Seasonal profile and level of CD4+ lymphocytes in the occurrence of cryptosporidiosis and cistoisosporidiosis in HIV/AIDS patients in the Triângulo Mineiro region, Brazil

Perfil sazonal e nível de linfócitos CD4+ na ocorrência de criptosporidiose e cistoisosporidiose em pacientes HIV/AIDS na região do Triângulo Mineiro, Brasil

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ABSTRACT

Patients with AIDS are particularly susceptible to infection with intestinal coccidia. In this study the prevalence of infections with Cryptosporidium sp and Cystoisospora belli were evaluated among HIV/AIDS patients in the Triângulo Mineiro region, Brazil. Between July 1993 and June 2003 faecal samples from 359 patients were collected and stained by a modified Ziehl-Neelsen method, resulting in 19.7% of positivity for coccidian (8.6% with Cryptosporidium sp, 10.3% with Cystoisospora belli and 0.8% with both coccidian). Patients with diarrhoea and T CD4+ lymphocyte levels < 200 cells/mm³ presented higher frequency of these protozoans, demonstrating the opportunistic profile of these infections and its relationship with the immunological status of the individual. It was not possible to determine the influence of HAART, since only 8.5% of the patients positive for coccidian received this therapy regularly. Parasitism by Cryptosporidium sp was more frequent between December and February and thus was characterised by a seasonal pattern of infection, which was not observed with Cystoisospora belli.

Key-words: Cryptosporidium sp. Cystoisospora belli. Isospora belli. HIV/AIDS.

RESUMO

Pacientes com AIDS são particularmente susceptíveis a infecção por coccídios intestinais e nesse estudo foi avaliada a frequência de Cryptosporidium sp e Cystoisospora belli entre pacientes HIV/AIDS na região do Triângulo Mineiro, Brasil. No período de julho de 1993 a junho de 2003, amostras de fezes de 359 pacientes foram submetidas à coloração pelo método de Ziehl-Neelsen modificado, sendo detectada a presença de coccídios em 19,7% destas (8,6% de Cryptosporidium sp, 10,3% de Cystoisospora belli e 0,8% de ambos coccídios). Pacientes com diarréia e níveis de linfócitos T CD4+ < 200 células/mm³ apresentaram maior frequência destes protozoários, demonstrando o perfil oportunista destas infecções e a relação com o status imunológico do indivíduo. Não foi possível determinar a influência da HAART, pois apenas 8,5% dos pacientes positivos para coccídios faziam uso regular desta terapia. Parasitismo por Cryptosporidium sp foi mais freqüente no período compreendido de dezembro a fevereiro caracterizando padrão sazonal desta infecção, fato não observado com Cystoisospora belli.

Palavras-chave: Cryptosporidium sp. Cystoisospora belli. Isospora belli. HIV/AIDS.

A high prevalence of gastrointestinal infections has been observed in AIDS patients since the first cases were described, especially those caused by opportunistic parasites. The rapid dissemination of human immunodeficiency virus (HIV) has increased considerably the incidence of these entomopathogens, especially Cryptosporidium sp and Cystoisospora belli, which are responsible for potentially severe diarrhoeic profiles with morbidity directly related to the degree of immunodepression20,24. The clinical profile may be presented as a self-limiting infection, acute or chronic diarrhoeic syndromes, with debilitating diarrhoea.
frequently accompanied by weight loss, dehydration, abdominal pain and poor absorption syndrome\textsuperscript{1, 7-11}. The chronic nature of these infections contributes to the increase in morbidity and mortality in these patients. Cryptosporidiosis may be associated with gastrointestinal syndrome and respiratory infections and recurrences are common. Infection rates vary from 0.6 - 85\% for Cryptosporidium\textsuperscript{12-15} and 0.2 - 20\% for Cystoisospora belli\textsuperscript{16,17}.

In Brazil, the prevalence of cryptosporidiosis in HIV/AIDS patients varies between 6.4-9.1\% while for cystoisosporidiosis the rate is 4.4-18.0\%\textsuperscript{12,18,19,20}. Cystoisosporidiosis is estimated to be the second most important gastrointestinal protozoan disease in AIDS patients, exceeded only by cryptosporidiosis\textsuperscript{21}.

In this study the frequency and seasonal profile of cryptosporidiosis and cystoisosporidiosis were evaluated among HIV/AIDS patients attended at the Hospital Escola da Universidade Federal do Triângulo Mineiro (UFTM). Possible associations based on examination of clinical and laboratory data of the patients were also investigated.

**MATERIAL AND METHODS**

Data were examined from previous studies on Cryptosporidium sp and Cystoisospora belli in faecal samples (diarrhoeic or not) from HIV/AIDS patients attended in the outpatients clinic or infirmary of the UFTM Department of Infectious and Parasitic Diseases between July 1993 - June 2003. This institution provides the regional reference centre for treatment of HIV/AIDS patients in Uberaba and the surrounding region. The examinations were performed in the parasitology laboratory, where the samples were submitted to sedimentation by the formalin-ether (Ritchie’s) method. Faecal smears were made form the sediment, stained by a modified Ziehl-Neelsen method and examined under the 100X objective of the light microscope to look for Cryptosporidium sp and Cystoisospora belli. Samples were considered to be negative when no oocysts were detected after observation of at least 100 microscopic fields.

Clinical data, quantification of T CD4\textsuperscript{+} lymphocytes, confirmatory examination of HIV infection and use of antiretroviral therapy (HAART) were obtained from the participants.

**RESULTS**

In all 638 faecal samples were examined from 359 patients (1.7 samples/patient). The ages of members of the study group varied from 2 - 72 with a mean of 33.3 (± 10.42). Males comprised 241 (67.2\%) of the participants and females 118 (32.8\%).

Coccidian were detected in 71 (19.7\%) of the patients, with 31 (8.6\%) presenting Cryptosporidium sp alone, 37 (10.3\%) infected with only Cystoisospora belli and 3 (0.8\%) harbouring both coccidian. The mean age of patients positive for coccidian was 32.42 (± 8.62), 48 (67.6\%) being male and 23 (38.4\%) female.

Diarrhoeic syndrome as a clinical indication of coccidian in the faeces was reported in 252 (70.2\%) of the cases. Of these, 54 (21.4\%) were positive for coccidian, 22 (8.7\%) harbouring Cryptosporidium sp, 29 (15.5\%) Cystoisospora belli and 3 (1.2\%) both coccidian. In the group of patients without diarrhoea, prevalence of coccidian was 15.8\% (17/107), with 9 (8.4\%) positive for Cryptosporidium sp and 8 (7.4\%) for Cystoisospora belli (Table 1).

T CD4\textsuperscript{+} lymphocytes were quantified in 64 (17.8\%) of all patients and a mean value of 194.9 ± 215.3 cells/mm\textsuperscript{3}. Of these patients, 50 (78\%) presented diarrhoea and 14 (21.8\%) coccidian in the faeces, with 7 (50\%) positive for Cryptosporidium sp, 6 (43\%) with Cystoisospora belli and 1 (7\%) with both coccidian (Table 2).

Eleven of the 14 (78.6\%) patients who presented coccidian in the faeces had diarrhoea and a T CD4\textsuperscript{+} lymphocyte count equal to or less than 200 cells/mm\textsuperscript{3}. Of these patients, 32 (78.6\%) presented diarrhoea and 37 (91.4\%) with diarrhoea no coccidian, 6 (15\%) with diarrhoea and 32 (85.7\%) without diarrhoea and 37 (91.4\%) with diarrhoea and 3 (7.8\%) without diarrhoea and 3 (7.8\%) coccidian in the faeces, with 7 (50\%) positive for Cryptosporidium sp, 6 (43\%) with Cystoisospora belli and 1 (7\%) with both coccidian (Table 2).

The mean T CD4\textsuperscript{+} lymphocyte count was 215.3 cells/mm\textsuperscript{3}, 48 (67.6\%) being male and 23 (38.4\%) female.

<table>
<thead>
<tr>
<th>Intestinal coccidian</th>
<th>With diarrhoea</th>
<th>Without diarrhoea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n%</td>
<td>n%</td>
</tr>
<tr>
<td>Cryptosporidium sp</td>
<td>22</td>
<td>8.7</td>
</tr>
<tr>
<td>Cystoisospora belli</td>
<td>29</td>
<td>15.5</td>
</tr>
<tr>
<td>Both coccidian</td>
<td>3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Table 1 - Frequency of intestinal coccidian in faecal samples of HIV/AIDS patients presenting diarrhoea or not.

Table 2 - Cryptosporidiosis, Cystoisosporidiosis and diarrhoea in HIV/AIDS patients and their relationship with T CD4+ lymphocyte counts.

<table>
<thead>
<tr>
<th>T CD4\textsuperscript{+} counts</th>
<th>Patients with coccidian</th>
<th>Patients without coccidian</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with diarrhoea</td>
<td>without diarrhoea</td>
</tr>
<tr>
<td>&lt;50</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>51 - 200</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>201 - 400</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>401 - 600</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>601 - 800</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&gt; 800</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

| Total                            | 6           | 6                       | 1           | 1                       | 0           | 0                       | 57          | 13                      | 64                      |

| Table 2 - Cryptosporidiosis, Cystoisosporidiosis and diarrhoea in HIV/AIDS patients and their relationship with T CD4+ lymphocyte counts. |
The prevalence of Cystoisospora belli in the Triângulo Mineiro (8.6%) only exceeded that reported in the Ribeirão Preto region (6.4%)\(^2\). It was lower than the values found for HIV/AIDS patients in the cities of Uberlândia (15%)\(^3\), São Paulo (12.1%)\(^4\), Santos (19.1%)\(^11\) and Campinas (18%)\(^2\). In relation to Cystoisospora belli, the frequency (10.3%) was higher than that detected in São Paulo (5.7%)\(^9\) or Ribeirão Preto (4.4%)\(^2\), similar to rates in Rio de Janeiro (10.1%)\(^19\) and Santos (9.9%)\(^13\) and only lower than that in Campinas (18%)\(^1\).

When occurrence of diarrhoeic syndrome was evaluated as an indicator of the presence of these coccidian within the study population, a higher positivity was observed in patients with diarrhoea (21.4%), than in non-diarrhoeic individuals (15.8%). Similar results were reported by Rigo & Franco (2000), who found higher rates of both cryptosporidiosis (18.3%) and cystoisosporidiosis (6.6%) in HIV patients with diarrhoea than in non-diarrhoeic individuals (1.6%). According to these authors, this data illustrates the risk of overestimating the frequency of Cryptosporidium sp and Cystoisospora belli infection rates when only patients with diarrhoea are included in the study population.

In immunocompromised patients a T CD4+ lymphocyte level above 200 cells/mm\(^3\) is considered to be a limiting factor for opportunistic infections. Despite T CD4+ lymphocyte levels being quantified in only 17.8% of patients, the opportunistic character of intestinal coccidial infections could be perceived in 78.6% of the patients which presented coccidian in the faeces showing T CD4+ lymphocyte counts ≤ 200 cells/mm\(^3\). This fact was closely related to the cases of cryptosporidiosis, corroborating results already reported in the literature\(^1\). This association was also detected in cases of cystoisosporidiosis, although the high standard deviation in the T CD4+ lymphocyte counts observed in patients with Cystoisospora belli, demonstrated that this coccidioid is less than from the host immune response than Cryptosporidium.

According to the literature, the numbers of opportunistic infections in HIV/AIDS patients\(^13\) has diminished considerably since the introduction of HAART in 1996, including cases of cryptosporidiosis. However, in this study it was impossible to determine the influence of this therapy on infections with Cryptosporidium sp and Cystoisospora belli, since only 8.5% of patients which presented coccidian regularly received HAART.

In the Triângulo Mineiro region, as well as other parts of the world\(^16\)\(^17\)\(^25\), a seasonal pattern was noted for Cryptosporidium sp, with a higher occurrence during the hottest months of the year (January-March). Although frequency of cystoisosporidiosis cases was somewhat higher in the months of June - August, it was not possible to show seasonal behaviour of infection by this coccidian in the studied area, and this remains to be established elsewhere\(^1\).

The survey of Cryptosporidium sp and Cystoisospora belli in HIV/AIDS patients attended the UFTM, from June 1993 - June 2003, showed that these parasites occur at a similar frequency in the studied population and that are closely linked to the presence of diarrhoeic syndrome (particularly in cryptosporidiosis), with a drop in T CD4+ lymphocyte counts. However, the influence of

Only 8.5% (6/71) of the patients who presented coccidian in the faeces were receiving HAART.

The frequency distribution of coccidian per year of observation showed a higher occurrence of patients infected with Cryptosporidium sp during 1997 (23%) and with Cystoisospora belli in 2000 (18.8%) (Figure 1). When seasonal distribution of the two coccidian in HIV/AIDS patients over a 10-year period was evaluated, seasonal distribution of Cryptosporidium sp was found to peak between December and February. A slight rise in Cystoisospora belli infections was seen from June - August (Figure 2).

DISCUSSION

Factors such as immunological status of the patient, use or not of highly active antiretroviral therapy (HAART), level of basic sanitation and climatic diversities of the different regions, as well as methodological differences between the various studies, have all been used to explain differences in prevalence values of these coccidioses in the literature.
T CD4+ lymphocytes in infection with *Cryptosporidium parvum* needs to be better evaluated due to the reduced number of samples screened in the present study and to the possible recurrence of cases after treatment. These factors may be related to the immune response of the patient or even the presence of extraintestinal cysts, which are less susceptible to treatment.10-15.

ACKNOWLEDGEMENTS

Dr. Alexander Bruce for English review.

REFERENCES


