CRYPTOSPORIDIOSIS IN HIV-INFECTED VENEZUELAN ADULTS IS STRONGLY ASSOCIATED WITH ACUTE OR CHRONIC DIARRHEA

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Abstract. A cross-sectional study was undertaken to determine the prevalence of cryptosporidiosis and its clinical and laboratory pattern in Venezuelan HIV-infected patients (N = 397). At enrollment, they underwent thorough clinical history and physical examination and provided stool specimens for the identification of Cryptosporidium sp. and other parasites. Cryptosporidium sp. was identified in 59 subjects (15%). This infection was strongly associated with acute and chronic diarrhea, weight loss, CD4+ counts below 100 cells/mm³, older age in patients with leukopenia, and more than 5 stools per day when CD4+ counts were below 100 cells/mm³. In addition, patients with Cryptosporidium infection were less likely to be coinfected with Isospora belli (OR = 0.05, P = 0.001). In fact, results of the current study confirm the worldwide importance of cryptosporidiosis as a clinically significant opportunistic infection associated with an advanced stage of immunosuppression.

INTRODUCTION

Cryptosporidium sp. is considered an emerging pathogen by the U.S. Centers for Disease Control and Prevention. This parasite has been reported worldwide, especially infecting immunocompromised persons, and its prevalence varies according to different regions. A prospective long-term study from Europe suggested that 3–4% of those infected with human immunodeficiency virus (HIV) will have cryptosporidiosis when diagnosed with HIV infection and an equal number will develop it later in the course of their disease. No specific effective treatment exists for cryptosporidiosis, and an intact immune system is the major factor to resolve the infection. In this context, immune system restoration induced by highly active antiretroviral therapy (HAART) emerges as crucially important in those patients with cryptosporidiosis. Unfortunately, cryptosporidiosis remains a clinically significant opportunistic infection in HIV-infected patients without access to HAART, particularly in developing tropical regions. However, few data exist on the clinical characterization and the epidemiologic aspects of this disease in these countries. We therefore undertook a cross-sectional study of HIV-infected patients to provide some insight into the actual prevalence of cryptosporidiosis in Venezuela and to describe risk factors associated with this infection.

MATERIALS AND METHODS

Study population. This cross-sectional study took place between July 1997 and August 2002. Consecutive adult patients with confirmed HIV infection referred from different health centers to the Parasitology Department of the Faculty of Medicine of Universidad Central de Venezuela, Caracas, were included. These patients were referred to our laboratory as a part of their baseline investigations irrespective of their symptoms. Their informed consent was obtained, and the Research Unit of the “José María Vargas” School of Medicine and the Consejo de Desarrollo Científico y Humanístico (CDCH) of the Universidad Central de Venezuela approved the project and its ethical aspects.

Clinical evaluation. At enrollment, each participant underwent a thorough clinical history and physical examination and provided laboratory tests when available, such as blood count determination, CD4+ lymphocyte counts, and viral load within 6 months of enrollment. Information obtained via a questionnaire included demographic characteristics, current symptoms, and previous opportunistic infections and treatments.

Stool evaluation. Subjects provided three stool specimens from three different days. All participants were informed about the adequate techniques for sample collection, transport, and preservation. Fecal specimens were processed immediately after they were received. Each sample was divided into 4 portions to perform different coprological analysis: direct method using saline and lugol solutions mainly for the diagnosis of protozoa, Kato for the detection of helminths, Baermann for Strongyloides stercoralis, and Kinyoun stain for coccidian parasites. Participants were split in two groups: HIV-infected patients with cryptosporidiosis and all the remaining HIV-infected patients.

Definitions. A patient was considered infected with Cryptosporidium sp. if this parasite was detected in one or more stool specimens. A stool was considered positive for Cryptosporidium sp. if typical oocysts of 5 μm were observed with the Kinyoun technique. Diarrhea was defined as 3 or more unformed stools in a 24-hour period. A diarrheic episode was defined as acute when it was present for a period shorter than 3 weeks and as chronic if its duration was longer than three weeks. Weight loss was assessed in this study as a subjective appreciation of participants. It was considered significant when referred patients lost more than 10% of their baseline body weight within the period associated with diarrhea. Leukopenia was defined as less than 4,000 leukocytes × 10⁹/L blood. Relative eosinophilia was defined as more than 4 eosinophils in 100 leukocytes from peripheral blood. The relative value was considered to be due to the presence of leukopenia in most of the patients.

Statistical analysis. All numerical variables were summarized using mean and 95% confidence intervals if normally distributed or geometric mean if not. Median and inter-
quartile range (IQR) were used for those variables that included “0” and were not normally distributed. Categorical data were presented as proportions. Numerical data was also summarized into categories. The Student’s t test was used to compare the means of continuous variables, whereas categorical variables were compared using Fisher’s exact test or \( \chi^2 \) test.

Univariate and multivariate logistic regression analysis were performed to calculate odds ratios and likelihood ratio tests with Cryptosporidium infection as the main outcome. All variables were included in the crude analysis, even other parasite infections. Only those that were statistically significant in the crude analysis were analyzed in the multivariate model. They were checked also for interactions (effect modification). The general significance level was set at \( P \) value below 0.05. All statistical analysis was performed using Stata 7.0 software.

RESULTS

**Descriptive analysis.** During the study period, 397 HIV-infected patients were enrolled. They came to our service referred from other health care centers. There were 346 (87%) men and 51 (13%) women with ages ranging from 17 to 63 years, with a geometric mean of 35.8 years (95% CI: 33.8–35.8). Most of the patients came from urban communities; only 11 (3%) came from rural areas. There were 122 (30%) patients with acute diarrhea, 178 (45%) with chronic diarrhea, and 97 (24%) without diarrhea. An evaluation of T-lymphocyte populations revealed that 218 (55%) had CD4 count lower than 100 cells/mm\(^3\). Laboratory characteristics of the stool samples showed that 20 of 59 (34%) of Cryptosporidium –infected patients, CD4 lymphocytes ranged from 4 to 412 cells/mm\(^3\), with a median of 52 cells/mm\(^3\) (95% CI: 2,818.63–4,530.08 cells/mm\(^3\)), whereas the geometric mean for white blood cells for controls was 3,573 cells/mm\(^3\) (95% CI: 2,818.63–4,530.08 cells/mm\(^3\)), whereas the geometric mean for white blood cells for controls was 4,889.25 cells/mm\(^3\) (95% CI: 4,453.85–5,367.23) \( P = 0.006 \). In Cryptosporidium-infected patients, CD4 lymphocytes from 4 to 412 cells/mm\(^3\), with a median of 52 cells/mm\(^3\) (IQR: 15–161 cells/mm\(^3\)). In comparison, the control group had a median of 144 cells/mm\(^3\) (IQR: 44–300 cells/mm\(^3\)) \( P = 0.006 \). Laboratory characteristics of the stool samples showed that 20 of 59 (34%) of Cryptosporidium–infected patients had mixed infections with other parasites. The most common coinfections were Blastocystis hominis in 11 (19%) and Strongyloides stercoralis in 4 (7%).

**Univariate analysis.** In the univariate analysis, patients older than 35 years had an increased risk for cryptosporidiosis (OR = 2.4, 95% CI: 1.3–4.5, \( P = 0.003 \)). Also, the presence of diarrhea (OR = 6.8, 95% CI: 2.1–22.2, \( P < 0.001 \)), more than 5 stools per day (OR = 3.2, 95% CI: 1.2–8.4, \( P = 0.015 \)), weight loss (OR = 2, 95% CI: 1.1–3.5, \( P = 0.017 \)), absence of eosinophilia (OR = 0.4, 95% CI: 0.2–0.9, \( P = 0.015 \)), leukopenia (OR = 2.1, 95% CI: 1.1–4.0, \( P = 0.026 \)), and CD4 count < 100 cells/mm\(^3\) (OR = 2.2, 95% CI: 1.1–4.7, \( P = 0.035 \)) were significantly predictive for the risk of cryptosporidiosis.

**Multivariate analysis.** In the multivariate analysis (Table 2), cryptosporidiosis was used as the main outcome, and age, diarrhea, number of evacuations per day, weight loss, leukopenia, absence of eosinophilia, CD4 < 100 cells/mm\(^3\), and coinfection with I. belli were analyzed as explanatory variables. Patients with cryptosporidiosis had a higher risk of developing diarrhea, CD4 count lower than 100 cells/mm\(^3\), and weight loss. Additionally, patients with Cryptosporidium were less likely to have eosinophilia and to be coinfected with I. belli. As well, Cryptosporidium–infected patients were 2.6 times more likely to have 35 years of age or more with a marginal \( P \) value of 0.058. After adjusting all these ORs by each other, we found an effect modification between leukopenia and age. Those Cryptosporidium cases with leukopenia were more likely to be older than 35 years. An effect modification was found between number of stools per day and CD4 count. Patients with cryptosporidiosis and CD4 count lower than 100 cells/mm\(^3\) had a 26-fold higher risk of having more than 5 stools per day. There was an interaction between I. belli coinfection and eosinophilia. Those Cryptosporidium cases with coinfection with I. belli were more likely to have 5% of eosinophils or more. Cryptosporidium cases without eosinophilia were less likely to have coinfection with I. belli.

**DISCUSSION**

Our data demonstrate that Cryptosporidium infection is common among HIV-infected patients living in Caracas with...
Cryptosporidium

I. belli

It was observed too that CD4+ lymphocytes usually occur in patients with CD4 counts of < 150 cells/mm3. In our study, there was an interaction between age and leukopenia showing that this age-related risk is probably due to an increased cumulative probability for an advanced immunosuppression in HIV-infected patients.

In relation to the main clinical features of this infection, it was found that the prevalence of watery diarrhea in this patient population was very high, either acute or chronic, with a frequency of more than 5 evacuations daily. In previous studies, it has been described that chronic diarrhea is more common than acute diarrhea in patients with cryptosporidiosis.

In our study, there was also a significant proportion of patients with acute diarrhea. One explanation for this could be that these patients were seen immediately after the first symptoms, had access to an appropriate health care unit, and/or had access to a specialized laboratory for the diagnosis of parasitic infections, as has been described before for patients infected with I. belli.

It has been reported that Cryptosporidium-infected patients can have a 10% drop in body weight and severe malabsorption too. Although weight loss was assessed in this study as a subjective appreciation of participants, a significant association was found between this variable and cryptosporidiosis.

Analyzing laboratory results, we found that patients with cryptosporidiosis do not have eosinophilia. Our previous studies have shown that in patients infected by other intestinal coccidia such as I. belli, eosinophilia was strongly associated with isosporiasis. It was observed too that CD4+ count reaches statistical significance in the multivariate analyses as a predictor for the risk of cryptosporidiosis. Moreover, the media of CD4+ counts for patients with cryptosporidiosis was 52 cells/mm3, confirming previous observations showing that Cryptosporidium usually occurs in patients with CD4+ counts of < 100 cells/mm3. It has also been described that in patients with CD4+ counts of < 150 cells/mm3, Cryptosporidium produces a large volume of watery diarrhea that is usually progressive.

The presence of other copathogens was observed. However, after the statistical analysis, it seems that Cryptosporidium-infected patients are less likely to get isosporiasis at the same time. This current finding is consistent with other reports that have found lower prevalence of mixed infection by Cryptosporidium and Isospora compared to single infection with one of these two parasites, even though a similar advanced state of immunosuppression is a main risk factor for both and that they share an oral route of transmission.

On the other hand, a previous study has shown a protective immune response of Isospora felis–infected mice against Babesia microti infection due to cell-mediated immunity. However, whether Cryptosporidium-infected gastrointestinal epithelial cells elicit host immune responses remain to be assessed.

In summary, these data indicate that the prevalence of cryptosporidiosis is high among HIV-infected patients living in Caracas. Therefore, we think that the presence of this infection should be suspected in patients from tropical countries not only with chronic watery diarrhea but also with acute diarrhea and weight loss, especially in those with lower CD4+ counts. Consequently, it is recommended to consider stool examination for Cryptosporidium as a routine in the diagnosis of cryptosporidiosis.
of causes of diarrhea in HIV-infected patients. Further studies are necessary to define all of the risk factors for this disease.

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REFERENCES