INFECTION BY HUMAN IMMUNODEFICIENCY VIRUS-1 IS NOT A RISK FACTOR FOR AMEBIASIS

PATRICIA MORÁN, FERNANDO RAMOS, MANUEL RAMIRO, OCTAVIO CURIEL, ENRIQUE GONZÁLEZ, ALICIA VALADEZ, ALEJANDRO GÓMEZ, GABRIELA GARCÍA, EMMA I. MELENDRO, AND CECILIA XIMÉNÉZ*

Departmento de Medicina Experimental, Facultad de Medicina, UNAM, México, D.F.; Hospital Regional 1cro de Octubre, ISSSTE, México, D.F.; Clínica Lomas Altas, México, D.F.; Coordinación de Investigación en Salud, IMSS, México, D.F.

Abstract. The purpose of this study was to determine whether HIV-1 infected patients in our community were more susceptible to Entamoeba histolytica and Entamoeba dispar infection than non-HIV-infected individuals. The prevalence and frequency of invasive amebiasis was determined in 203 HIV+AIDS subjects and 140 close relatives or sexual partners, all of whom were HIV-. Anti-E. histolytica antibodies (IgG, IgA) were assessed as indicators of E. histolytica invasive infection. Polymerase chain reaction (PCR) was used for the characterization of the Entamoeba species. The prevalence estimated with PCR data showed that E. histolytica infection was more common in the HIV+/AIDS group (25.32%), than in HIV- contacts (18.46%). E. histolytica + E. dispar infection was more frequent in HIV+/AIDS patients (13.3%), than in HIV- contacts (0.7%). E. histolytica and/or E. dispar infection was highly prevalent in HIV+/AIDS patients (34.1%) without evidence of recent or current invasive disease. Contacts of HIV+/AIDS patients who were infected with E. histolytica were asymptomatic cyst passers. Our results suggest that E. histolytica strains prevalent in the studied community appear to be of low pathogenic potential.

INTRODUCTION

Intestinal parasites are among some of the most important causal etiologic agents of diarrhea in patients with the acquired immunodeficiency syndrome (AIDS), both in developed and underdeveloped countries. The most frequent opportunistic parasites in HIV-infected individuals are intracellular protozoa (Isospora belli, Cryptosporidium parvum, Cyclospora). However, infection with other extracellular parasites considered nonopportunistic, but otherwise pathogenic for humans, are also related to diarrheal disease in AIDS patients. Among these parasites are Entamoeba histolytica, Giardia lamblia, Strongyloides stercoralis, and Ascaris lumbricoides. Parasites can cause diarrhea in HIV-infected patients and can play a role in the progression of HIV infection in AIDS patients, as well as in asymptomatic HIV-infected individuals. Several studies report stimulation of the HIV replication process when host resistance mechanisms against HIV are impaired.

The adaptive cellular immune response, which is seriously damaged in AIDS patients, is a central mechanism of resistance against the parasite. However, the effect of HIV infection on susceptibility for amebic infection or invasive amebic disease is unknown. There are reports concerning the occurrence of invasive amebiasis in endemic areas. In the developing world, including Mexico, parasite prevalence is particularly high, and many cases coincide with the HIV epidemic. The anti-E. histolytica antibody seroprevalence in Mexico is 8.41%. and intestinal infection with E. histolytica/Entamoeba dispar is 5–7% in urban communities and up to 30% in rural populations. These numbers highlight the high exposure to these protozoa in Mexico. Previous data showed a prevalence of 30% for E. histolytica/E. dispar infection in homosexual men; however, invasive amebic disease appeared to be uncommon.

To determine whether HIV-1 infected (HIV+) patients in Mexico City are more susceptible to E. histolytica or E. dispar infection than non-HIV-infected individuals (HIV-), we studied the prevalence and frequency of amebic invasive disease in groups of HIV+ patients who met the Centers for Disease Control and Prevention Surveillance Criteria for AIDS. These individuals comprised patients seen at the AIDS Clinic at the Hospital Regional 1cro de Octubre, ISSSTE, in Mexico City from April 1999 to April 2000. Simultaneously, HIV- close relatives or sexual partners of AIDS patients were included as second population. We also studied the anti-E. histolytica antibody response in both serum (IgG) and saliva (IgA) samples and characterized Entamoeba found in fecal specimens using polymerase chain reaction (PCR).

MATERIALS AND METHODS

Subjects. This study was previously assessed and approved by the Ethics Committee at the Hospital Regional 1cro de Octubre, ISSSTE, in Mexico City, in accordance with the Mexican General Health Law for research in humans, which is based on the Declaration of Helsinki. Three hundred forty-three subjects were included in the study after signing a written informed consent (203 HIV+/AIDS and 140 HIV- contacts). The HIV+/AIDS patients were on a triple antiviral medication scheme. For the purpose of comparing two groups of individuals exposed to the same E. histolytica/E. dispar infectious sources, we included 203 HIV+/AIDS patients in the experimental group and 140 healthy, non-HIV-infected close relatives or sexual partners of HIV+/AIDS patients.

Consecutive HIV+/AIDS patients were enrolled in the 12-month period from January 2000 to February 2001 and clinically examined. Laboratory tests included microscopic examination of three consecutive stool samples, CD4+ lymphocyte T-cell counts, measuring of viral load, and PCR characterization of E. histolytica and E. dispar species. Anti-amoeba antibody response was also assessed both in saliva and in serum samples.

Detection of parasites. Detection of parasites was performed both through microscopic examination of fresh stool samples previously stained with iodine solution (4%) at 40x and afterwards using the flotation technique in a zinc-sulfate gradient (d = 1.192° Bulk).
DNA extraction and PCR. DNA was extracted from cysts present in stool samples placed in the zinc-sulfate gradient. Cysts were transferred to 2-mL microcentrifuge tubes, washed four times with 0.15 M NaCl, and suspended in 300 μL of lysis buffer (100 mM EDTA, SDS 0.25%, pH 8).

The tubes were frozen three times in ethanol-dry ice and thawed in a 37°C water bath. Finally, 3 μL of 20 mg/mL proteinase K (Sigma Chemical Co., St. Louis, MO) was added, and the sample was incubated for 1 hour at 55°C. After digestion with proteinase K, lysates were adjusted to 0.7 M NaCl and 1% CTAB (Sigma Chemical Co.). The mixture was incubated at 65°C for 20 minutes. DNA was extracted with chloroform and phenol/chloroform, followed by precipitation with ethanol. Precipitated DNA was suspended in water and placed in a Sephadex G-25 spin column (Pharmacia-Biotech, Uppsala, Sweden).

The DNA extracted was submitted to PCR amplification assay under standard conditions (AmpliTaq Kit, Perkin Elmer Applied Biosystems, Foster City, CA) for 35 one-minute cycles at 94°C, for 1.5 minutes at 55°C, and 2 minutes at 72°C in a DNA thermocycler (Perkin Elmer Applied Biosystems). For characterization of E. histolytica or E. dispar species, species-specific DNA sequences of rRNA genes for small ribosomal subunits were amplified using Psp5'-3' primer for E. histolytica and NPs 5'-3' for E. dispar detection. Both amplification products were 876 bp in size. These products were visualized in 1.2% agarose gels in TBE buffer. Gels were stained with ethidium bromide and photographed for later analysis.

CD4⁺ lymphocyte T-cell counts. Quantification of CD4⁺ T-cell lymphocytes was performed through cytofluorometry using T-cell monoclonal antibodies. Patients were grouped according to preestablished CD4⁺ T-cell lymphocyte count ranges based on the stage of HIV infection. In some cases, viral loads were measured at different periods throughout follow-up.

Anti-amebic antibody detection. Anti-amebic antibody detection was performed in both saliva and serum samples following the ELISA technique as previously described.

For the detection of anti-amebic IgA, saliva samples were initially titrated for total IgA quantification and then all samples were adjusted to 50 μg/mL. Serum samples were diluted 1:1000 for anti-amebic IgG detection.

The antigen used in ELISA was a membrane-enriched extract (1 μg/well) obtained from axenically cultured E. histolytica HM1:IMSS as previously described. Afterwards, 50 μL/well of serum or saliva dilution was added to each microtiter plate, blocking previously the free antigen spaces with 3% PBS-BSA and subsequently incubated for 2 hours at room temperature. Plates were then washed once with PBS-BSA, added to 0.5% Tween-20 (Sigma Chemical Co.) (PBS-BSA-Tw), and twice with PBS-Tw. Anti-human IgA or IgG heavy chain-specific antibodies coupled with peroxidase (Zymed Laboratories, San Francisco, CA) were used for the detection of the antigen-antibody reaction (50 μL/well of 1:1,000 dilution). The antibodies were incubated overnight at 4°C and mildly shaken. After three consecutive washes (3 minutes each) with PBS-BSA-Tw, 50 μL/well of substrate solution was added [0.1 M citrate buffer pH 4.5 (10 mL) added with 10 mg o-phenylenediamine and 4 μL of 30% H₂O₂ solution] and allowed to react for 3 minutes; then, the reaction was stopped with 1 M H₂SO₄ (200 μL/well). Plates were read at 490 nm in a Micro-ELISA reader. An ELISA test for the presence of anti-amebic antibodies was considered positive when the O.D. value was above the cutoff point defined as the mean of the respective O.D. values obtained from nonparasitized control individuals (HIV⁻) plus two standard deviations (IgG = 0.29 and IgA = 0.53).

Statistical analysis. The statistical analysis was performed using SPSS 10.0 version software (SPSS, Chicago, IL). A descriptive exploratory analysis of the data was performed to assess the distribution of variables (age, gender, marital status, and sexual preferences) in HIV⁻/AIDS (experimental) and HIV⁻ (control) groups. χ² and/or χ² for linear trend testing were used to determine the level of association of E. histolytica infection to the HIV⁻/AIDS condition and study group’s immune status. Fisher’s exact test was used to evaluate the association between anti-E. histolytica antibodies and E. histolytica infection in both groups.

RESULTS

Characteristics of the study populations. Three hundred forty-three individuals were studied: 203 were HIV⁻/AIDS patients, and 140 were HIV⁻ contacts. In the HIV⁻/AIDS group the mean age was 42.3 ± 10.2 years, and 184 (91%) were males. The HIV⁻ contacts included 59 males (42%) and 81 females with an overall mean age of 33.0 ± 21.3 years. This group was recruited from close relatives or sexual partners of HIV⁻/AIDS patients. Only 12 (9%) contacts were active homosexuals compared with 137 (67%) in the HIV⁻/AIDS cohort.

Clinical categories of HIV⁻/AIDS patients were based on peripheral T-CD4⁺ lymphocyte cell counts and clinical manifestations (Centers for Disease Control and Prevention [CDC], Atlanta, GA). Thirty-three percent of the HIV⁻/AIDS patients were classified as stage A, 41.9% were stage B, and 25.1% were stage C. Eighty percent of the patients were diagnosed as HIV⁻ and/or AIDS 5 years prior to their participation in the current study.

Prevalence of parasites infection. Table 1 shows the prevalence of different intestinal parasites was determined by microscopic examination of stool samples. The most common parasites were protozoa, some of them potential pathogens for humans (e.g., G. lamblia and E. histolytica/E. dispar). Thirty-five (17.2%) HIV⁻/AIDS patients excreted at least one intestinal parasite compared with 37 (26.4%) HIV⁻ contacts. (P = 0.04). With exception of Endolimax nana, which

<table>
<thead>
<tr>
<th>Parasite frequency</th>
<th>HIV⁻/AIDS (N = 203)</th>
<th>HIV⁻ contacts (N = 140)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastocystis hominis</td>
<td>4 (1.9%)</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>Entamoeba coli</td>
<td>15 (7.3%)</td>
<td>14 (10.0%)</td>
<td>0.39</td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td>4 (1.9%)</td>
<td>1 (0.7%)</td>
<td>0.34</td>
</tr>
<tr>
<td>Entamoeba histolytica/Entamoeba dispar</td>
<td>12 (5.9%)</td>
<td>4 (2.9%)</td>
<td>0.188</td>
</tr>
<tr>
<td>Enteromonas hominis</td>
<td>3 (2.0%)</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>Endolimax nana</td>
<td>13 (6.4%)</td>
<td>28 (20.0%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Isospora belli</td>
<td>1 (0.4%)</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>Iodamoeba butschlii</td>
<td>3 (1.4%)</td>
<td>1 (0.7%)</td>
<td>0.51</td>
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</tbody>
</table>

Differences in frequency of parasitic infection between groups were statistically significant (P = 0.06); ND, not determined.
was more common in HIV\(^-\) contacts, the frequency of other protozoa was similar in both studied groups (Table 1). The *E. histolytica*/*E. dispar* complex was detected in 5.9% of HIV\(^+\)/AIDS patients and in 2.9% of contacts. Although not statistically significant, there was a twofold increase in frequency of *E. histolytica*/*E. dispar* in HIV\(^+\)/AIDS patients.

**PCR characterization of Entamoeba species.** In 158 HIV\(^+\)/AIDS patients, the *E. histolytica* alone was detected in 12% (\(N = 19\)) of the analyzed stool samples, while *E. dispar* alone was found in 8.9% (\(N = 14\)), and both *Entamoeba* species were detected in 13.3% (\(N = 21\)) of patients (Table 2). In 130 HIV\(^-\) contacts, 17.7% (\(N = 23\)) were positive for *E. histolytica*. *E. dispar* was detected in 3.1% (\(N = 4\)) and *E. histolytica + E. dispers* were detected in 0.7% (\(N = 1\)). Because *E. histolytica* and *E. dispers* share biological cycles, ecological niches and transmission mechanisms in humans, HIV\(^+\)/AIDS patients colonized with *E. histolytica* and/or *E. dispers* could be considered as a single group. The aim of the analysis was to define risk factors that may be associated with the presence of *E. histolytica* and/or *E. dispers* intestinal infection. Prevalence of *E. histolytica* (including *E. histolytica* and *E. histolytica + E. dispers*) in HIV\(^+\)/AIDS patients was higher (25.3%) than in contacts (18.4%), but not significantly so (\(P = 0.16\)). However, the prevalence of *Entamoeba* infection (Eh\(^+\)/Eh\(^+\)Ed\(^+\)Ed) was significantly greater (\(P = 0.019\)) in HIV\(^+\)/AIDS patients (34.1%, 54 of 158) compared with HIV\(^-\) contacts (21.5%, 28 of 130). Further analysis of the separate *Entamoeba* species showed that this difference was due to greater prevalence of *E. dispar* in HIV\(^+\)/AIDS patients (22.2%, 35 of 158) versus HIV\(^-\) contacts (3.8%, 5 of 130) (\(P = 0.001\)). When categorized in this fashion, *E. histolytica* and/or *E. dispar* colonization was not significantly associated with CD4 lymphocyte count, the stage of disease, or viral load, even though there is a trend toward a greater percent of infected patients with worsening HIV disease (Table 3).

**Association between anti–*E. histolytica* antibodies and *E. histolytica* infection.** Serum anti-amebic antibodies may serve as markers for *E. histolytica* infection. Anti-amebic antibody response, in serum and saliva were, measured in both study populations. Anti-*E. histolytica* serum IgG was found in 59.4% (23 of 37) of HIV\(^+\)/AIDS patients colonized with *E. histolytica*, and 49% (53 of 108) of those who were not. In the HIV\(^-\) contacts only 2 of 6 *E. histolytica–*colonized individuals (33.3%) had high IgG levels versus 13 of 29 (45.0%) noncolonized individuals.

Specific salivary IgA was found in 39.5% (15 of 38) of *E. histolytica–*colonized HIV\(^+\)/AIDS patients versus 40.2% (43 of 107) of non-*E. histolytica–*parasitized patients. In HIV\(^-\) contacts, the occurrence of secretory IgA was essentially the same in *E. histolytica–*colonized (2 of 4 [50%]) and non-*E. histolytica–*colonized (17 of 33 [51.5%]) individuals. None of the associations between the presence of specific antibody and *E. histolytica* colonizations was statistically significant.

**DISCUSSION**

The aim of the current study was to determine the prevalence of enteric protozoa *E. histolytica* and *E. dispers* in HIV\(^+\)/AIDS patients and their contacts in Mexico City. Further, the immunologic status and clinical stages of HIV infection were examined for a potential association with *E. histolytica* and/or *E. dispers* intestinal colonization. Intestinal parasites have been identified as causal agents of diarrhea in HIV\(^+\)/AIDS patients in the industrialized world, an occurrence that is even more likely in the developing world where intestinal parasites

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**TABLE 2**

<table>
<thead>
<tr>
<th>Study groups</th>
<th>HIV(^+)/AIDS ((N = 158))</th>
<th>HIV(^-) contacts ((N = 130))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>19 (12.0%)</td>
<td>23 (17.7%)</td>
<td>0.17</td>
</tr>
<tr>
<td><em>E. histolytica + E. dispar</em></td>
<td>21 (13.3%)</td>
<td>1 (0.7%)</td>
<td>ND</td>
</tr>
<tr>
<td><em>E. dispers</em></td>
<td>14 (8.9%)</td>
<td>4 (3.1%)</td>
<td>ND</td>
</tr>
<tr>
<td>Other protozoa</td>
<td>18 (11.4%)</td>
<td>44 (33.9%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Negative</td>
<td>86 (54.4%)</td>
<td>58 (44.6%)</td>
<td>0.097</td>
</tr>
</tbody>
</table>

ND, not determined.

---

**TABLE 3**

<table>
<thead>
<tr>
<th>Gender</th>
<th><em>E. histolytica</em> infection and immunological status in HIV(^+)/AIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. histolytica</em> infection (Eh(^+)/Eh(^+)Ed(^+)Ed)</td>
</tr>
<tr>
<td></td>
<td>(N = 54)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Females</td>
</tr>
<tr>
<td></td>
<td>Males</td>
</tr>
<tr>
<td>Sexual preference</td>
<td>Homosexual</td>
</tr>
<tr>
<td></td>
<td>Heterosexual</td>
</tr>
<tr>
<td></td>
<td>Bisexual</td>
</tr>
<tr>
<td>Viral load (copies/mL)</td>
<td>(0–10,000)</td>
</tr>
<tr>
<td></td>
<td>(10,001–30,000)</td>
</tr>
<tr>
<td></td>
<td>(&gt; 30,000)</td>
</tr>
<tr>
<td>CD4 lymphocyte count (cells/mm(^3))</td>
<td>(\geq 500)</td>
</tr>
<tr>
<td></td>
<td>200–499</td>
</tr>
<tr>
<td></td>
<td>(&lt; 200)</td>
</tr>
<tr>
<td>Clinical categories</td>
<td>A1–A2</td>
</tr>
<tr>
<td></td>
<td>B1–B2</td>
</tr>
<tr>
<td></td>
<td>A3, B3, C1–C3</td>
</tr>
</tbody>
</table>

* for linear trend.
(protozoa or helminthes) are highly endemic. Indeed, increased prevalence of intestinal parasitic diseases have been documented in HIV-infected individuals and in some cases are related to the progression of HIV infection. Mexico is considered highly endemic for E. histolytica infection, including some geographic regions where the infection is especially prevalent.

The current study describes two closely related populations, HIV+/AIDS patients and their HIV− contacts, and show that both groups were equally likely to have intestinal parasitic infections. Opportunistic parasite infections (Blastocystis hominis, Isospora belli) were detected in only 5 HIV+/AIDS patients. We also observed an equally high frequency of multiparasitism in both groups (Table 1). These two observations are consistent with previous studies conducted in other developing countries.

We speculated that both groups would be equally exposed to E. histolytica and/or E. dispar infection given their close physical contact and similar environments but might differ in their susceptibility to infection. There are previous reports where the apparent association between HIV infection and E. histolytica/E. dispar protozoa is unlikely to be the result of sociodemographic characteristics. Studies conducting multivariate analysis while controlling for different variables including sexual behavior, showed that E. histolytica/E. dispar infection was more frequently diagnosed in men who have sex with men, than in persons with exposure to other parasites.

In our study, the prevalence of E. histolytica and/or E. dispar colonization was significantly greater in HIV+/AIDS patients than in non-HIV infected contacts (P = 0.019); however, this difference was a consequence of higher prevalence of E. dispar infection in the HIV+/AIDS group (22%) versus (3.8%) in non-HIV-infected contacts (P = 0.001) (Table 2).

On the other hand, E. histolytica and/or E. dispar infection were not significantly related to HIV status, sexual behavior, or gender (Table 3). However, there was a trend toward a higher percent of infected individuals as the HIV infection progressed.

Previous studies show that the role of sexual behavior in the higher frequency of E. histolytica/E. dispar infection in homosexual communities is controversial. Increased fecal-oral contact may place these individuals at higher risk for HIV as well as amebic infection. However, the association between HIV and amebic infection may be more relevant in communities with low-background transmission of E. histolytica, such as homosexual communities in industrialized countries. In underdeveloped countries, like Mexico, with a high prevalence for amebic infection, the observed association may be related to the higher exposure to the parasite. In our study, we explored the association of amebic infection in two different populations (HIV+/AIDS and HIV− individuals) exposed to the same environment and parasitic infectious sources. When the Entamoeba species was characterized through PCR assays, HIV+/AIDS patients and HIV− contacts showed a similar prevalence for the E. histolytica species both as a single species or detected together with the E. dispar species (P = 0.16) (Table 2). However, as we previously mention, prevalence of E. histolytica and/or E. dispar was greater in HIV+/AIDS group than in HIV contacts (P = 0.019).

We further examined the susceptibility of HIV+/AIDS patients to E. histolytica and/or E. dispar infection as related to the immune status. In our study, no significant association was found between infection with E. histolytica and/or E. dispar, and the stage of the HIV infection, including T-CD4+ lymphocyte cell counts (Table 3). However, our results can be potentially biased due to the limited number of individuals in the study, and the effect of this circumstance on the statistical analysis. Additional studies may reveal significance in cases where trends or no differences were seen. Interestingly, none of the individuals infected with the E. histolytica (whether HIV+ or HIV−) had diarrhea or invasive amebiasis within 12 months before the study. Further, the presence of secretory or systemic anti-amebic antibody response was not associated with E. histolytica invasive infection in either study population. These results agree with other reports where the seropositivity of the population exposed to E. histolytica does not correlate with the presence of intestinal colonization. In Japan, nearly all showed high levels of serum anti-amebic antibodies in a population where intestinal and/or amebic liver abscesses were present in 14 of 30 individuals infected with the E. histolytica species.

These data confirm the high prevalence of E. histolytica strains in Mexico, particularly in Mexico City. However, during the 12 months of the study, no one developed a clinical event attributable to E. histolytica infection. This suggests that the study populations were colonized with E. histolytica strains of low pathogenic potential. Recently, the polymorphic structure of E. histolytica has been described. Preliminary data concerning the polymorphism of serine-rich protein and chitinase loci of E. histolytica and E. dispar in isolates obtained from patients with invasive amoebiasis and from asymptomatic cyst passers from different geographic areas in Mexico suggested a complex geographic distribution of different strains of both Entamoeba species. However, no correlation was found between a particular polymorphic pattern of a given E. histolytica isolate and the invasive or commensal behavior within the human host.

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Reprint requests: Dr. Cecilia Ximénez, Depto. de Medicina Experimental, Facultad de Medicina, UNAM, Dr. Balmis 148, Col. Doctores, C.P. 06726, México D.F., México. Telephone: +5255-56-23-26-66, Fax: +5255-56-23-26-79, E-mail: eximenez@servidor.unam.mx.
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