MICROSPORIDIOSIS IN VENEZUELA: PREVALENCE OF INTESTINAL MICROSPORIDIOSIS AND ITS CONTRIBUTION TO DIARRHEA IN A GROUP OF HUMAN IMMUNODEFICIENCY VIRUS-INFECTED PATIENTS FROM ZULIA STATE

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Abstract. Microsporidia are recognized as a cause of morbidity among patients infected with the human immunodeficiency virus (HIV). Infection rates for intestinal microsporidiosis in HIV-infected patients from Venezuela are unknown. To determine the prevalence and pathogenic role of microsporidia in these patients in northwestern Venezuela, a case control study was conducted in 103 outpatients (mean ± SD age = 37.3 ± 5.6 years). Microsporidia were detected using unconcentrated formalin-fixed stools examined by Weber’s chromotrope-based staining method. For identification of coccidia, modified Ziehl-Neelsen carbolfuchsin staining of formalin-ether concentrates were used, and for other pathogenic parasites, iron hematoxylin–stained smears and formalin-ether concentrates were examined. Microsporidial infections were detected in 14 (13.6%) of 103 patients and 39 (37.9%) had other parasitic pathogens. No significant difference was noted in the occurrence of the infection in patients with diarrhea (13 of 74, 17.6%) and controls (1 of 29, 3.4%) (P = 0.118). Nevertheless, this result may be due to the small sample size (n = 14) of infected individuals. The proportions of other pathogens in patients with or without diarrhea were not significantly different (P = 0.828). Microsporidiosis is common among the HIV-infected population in northwestern Venezuela. However, its pathogenic role in these patients is uncertain and warrants further investigation.

INTRODUCTION

Chronic diarrhea is common in patients with human immunodeficiency virus (HIV) infection,1 however, in up to 50% of patients, no enteric pathogen can be identified.2–3 The two main explanations proposed to account for these unexplained diarrhea are intestinal infection with HIV that has been associated with enteropathy in patients with acquired immunodeficiency syndrome (AIDS)4 and infections with as yet unidentified enteric organisms. Recent studies have focused on the role of microsporidia as a cause of chronic diarrhea in patients with AIDS. These organisms are considered to be a cause of emerging and opportunistic infections in immunodeficient individuals worldwide.5,6 The prevalence of microsporidial infection in HIV-infected patients is relevant; in those with AIDS infection rates up to 50% have been reported and an approximate overall prevalence of 15% has been estimated.6 In Latin America, percentages of infection from 2.9% to 33% in HIV-infected patients have been found.7–12

Recent immunologic evidence suggests that latent infections may be quite common in the general population.13–15 In symptomatic immunocompetent individuals, a self-limited diarrhea is the most common clinical manifestation. In patients with AIDS, microsporidial infection is recognized as an increasingly important cause of morbidity and is responsible for significant gastrointestinal and disseminated diseases.5,6 Many reports have associated this infection with chronic diarrhea in AIDS patients throughout the world,5,16–20 and it is considered the most common intestinal infection associated with gastrointestinal symptoms in these patients.5 The factors that predispose these people to chronic microsporidiosis rather than self-limited illness appear to be immunologic.6 In fact, persistent diarrhea, malabsorption, and wasting, which are the most common clinical manifestations associated with the infection in patients with AIDS, are observed in those with ≤ 100 CD4 cells/mm3 16–20. Despite several studies of patients with gastrointestinal illness attributed to enteric microsporidia, some reports have concluded that these parasites have limited pathogenicity, even in patients with CD4 cell counts ≤ 100.21–23

Limited information is available on the potential etiologies of diarrhea in HIV-infected patients from the developing world. In Venezuela, the extent of microsporidial infection and its effects on human health are unknown; the literature concerning this infection is scarce. A recent study of 35 HIV-infected subjects from the southeastern area of this country showed no intestinal microsporidia.24 However, this result might be due to the small sample size or the immunologic status of the patients studied because the widespread occurrence of intestinal microsporidiosis in HIV-infected patients5,6 and the frequency of the infection in the general population13–15 suggest that microsporidia are ubiquitous organisms found throughout the environment.

In developing countries, diarrhea is quite common, occurring in 60–90% of HIV-infected individuals.25 Therefore, studies concerning microsporidia in these patients are a compelling issue in Venezuela where high prevalences of intestinal parasites have been reported.26–28 The objective of this study was to assess the prevalence of intestinal microsporidiosis and its contribution to diarrheal illness in a group of HIV-infected patients from Zulia State, Venezuela.

MATERIALS AND METHODS

Patient population. The study population included 103 consecutive HIV-infected adult patients attending the Unidad Regional de Inmunología Clínica in Maracaibo, the capital city of Zulia State in northwestern Venezuela. This unit is a
referral center for these patients and provides HIV testing. The HIV serostatus is determined by a repetitively enzyme immunoassay and confirmed by Western blotting. The patients were referred to us by the unit staff for parasitologic and medical assistance or were recruited directly by the investigators. Informed consent was obtained from all participants before clinical and parasitologic studies, and all aspects of the study were reviewed and approved by the Academic Committee of the Postgraduate Institute for the Study of Immunoimmunology, Faculty of Medicine, Universidad del Zulia.

**Clinical evaluation.** Basic demographics, presumed modes of HIV exposure, clinical categories of HIV infection, as defined by the Centers for Disease Control (CDC).29 Symptoms relating to enteric disease, recent (within one month) CD4 cell counts, and history of anti-infected drugs were reviewed in the medical records supplied by physicians who referred the patients and evaluated information obtained in interviews of the participants. Each patient underwent a physical examination.

Patients were classified into cases or controls on the basis of clinical findings. Cases included patients with diarrhea defined as the presence of three or more stools per day that were looser than usual for a period of three or more days before examination. Chronic diarrhea, continuous (two or more loose stools per day for ≥1 month) or intermittent (episodes of two or more loose stools per day alternating with episodes of formed stools for ≥1 month), was defined according to the CDC.29 Control patients were those who had no current or history of diarrheal disease during the preceding month.

**Stool examination.** The patients were given stool containers and asked to return three fresh specimens during a period of 10 days. Immediately after fecal collections, a portion of each specimen was preserved in 10% formalin solution. For identification of microsporidia, unconcentrated formalin-fixed stools from 103 patients were examined by the Weber’s chromotrope-based staining method.30 Each stained smear was examined by light microscope under oil immersion at a magnification of 100× for at least 15 minutes before it was considered negative. For identification of Cryptosporidium spp. and Cyclospora cayetanensis infections, modified Ziehl-Neelsen carbol-fuchsin staining of formalin-ether concentrates was used.31 For other potential pathogenic parasites, we examined iron-hematoxylin–stained smears and formalin-ether concentrates.32 Investigations for bacterial and viral pathogens were not conducted.

**Statistical analysis.** For normally distributed continuous variables, means were compared by the Student’s t-test. Categoric variables were compared by the chi-square test and Fisher’s exact test, where appropriate. A P value <0.05 was considered statistically significant.

**RESULTS**

Of the 103 HIV-infected patients enrolled, 95 were males and 8 were females. The age range was 23–52 years (mean ± SD age = 37.3 ± 5.6 years). They came from different areas of Zulia State; most (83 of 103, 80.6%) resided in poor sectors. The risk factors for acquisition of HIV infection included 72.8% (75 of 103) with homosexual or bisexual behavior and 27.2% (28 of 103) with heterosexual behavior with or without a history of transfusion or intravenous drug use. Fifty nine (57.3%) of the patients had AIDS. The mean ± SD CD4 cell count of all studied individuals was 159.5 ± 108.8 cells/mm³ (range = 10–414 cells/mm³). All participants were receiving antiretroviral therapy, 11 (10.7%) had recently taken alendazole at a single dose of 400 mg for the treatment of intestinal parasites other than microsporidia, and 16 (15.5%) were receiving or had received other anti-infected drugs. Of the 103 patients, 74 (71.8%) had diarrhea (cases) and 29 (28.2%) did not have gastrointestinal illness (controls). In the cases, stools were loose to watery; most (49 of 74, 66.2%) had persistent (continuous or intermittent) diarrhea for 2 to 8 months and all experienced weight loss averaging 18 kg during this period.

Comparison of cases and controls (Table 1) showed two statistically significant differences. The CD4 cell counts were significantly lower (133.8 cells/mm³ versus 226.3 cells/mm³; P < 0.01) and the proportion of AIDS patients was significantly higher (66.2% versus 34.5%; P < 0.01) in cases than in controls.

Most (90/103, 87.4%) of the patients submitted three stool specimens; 289 samples were examined. Microsporidial infections were identified in 14 (13.6%) of 103 patients. Only 5 of 14 infected patients had enough spores to be readily identified. In the remainder, they were scarce and their identification was time-consuming. In three of the patients who had loose fecal specimens, the infection was not detected in the initial examination. The infected patients came from 10 different areas of Zulia State. High overall infection rates were found with one or more pathogenic parasites (46 of 103, 44.7%) and pathogens other than microsporidia (39 of 103, 37.9%).

The results of stool examinations in cases and controls are shown in Table 2. The prevalence of microsporidia among cases and controls (13 of 74, 17.6% versus 1 of 29, 3.4%; P = 0.118) and the prevalence of parasitic pathogens other than microsporidia among cases and controls (29 of 74, 39.2% versus 10 of 29, 34.5%; P = 0.828) were not significantly different.

Of 14 patients with microsporidiosis, a high percentage (10 of 14, 71.4%) were men with homosexual or bisexual behaviors. However, no significant difference was noted in the prevalence of these behaviors in patients with or without the infection (10 of 49, 20.4% versus 6 of 59, 10.2%; P = 0.843). Ten (71.4%) of 14 patients had AIDS. The mean ± SD CD4 cell counts of 14 patients with microsporidiosis was 140.2 ±

### Table 1

<table>
<thead>
<tr>
<th>Category</th>
<th>Cases (n = 74)</th>
<th>Controls (n = 29)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD age, years</td>
<td>37.4 ± 5.7</td>
<td>36.9 ± 5.5</td>
<td>0.439</td>
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<tr>
<td>Sex, males/females</td>
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<td>28/1</td>
<td>0.538</td>
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<td>Homosexual or bisexual men, no. (%)</td>
<td>54 (80.6)</td>
<td>21 (75.0)</td>
<td>0.850</td>
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<td>AIDS, no. (%)</td>
<td>49 (66.2)</td>
<td>10 (34.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean ± SD CD4 count</td>
<td>133.8 ± 75.8</td>
<td>226.3 ± 149.0</td>
<td>&lt;0.01</td>
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<tr>
<td>Parasitic pathogens other than</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>microsporidia, no. (%)</td>
<td>29 (39.2)</td>
<td>10 (34.5)</td>
<td>0.828</td>
</tr>
<tr>
<td>Alendazole-treated patients, no. (%)</td>
<td>9 (12.2)</td>
<td>2 (6.9)</td>
<td>0.671</td>
</tr>
</tbody>
</table>

* HIV = human immunodeficiency virus. AIDS = acquired immunodeficiency syndrome.
82.9 cells/mm$^3$ (range = 30–358 cells/mm$^3$). There was no significant differences between CD4 cell counts of patients with or without microsporidia (140.2 ± 82.9 cells/mm$^3$ versus 165.7 ± 114.5 cells/mm$^3$; $P = 0.960$). However, significant lower CD4 cell counts were observed in 13 patients with the infection and diarrhea compared with the remainder patients (123.5 ± 56.4 cells/mm$^3$ versus 165.7 ± 114.5 cells/mm$^3$; $P < 0.05$). Eight (61.5%) of these 13 patients had chronic diarrhea (continuous or intermittent) for 3 to 7 months and weight loss averaging 16 kg during this period. Half of the patients (7 of 14) had microsporidia as the sole parasitic pathogen, and five had chronic, watery, non-bloody diarrhea, weight loss, and CD4 cell counts ≤ 100 cells/mm$^3$. Of the 14 patients with microsporidia, one had taken albendazole; no significant differences were observed in the proportions of albendazole-treated patients with and without the infection (1 of 14, 7.1% versus 10 of 89, 11.2%; $P = 0.996$) and with or without diarrhea (9 of 74, 12.2% versus 2 of 29, 6.9%; $P = 0.671$).

**DISCUSSION**

This study is one of the few on parasitic etiologies of diarrhea in the HIV-infected population in Venezuela and appears to be the first to demonstrate enteric microsporidial infection in this population. Our data further support the observation that chronic diarrhea is a common manifestation of infection with HIV. In this study, 71.8% of the patients had diarrhea illness and in most (66.2%) it was chronic. Case patients had more advanced HIV disease as assessed by significantly higher prevalence of AIDS ($P < 0.01$) and lower CD4 cell counts ($P < 0.01$).

The overall infection rate (14 of 103, 13.6%) with microsporidiosis among HIV-infected patients in this report was similar to the estimated total prevalence (15%) of the infection in AIDS patients worldwide$^8$ and was within the range of prevalence rates (3.9–33.3%) reported in Latin America.$^2$–$^12$ The fact that the 14 patients with microsporidia came from 10 different sectors of Zulia State suggests that the organisms are widely distributed in the region. In our patients, microsporidial infection was common, particularly in the case group in which a high infection rate (17.6%) was noted. Since non–HIV-infected controls matched to the patients were not used in our study, it is not clear if the frequency of microsporidia observed is truly associated with HIV infection or merely reflects a high risk of exposure to the parasites by a large part of the general population. In Venezuela, most of the people lives in poverty with favorable conditions for the survival and transmission of intestinal parasites, which remain highly endemic.$^{26–28}$ Most (80.6%) of our patients resided in poor sectors. Although background rates of microsporidiosis in Venezuela are unknown, the high endemicity of intestinal parasites in Zulia State$^{26,27}$ and evidence that latent microsporidial infections are common in healthy populations$^{13–15}$ suggest that microsporidiosis in our patients might be a latent infection, rather than an opportunistic infection, that would become symptomatic with the progression of immunosuppression of the patients. We observed microsporidia in a patient without diarrhea with a CD4 cell count of 358 cells/mm$^3$, which is above the severely immunodeficiency range of ≤ 100 cells/mm$^3$ reported in HIV-infected patients with microsporidiosis.$^{16–20}$

In this study, we observed a greater prevalence of microsporidial infection in the cases. Nevertheless, there was no association between the presence of the organisms and diarrhea ($P = 0.118$). Although this finding is consistent with findings of other reports,$^{1–23}$ it was unexpected since the infection has been strongly linked with diarrhea and wasting in numerous HIV-AIDS patients with characteristic histopathologic and functional abnormalities.$^{16–20}$ Possible explanations for the absence of an association between microsporidiosis and diarrhea in this study might include one or more of the following: 1) the small sample size ($n = 14$) of the infected subjects could have limited the statistical power of the analysis; 2) patients with microsporidia had a mean ± SD CD4 cell count of 140 ± 82.9 cell/mm$^3$, which is above the severely immunodeficient range of ≤ 100 cells/mm$^3$, as previously reported in HIV-infected patients with microsporidiosis treated with albendazole.$^{16–20}$ 3) infection was not sufficiently intense to cause disease, as previously suggested; $^{21}$ 4) the association may be detected after several stool examinations; $^7$ and 5) use of albendazole in 10.7% of the patients and antiretroviral therapy in all of them may be directly or indirectly preventing microsporidial infections.$^{34}$ However, the single dose of albendazole used by 11 patients was very low and did not appear to play an important role against the infection because no significant differences were found in the proportions of albendazole-treated patients with or without microsporidia ($P = 0.996$) and with or without diarrhea ($P = 0.671$).

Although no association was found between microsporidiosis and diarrheal illness in our patients, bacteria and viruses would likely account for the symptoms in some of the patients. However, we did not investigate the presence of these pathogens. Five of the seven patients with microsporidia as the sole parasitic pathogens had chronic, watery, non-bloody diarrhea and weight loss, as described previously in microsporidiosis,$^{17}$ and ≤ 100 CD4 cells/mm$^3$. Therefore, it seems likely that there was a causal relationship between the infections and symptoms, at least in these patients. The significantly lower CD4 cell counts ($P < 0.05$) observed in the patients with diarrhea and microsporidiosis compared with the remaining patients suggest that the pathogenic role of the parasite may be a late-occurring event in HIV-infected patients.

The absence of an association of diarrheal disease with

| Table 2 | Prevalence of intestinal microsporidia and other parasitic pathogens in 103 HIV-infected patients from Venezuela$^*$ |
| --- | --- | --- | --- | --- | --- |
| Pathogenic parasite | Cases (n = 74) | Controls (n = 29) |
| | | No | % | No | % |
| Microsporidia | 13 | 17.6 | 1 | 3.4 |
| Cryptosporidium spp. | 22 | 29.7 | 4 | 13.8 |
| Isospora belli | 6 | 8.1 | 2 | 6.9 |
| Cyclospora cayetanensis | 5 | 6.8 | 1 | 3.4 |
| Giardia lamblia | 10 | 13.5 | 3 | 10.3 |
| Entamoeba histolytica/E. dispar | 5 | 6.8 | 2 | 6.9 |
| Dientamoeba fragilis | 2 | 2.7 | 0 | 0.0 |
| Trichuris trichiura | 6 | 8.1 | 2 | 6.9 |
| Ascaris lumbricoides | 5 | 6.8 | 2 | 6.9 |
| Strongyloides stercoralis | 6 | 8.1 | 1 | 3.4 |
| Hymenolepis nana | 3 | 4.0 | 2 | 6.9 |
| Pathogens other than microsporidia | 29 | 39.2 | 10 | 34.5 |
| One or more pathogens | 36 | 48.6 | 10 | 34.5 |

$^*$ HIV = human immunodeficiency virus.
parasitic pathogens other than microsporidia suggests that other pathogens did not have a role in causing the diarrhea. This finding is consistent with the observation that in this region at the community level, most individuals infected with pathogenic parasites, including Cryptosporidium spp., are asymptomatic carriers, which is an unusual situation in developed countries.

This report may also provide insights into the epidemiology of enteric coccidia. Our findings confirm that Cryptosporidium spp. is the most common parasite in HIV-infected patients with diarrhea in Venezuela and high infection rates have been observed. The prevalence of C. cayetanensis was similar to that previously reported in AIDS patients from this area. Isospora belli, which is rare in the general population, was common in our patients. This result supports the observation that this parasite is more frequently found in the tropics.

In conclusion, our study shows that microsporidiosis is common among HIV-infected population in northwestern Venezuela. However, the role of microsporidia as enteropathogens in these patients is uncertain and warrants further investigation. This study provides new information on the epidemiology of microsporidia.

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