Intestinal parasitic infections in HIV/AIDS patients: epidemiological, nutritional and immunological aspects

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Abstract: This study applied a socioeconomic questionnaire designed to evaluate the frequency of intestinal parasites and characterize epidemiological, nutritional, and immunological variables in 105 HIV/AIDS patients – with and without parasitic infections, attending the Day Hospital in Botucatu, UNESP, from 2007 to 2008. Body mass index was calculated and the following tests performed: parasitological stool examinations; eosinophil, IgE, CD4+ T and CD8+ T lymphocyte cell counts; albumin test; viral load measure; and TNF-α, IFN-γ, IL-2, IL-5 and IL-10 cytokine levels. Results were positive for parasitic intestinal infections in 12.4% of individuals. Most patients had good socioeconomic conditions with basic sanitation, urban dwellings, treated water supply and sewage, good nutritional and immunological status and were undergoing HAART. Parasites were found at the following frequencies: *Entamoeba* – five patients (38.5%), *Giardia lamblia* – four (30.7%), *Blastocystis hominis* – three (23.0%), *Endolimax nana* – two (15.4%), and *Ascaris lumbricoides* – one (7.7%). There were no significant differences between the two groups for eosinophils, albumin, IgE, CD4+ T and CD8+ T lymphocytes, INF-γ, IL-2, or IL-10. Most patients also showed undetectable viral load levels. Significant differences were found for TNF-α and IL-5. These results show the importance of new studies on immunodeficient individuals to increase understanding of such variables.

Key words: HIV/AIDS, enteroparasites, nutrition, immunology, cytokines.

INTRODUCTION

The number of people living with the human immunodeficiency virus (HIV) has increased as a result of new infections each year and the beneficial effect of highly-active antiretroviral therapy (HAART) (1). Since the emergence of AIDS, clinical alterations related to the gastrointestinal tract have been highly prevalent, of which diarrheic conditions associated to parasitic infections are noteworthy (2). Many studies have shown that some intestinal parasites were frequently associated with severe diarrhea in HIV patients, both in developed and developing countries (2-8). Socioeconomic and cultural factors have carefully been studied for the impact that they may have on the onset of enteroparasitosis in general and in HIV-infected patients (9).

In the last few years, advances in HAART have led to improvements in immunological state with a resulting reduction in the frequency of certain intestinal parasites (10-13). Recent studies have also shown that prolonged use of HAART, particularly of protease inhibitors, has an important impact on user nutritional status. The relationship between infections caused by intestinal parasites and some nutritional indicators has been proven in numerous studies involving nutritional status, infection prevalence and intensity (14-20). Malnutrition has a serious influence on immunity aspects, including
the production of cytokines and antibodies, particularly Th2 profile (21-23). Recent research has also reported that intestinal helminth infections result in immune responses that involve cytokines produced by Th2 cells, with IgE production, eosinophilia, and mastocytosis (24-27).

Considering the high frequency of parasitic intestinal diseases in individuals with HIV or AIDS, many of which are opportunistic in character and seriously affect patients, new studies are required to investigate the relationship between such infections and the immunological response in these individuals, as well as the nutritional effects of these diseases in this population. Hence, this study aimed at evaluating the prevalence of enteroparasitosis in individuals with HIV or AIDS and at correlating the epidemiological, clinical, nutritional and immunologic variables which could be affected by the presence of intestinal parasites in these patients.

PATIENTS AND METHODS

Patients
One-hundred and five male and female individuals were selected. They were over 18 years old, had HIV diagnosis confirmed by western blott and were treated at the Specialized Outpatient Service and Day Hospital Professor Emeritus Domingos Alves Meira, UNESP, between March 2007 and June 2008. All were interviewed using a previously established protocol for collecting personal and socioeconomic data; they also underwent clinical, nutritional, and laboratory evaluation. This research project was approved by the Research Ethics Committee of Botucatu Medical School, according to approval document number 147/2007-CEP.

Clinical evaluation
Clinical and nutritional data were obtained by the researcher using a preestablished protocol which included personal information, and data on weight, appetite loss or gain, and the presence or absence of vomiting and diarrhea.

Nutritional evaluation
Weight and height were measured using a standardized technique (28). Body mass index (BMI) was then calculated according to World Health Organization guidelines (29, 30).

Laboratory evaluation
Samples for laboratory analysis were collected as follows: three 10 g feces samples were collected on alternate days following TF-test® (Immuoassay, USA) kit instructions, and a 2-mL blood sample was collected for eosinophil count; IgE dosage; CD4+T cell count; viral load and cytokine quantification.

Parasitological evaluation of feces
Examination for enteroparasitosis diagnosis was performed using the TF-test® kit (Immuoassay, USA) for protozoan, larvae, and helminth investigation. Three samples of feces were collected on alternate days and processed according to manufacturer’s instructions. Each kit has three collection tubes containing formalin, which enable material preservation and dilution. The processing technique included agitating collection tubes to homogenize fecal material, followed by the addition of one drop of neutral colorless detergent, which lysed fat molecules. Later, 3 mL of ethyl acetate was pipetted, which eliminated fats and much of the detritus, thus providing clean sediment. Next, the three collection tubes were fitted in the set of filters, and the system was centrifuged at 1,500 rpm for two minutes. Supernatant was then discarded and the sediment placed on a slide stained with 2% Lugol’s solution and analyzed using a microscope.

Evaluation of eosinophils
Global and relative eosinophil counts were performed by using an automated hematology counter ABX Pentra 120® (Horiba ABX Diagnostics, France). This equipment analyzed eosinophils from a reagent (Eosinofix) which lysed the red cells with reagent action on the cytoplasmic membranes. The leukocytes maintained their original size, and only eosinophils were stained for optical separation. The reference interval was 0 to 500 cells/mm³.

Albumin evaluation
Serum albumin level was measured by the dry chemistry colorimetry method. The reference value was 3.5 g/dL to 5.0 g/dL.

IgE determination
Serum or heparinized plasma and EDTA or frozen samples were used. Analysis was performed by means of BN systems particle-
enhanced immunonephelometry, using the N latex IgE mono assay (Dade Behring Marburg GmbH, EUA). In adults, concentrations of approximately 100 UI/mL can be considered the upper limit of the reference range.

**CD4+ T lymphocyte count**
Quantification of CD4+ T lymphocyte subpopulations in peripheral blood was performed by identifying surface antigens using flow cytometry. The Tritest™ anti-CD4-FITC/CD8 PE/CD3 PerCP kit (BD Biosciences, USA) and truCount™ (BD Biosciences, USA) tubes were used, thus enabling cell count in absolute figures by FACSCalibur™ flow cytometer (BD Biosciences, USA).

**Viral load count**
The Versant® HIV-1 RNA 3.0 bDNA system (Bayer Corporation, USA) test was used for direct quantification of type-1 human immunodeficiency virus (HIV-1) RNA in plasma from individuals with HIV-1 by amplification of the signal emitted by the nucleic acid, using a Bayer System 340 bDNA® Analyzer (USA).

**Cytokine quantification**
Levels of TNF-α, INF-γ, IL-2, IL-5, and IL-10 cytokines were determined by immunoenzymatic assay (ELISA) using commercial kits (R&D Systems, USA) according to manufacturer's instructions. Initially, 96-well microplates were sensitized with specific monoclonal anti-TNF-α, anti-INF-γ, anti-IL-2, anti-IL-5, and anti-IL-10 antibodies. Next, positive and negative controls and samples were added (dilution 1:2), and the plates were incubated at ambient temperature for two hours. Then, four washouts were performed with detergent solution containing 2-chloroacetamide (0.1%) and detergent solution containing 0.05% Tween 20 in PBS, pH 7.4. This procedure was repeated until the phase prior to substrate addition. Later, the biotin-coated plates received peroxidase-bonded streptavidin.

After the incubation period, substrate, formed by hydrogen peroxide (0.02%) and tetramethylbenzidine (2%), was added to the plate wells. Reaction was interrupted at ambient temperature using 50 µL of 1 M sulphuric acid. Results were evaluated by measuring optical-density (OD) at a wave length of 450 nm using an automated ELISA reader (Titertek Multiskan). Cytokine concentrations, in pg/mL, were calculated from the OD results applied to a standard curve of recombinant cytokine used as control, with 5 pg/mL as the minimum value detectable by the technique.

**Statistical Analysis**
A linear generalized model with negative binomial error was used to compare intestinal parasite frequencies.

Analysis of differences between positive and negative individuals (with or without intestinal parasites) in relation to eosinophil, IgE, CD4+ T, and CD8+ T values used a generalized linear model with negative binomial error adjusted for each one, thus obtaining p values.

Fisher’s exact test was used to study the association between detectable viral load and the presence of intestinal parasites.

The Kruskal-Wallis test was applied for albumin, BMI, and each cytokine, as it was not possible to adjust a normal distribution of errors to these variables, or they did not have constant variance. SAS Version 9.1 was used for statistical analyses.

**RESULTS**

**Characterization of the Studied Population**
Of the 105 participants, 54 (51.43%) were male with no statistical difference in gender distribution (p = 0.8522). Mean age of non-infected and infected individuals was 40.19 ± 10.39 and 44.15 ± 10.86 years, respectively. Mean monthly per capita income was approximately two minimum salaries (approximately US$ 500 per month). There was a 90% predominance of white skinned individuals; only 2% were illiterate; 95.23% lived in urban areas with basic sanitation; 26.66% had a vegetable garden as a food sources, and 88.57% used HAART.

**Clinical Evaluation**
All individuals denied the presence of diarrhea, or changes in appetite or weight over the last few months.

**Nutritional Evaluation**

**Analysis of albumin serum levels**
Albumin serum levels varied from 2.6 to 6.8 g/dL. There was no significant difference between groups (p = 0.2709).
BMI analysis
Patient BMI levels varied between 17 and 43.28 kg/m² (Table 1). There was no significant difference between groups ($p = 0.3946$).

Nutritional profile
Nutritional profile characteristics of study individuals were described according to BMI/WHO nutritional classification. Of the individuals without intestinal parasites, six (6.97%) showed low weight (BMI: < 18.5 kg/m²), 43 (50%) were within normal limits (BMI: 18.5 to 24.9 kg/m²), 27 (31.39%) were pre-obese (BMI: 25.0 to 29.9 kg/m²), seven (8.13%) were obese I (BMI: 30.0 to 34.9 kg/m²), two (2.32%) were obese II (BMI: 35.0 to 39.9 kg/m²), and one (1.16%) was obese III (> 40.0 kg/m²). Of the individuals with intestinal parasites, none showed low weight (BMI: < 18.5 kg/m²), five (50%) were within normal limits (BMI: 18.5 to 24.9 kg/m²), four (40%) were pre-obese (BMI: 25.0 to 29.9 kg/m²), one (10%) was obese I (BMI: 30.0 to 34.9 kg/m²), and none was obese II (BMI: 35.0 to 39.9 kg/m²) or obese III (> 40.0 kg/m²).

Evaluation of intestinal parasites
Of the 105 analyzed individuals, 92 (87.61%) showed negative results, and 13 (12.38%) showed positive results. *Entamoeba coli* was found in five (38.5%) samples, followed by *Giardia lamblia* in four (30.7%), *Blastocystis hominis* in three

Table 1. Median albumin and BMI values in the 105 HIV/AIDS individuals treated at the Specialized Outpatient Service and Day Hospital Professor Emeritus Domingos Alves Meira, UNESP, 2007-2008. The $p$ values obtained through the Kruskal-Wallis test to compare positive with negative individuals were 0.2709 and 0.3946 for albumin and BMI, respectively

<table>
<thead>
<tr>
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<th>Infected</th>
<th>Non-infected</th>
<th>$p &lt; 0.05$ values for Kruskal-Wallis test</th>
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<td>Albumin (g/dL)</td>
<td>4.41</td>
<td>4.4</td>
<td>0.2709</td>
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<td>BMI (kg/m²)</td>
<td>25.4</td>
<td>23.8</td>
<td>0.3946</td>
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BMI: body mass index

**Figure 1.** Number of intestinal parasites in HIV/AIDS individuals treated at the Specialized Outpatient Service and Day Hospital Professor Emeritus Domingos Alves Meira, UNESP, 2007-2008. The $p$ value obtained by adjusted negative binomial model was $< 0.0001$. 

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**Analysis of Eosinophil Serum Levels**

Serum eosinophil levels for all patients varied from 0 to 1.948 cells/mm$^3$. Statistical analysis showed no significant difference between groups ($p = 0.3198$) (Table 2).

**Analysis of IgE Serum Levels**

Serum IgE levels varied from 1.8 to 7.060 UI/mL. Statistical analysis showed no significant difference between groups ($p = 0.3147$) (Table 2).

**Analysis of CD4$^+$ T Cell Serum Levels**

Serum CD4$^+$ T cell levels varied from 21 to 1,749 cells/mm$^3$. Statistical analysis showed no significant difference between groups ($p = 0.0887$) (Table 2).

**Analysis of Viral Load**

From individuals with negative diagnosis for intestinal parasites, 73 showed undetectable viral load levels (< 50,000 copies/mL), and 11 showed a viral load > 50,000 copies/mL of blood. Form those with positive diagnosis, 11 showed undetectable viral load levels (< 50,000 copies/mL), and only one showed a viral load > 50,000 copies/mL of blood (Table 3).

**Quantification of Serum Cytokines**

Statistical analysis showed a significant difference in relation to TNF-α ($p = 0.0277$) and IL-5 ($p < 0.0001$) (Figure 2).

**DISCUSSION**

Studies relating AIDS patients to opportunistic infections have shown that HAART has contributed to HIV infection control and to the reconstitution of patient immune systems, leading to a reduction in the occurrence of opportunistic infections, including those caused by intestinal parasites (13-31).

Studies have connected serious or disseminated intestinal parasitic infections to immunosuppression, including AIDS, although several aspects remain obscure (12, 26, 32-37). This study aimed to better clarify the participation of intestinal parasites and their correlation with...
Various studies have associated intestinal parasites with education levels. In Brazil, this information has been used as an indirect measure of the socioeconomic situation, which shows, as regards HIV infection, an increased number of individuals with poorer education (38). In our study, 97.15% of the patients showed a good education level, thus better reflecting information and education levels in relation to various aspects, including parasitosis prevention. Rodrigues-Junior et al. (39) also reported schooling as an important variable in social stratification and concluded that the increased number of cases from social strata with poorer schooling is related to worse coverage by surveillance and care provision systems for individuals with less economically favored circumstances.

Another important aspect regarding the incidence of parasites is related to the presence or absence of basic sanitation. As concerns availability of treated water in the household, this study showed that 100% of the individuals dwelt in houses connected to the collective treated water supply network.

Fontbonne et al. (40) reported that housing type influences the number of different intestinal parasite species which affect members of a household. The majority of the population in our study lived in brick built houses in urban areas, thus agreeing with the authors that the role played by poverty can be seen by the fact that housing type influences the number of different species affecting household members. Only 26% of our population reported using a vegetable garden as a food source, thus suggesting this as another indicator of low intestinal parasite prevalence.

Another important aspect is the participation of nutritional and immunity status in the presence of intestinal parasites. The presence of opportunistic infections in HIV individuals as well as the effects of drug treatment may aggravate or precipitate malnutrition conditions in this disease (41). When evaluating HIV patients under HAART, Schwenk et al. (42) observed that such treatment prevented malnutrition onset. Other researchers investigating the impact of HAART, through biochemical and nutritional indicators, observed that patients gained weight; showed an increased number of CD4+T cells, reduced viral load, a reduced number of opportunistic infections, increased serum albumin, and consequently, a better quality of life (43).
Our study population displayed good adherence to treatment (88.5%), indicating increased survival and a reduced frequency in opportunistic diseases, including intestinal parasites. Several factors, such as service improvement and early diagnosis, in addition to HAART introduction, are involved in this scenario (44-47). Some studies have linked nutritional status to intestinal parasitic infections (14, 15, 48). Authors have reported that individuals with compromised nutritional status followed by immunosuppression may have larger numbers of intestinal parasites and more severe infections (49). Nutrition is important when evaluating parasitic infections, particularly in malnourished individuals, who are more susceptible to severe and disseminated forms. This was not observed in our study in which 50% were eutrophic and 31.39% were overweight in the two groups.

Intestinal parasitic infections play an important role in individuals in general, particularly in those with immunosuppression (12). Disseminated strongyloidiasis has been reported in patients with sepsis and severe immunosuppression, which can even lead to death (50). The frequency of intestinal parasites in our study was 12.38%. These findings agree with Cimerman et al. (12), who found a 12.3% frequency in both HIV-positive patients and HIV-negative individuals when evaluating patients in the post-HAART era.

Botero et al. (32) found a 32% frequency of parasites in a population with some type of immunological deficiency, including HIV infection. Reduced intestinal parasite prevalence has been observed in Brazil and worldwide since the early HAART years even when specific methods are included (51, 52). Bachur et al. (10) showed different frequencies for intestinal parasitic infections in the pre- and post-HAART eras. According to these authors, intestinal parasites were detected in 63.9% of patients from the pre-HAART era and 24% in the HAART era, thus showing a reduction in prevalence between the two periods. Cimerman et al. (2, 12) found a prevalence of 24.4% for opportunistic parasites in the pre-HAART era and 6.8% after HAART introduction, with higher rates in patients with CD4+T cells < 200/mm³. When comparing periods before and after HAART implementation, Nobre et al. (13) observed prevalences of 2.8% and 1.1%, respectively, low in both eras, but even lower after HAART use. Different percentages found by various authors, including in this study, can be explained in various ways: sample number, population studied, type of tests and HAART, among others, as well as differences in the varying sources of intestinal parasite exposure.

In the present study, the patients showed a good socioeconomic level and good education; they lived in urban areas with basic sanitation, and a minority used a vegetable garden. Most were eutrophic or overweight, thus showing good nutritional status. Most patients were using HAART, and none showed opportunistic infections.

Intestinal infections, caused by a large variety of infectious agents, particularly helminths, produce eosinophilia especially in the acute phase (53-55). However, patterns and levels are determined by the development, migration, and distribution of the parasite in the host as well as by immune response. Defense against many of these parasitic infections is mediated by the activation of Th2 cells, which results in IgE antibody production and eosinophil activation. Although less frequent, HIV infection may be accompanied by moderate eosinophilia (56-59). Cytokines produced by Th2 cells promote eosinophil activation and recruitment to sites of helminth infection, where they release toxic granular proteins which destroy the parasites (60). Our study population showed varying eosinophil levels although the two groups did not present any difference; this can be explained by the individuals having good clinical, nutritional, and immunological status.

As regards IgE, previous studies have described that synthesis of this immunoglobulin is an important component of immune resistance against helminthic infections (61, 62). High serum IgE in individuals with AIDS were seen at the beginning of the epidemic and confirmed years later by other studies (63-69). Some studies have found a relationship between high IgE levels and CD4+ T lymphocyte count < 300 cells/mm³ (64-65, 67). Association between increased IgE levels and faster progression to AIDS was observed by Rancinan et al. (69) in 1998. Total serum IgE levels were high in our study, particularly in infected patients, which suggests IgE participation in the induction and release process of histamine and other mediators in the immediate hypersensitivity reaction leading to helminth destruction (70).

In our study, both groups showed a mean
CD4⁺T lymphocyte count ≥ 400 cells/mm³. This indicates good immunological conditions, which explains, among other factors, the reduced number of parasitic infections, and is thus in agreement with the literature.

Most individuals in our study population had a viral load below the detection limit, thus showing control of the disease and good patient immunity levels. This shows that their disease was under control, with undetectable viral load and high CD4⁺T cell levels, which certainly contributed to the smaller number of parasitic infections. Few studies have reported the relationship between cytokines in individuals with HIV and parasitic intestinal infections. Some authors evaluated cytokines and their relationship with parasites such as *Schistosoma mansoni*, *Wuchereria bancrofti*, and *Trichinella spiralis* (71-79). No correlation between serum INF-γ, IL-2, and IL-10 levels and intestinal parasitic infections was seen. However, there was an increase in relation to the control of TNF-α and IL-5, also IL-5 has been associated with parasitic infections. TNF-α plays an inflammatory role, and it is also associated with cell loss during HIV infection. IL-5 is particularly important for the proliferation, growth, activation, and survival of eosinophils (80-84).

Therefore, the incidence of intestinal parasites in individuals with HIV/AIDS was very low. All patients had good socioeconomic and education levels, dwelt in urban areas with basic sanitation, and a minority used vegetable gardens. Most individuals were eutrophic or overweight; therefore of good nutritional status; most used HAART; and none had opportunistic infections. They showed good CD4⁺T cell levels and low viral load which were compatible with their good immunity status, serum eosinophil levels within normal limits, and IgE levels above normality in both groups. Increased IL-5 and TNF-α levels were observed, thus showing a tendency toward Th2 profile and suggesting that the presence of intestinal parasitic infections may be contributing to alterations in immune response.

Further studies should be conducted in regions where basic sanitation and socioeconomic levels are poor and there are reduced CD4⁺T cells for better comprehension of these variables.

**REFERENCES**


