore, no inference could be made. However, if the association between diarrhea and AIDS occurs because of an alteration in the immunologic system, this should not occur as a result of an immune dysfunction presented by LTCD4+ cells, as shown in another study in a HIV-1-seropositive Brazilian population[9]. One of the possible collateral effects of HAART, especially of the protease inhibitors, is diarrheic manifestation[14]. However, protease inhibitor therapy and viral load may also not be related to gastrointestinal dysfunction in the population analyzed.

In this study, it was verified that the protozoan *I. belli* was the most frequently found in HIV-1-seropositive patients; however, the indices were lower than 5%. Curiously, these data confirmed similar indices observed in a prospective study in this same population 7 years ago[22]. According to previous studies[1, 13], *I. belli* has been detected with low frequency in AIDS patients; this can occur because of the liberation of immature oocysts in a noncontinuous way, which may not correspond to the asymptomatic period and may not be eliminated in the first phase of the infection, when the signs and symptoms become evident[13]. Besides, there are some difficulties in the diagnoses of *I. belli* as there is a fluctuation in the gastrointestinal macrobiotic or enteropathogenic agents, probably because of chemoprophylactic therapy or treatment[14]. Furthermore, the specific techniques for the diagnosis of this protozoan are not routinely performed in the laboratories in this region.

Concerning the prevalence of *G. lamblia,* our study showed that, although this parasite was the second most commonly detected, it did not occur with excessive frequency, only in 3.5% of all diarrheic samples. This fact caught our attention because in some regions of São Paulo, up to 40% of the population carries this protozoan but without apparent symptoms[12]. On the other hand, the low frequency of this infection may be related to the fact that the elimination of the parasite forms of this protozoan is intermittent[23]. The low frequency of *G. lamblia* persists based on the national data, and is confirmed by all the data from prior studies about HIV-1-seropositive patients in the Northwest of São Paulo[22, 24]. Consequently, this retrospective study affirms the position that this protozoan does not present relevant expression in the HIV population in this part of São Paulo. Although *G. lamblia* was associated with diarrhea, the mechanisms by which this parasite disturbs and lowers intestinal absorption are still subjects for further investigation. We believe that these actions are multifunctional, involving both the pathogen and the host[25].

Cryptosporidium parvum has been found in 6% of 94% of HIV-1 patients in developing countries[26, 27], and according to the Brazilian Health Ministry, its official global prevalence is 0.7%[28]. The infection caused by *Cryptosporidium sp.* in HIV-1/AIDS patients has been attributed to the immune system reconstitution by HAART, even in patients without specific treatment for the parasite[29-31]. Besides, both the *in vivo* and the *in vitro* evidence shows that the control of cryptosporidiosis in patients under HAART is caused by protease inhibitors[32-34], which could be interfering with this protozoan`s aspartyl protease function[35]. In this study, only one case of cryptosporidiosis was identified in a non-diarrheic sample. With regard to previous data in the region of detection in adults (9%)[36] and children (62%)[22], we note that this profile can be related to age, which influences the distribution of the parasite, and the fact that the modified Ziehl-Neelsen stain does not normally detect this parasite as described for *I. belli*.

Ancylosotma duodenale, Taenia saginata, and *Strongyloides stercoralis* were detected in this study but with low frequency. Immune depression in human hosts has a less relevant impact on helminth parasites than on protozoa[37]. However, besides the chronic immune sequels, there is a hypothesis that Th2 lymphocyte polarization that follows helminths can have a relevant role in the HIV infection pathogenesis by the preferential virus application in these cells, increasing lymphocyte apoptosis and acting as a stimulus for viral co-receptors[38]. The pattern of *A. duodenale* infection has been verified, and although few studies have reviewed HIV patients that did not present a intestinal dysfunction, such as diarrhea, these hosts act as means of transportation through water and defecation. Besides, the nematode worms presented in the intestinal cells present low relevance because of the physical maintenance of the mucosa and immune local response[27]. In relation to cestodes, few studies associated immune depression to these helminths. In addition, they did not present any alterations in AIDS patients' clinical patterns. However, immune depression may benefit the presence of the parasite, reducing spontaneous cure. In the case of *T. solium*, immune depression and transplants can make cases of cysticercosis worse[39].

Undoubtedly, the particularities of *S. stercoralis* are well described in the medical literature. HIV-1 immune depression reinforces the infection caused by *S. stercoralis* and can be favorable to the dissemination of filarial larvae, which can reach the intestines, lungs, and other organs[40]. However, there is a controversy regarding the origin of this manifestation, whether it is related to the viral infection *per se* or arises from other factors that are inherent to HIV-1 patients, such as the use of steroids, inanition, or coinfection by HIV-1[41]. A recent study showed that HIV-induced immune depression hindered the multiplication of infectious larvae in the intestine, a necessary condition for hyperinfection[42]. Nevertheless, in tropical and subtropical countries, the occurrence of severe strongyloidiasis in the HIV-1 infected group is noticed and may, therefore, aggravate the infection by this virus[43]. Because of the reduced number of cases of *S. stercoralis* hyperinfection syndrome in AIDS patients, in 1987 the Center for Disease Control and Prevention (CDC) excluded this disease from the list of AIDS opportunist diseases.

Cryptosporidiosis, Strongyloidiasis, giardiasis, and isosporiasis are the most common parasitoses in patients infected by HIV-1 living in the Northwest of São Paulo. The results indicate that enteroparasitoses are the most important causes of death among infected patients, especially those caused by *I. belli* and *G. lamblia*. The diarrhea etiology for adults seropositive for HIV-1 in this region is not associated with HAART, but the intestinal clinical complications may be related to other enteropathogens, such as viruses, bacteria, fungi, and other emergent coccidian. In addition, the low prevalence of these enteroparasites may be linked to HAART since antiretroviral therapy can change the profile of parasite infections, probably because of the reconstitution of cell immunity and the action of these drugs on parasites[14] even in individuals with no specific therapy for enteric parasites[44]. Investigations are currently being undertaken in our laboratory to clarify the role of diverse enteropathogenic agents in noninfected HIV-1 individuals.

A possible limitation of this study may be related to factors, such as confusion, mainly due to incomplete forms or therapeutic administration prior to fecal parasitologic evaluation. Although the data analyzed are representative of a community of HIV-1 patients in the Northwest of São Paulo, they can be used in further studies for comparisons with other regions of Brazil and developing countries. Furthermore, the data provide important insights toward improving the understanding, prevention, and control of parasites in HIV-1 patients all over the world. Even with the recent developments in this field, enteroparasitoses are still a public health problem, mainly in developing countries. Besides, the presence of the above-mentioned parasites in HIV-1 patients can stimulate new cases of enteroparasitoses because the patients function as hosts.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

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