Article/Artigo

Detection of *Dientamoeba fragilis* in patients with HIV/AIDS by using a simplified iron hematoxylin technique

Detecção de *Dientamoeba fragilis* em pacientes com HIV/AIDS utilizando a técnica de hematoxilina férrica simplificada

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ABSTRACT

Introduction: Studies strongly indicate *Dientamoeba fragilis* as one of the causes of diarrhea in human immunodeficiency virus (HIV) patients. **Methods**: The objective of the present study was to evaluate the prevalence of *D. fragilis* associated with the causes of diarrhea in 82 HIV/AIDS patients hospitalized at the *Instituto de Infectologia Emílio Ribas* from September 2006 to November 2008. **Results**: In total, 105 samples were collected from 82 patients. Unprotected sex was the most frequent cause of HIV infection (46.3%), followed by the use of injectable or non-injectable drugs (14.6%). Patients presented with viral loads of 49–750,000 copies/mL (average: 73,849 ± 124,850 copies/mL) and CD4 counts ranging of 2–1,306 cells/mm^3 (average: 159 ± 250 cells/mm^3). On an average, the odds of obtaining a positive result by using the other techniques (Hoffman, Pons and Janer or Lutz; Ritchie) were 2.7 times higher than the chance of obtaining a positive result by using the simplified iron hematoxylin method. Significant differences were found between the methods (p = 0.003). **Conclusions**: The other techniques can detect a significantly greater amount of parasites than the simplified iron hematoxylin method, especially with respect to *Isospora belli, Cryptosporidium sp., Schistosoma mansoni*, and *Strongyloides stercoralis,* which were not detected using hematoxylin. *Endolimax nana* and *D. fragilis* were detected more frequently on using hematoxylin, and the only parasite not found by the other methods was *D. fragilis.*

Keywords: *Dientamoeba fragilis.* Diarrhea. AIDS. Simplified iron hematoxylin.

RESUMO

Introdução: Estudos indicam a *Dientamoeba fragilis* como uma das causas de diarréia em pacientes com HIV/AIDS. **Métodos**: Os objetivos deste estudo foram avaliar a prevalência de *D. fragilis* associadas com as causas de diarréia em pacientes com HIV/AIDS internados no Instituto de Infectologia Emílio Ribas (IIER). Oitenta e dois pacientes internados no IIER fizeram parte deste estudo de setembro de 2006 a novembro de 2008. **Resultados:** No total, 105 amostras foram coletadas a partir de 82 pacientes neste estudo. Sexo desprotegido foi à causa mais frequente para a aquisição do HIV (46,3%), seguido pelo uso de drogas injetáveis ou não injetáveis (14,6%). Relações heterossexuais foram os mais citados (19,5%). Pacientes apresentaram carga viral entre 49 e 750.000 (média de 7.849 ± 124.850) e CD4 variando de 2 a 1.306 (média de 159 ± 250). Em média, as chances de um resultado ser positivo com outras técnicas foram 2,7 vezes maiores do que a chance de um resultado positivo com hematoxilina férrica simplificada. Foram encontradas diferenças significativas entre os métodos (p=0,003). **Conclusões**: As outras técnicas são capazes de detectar uma quantidade significativa maior de parasitas em comparação com a hematoxilina férrica simplificada, especialmente em relação à *Isospora belli, Cryptosporidium sp., Schistossoma mansoni e Strongyloides stercoralis* que não foram encontrados utilizando a hematoxilina e a *Endolimax nana e D. fragilis* foram mais detectados pela hematoxilina férrica simplificada, principalmente a *D. fragilis* que não foi detectada pelos outros métodos.

Palavras-chaves: *Dientamoeba fragilis.* Diarréia. AIDS. Hematoxilina férrica simplificada.

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INTRODUCTION

Diarrhea is a common clinical manifestation in patients infected with the human immunodeficiency virus (HIV). It affects people in many underdeveloped countries, but it is also present in developed countries; therefore, it has an impact on the health sector[1,2].

Parasitic infections are commonly found in acquired immunodeficiency syndrome (AIDS) patients. From the studies on the etiology of diarrhea in AIDS, the study performed by Cimerman reported that parasites are the cause of diarrhea in up to 40% of these cases[3].

The involvement of the gut in the course of AIDS is of huge importance, not only because of its frequency but also primarily because of the associated morbidity[4].

Little is known about the genus *Dientamoeba*, which was first described in 1918 by Jepps and Dobell, or even about *Dientamoeba fragilis*, which has a worldwide distribution, causes irritable bowel syndrome, and is associated with allergic colitis and diarrhea in AIDS patients[2,5].

Dientamoeba fragilis is almost never investigated, and because there is no consensus on the characterization of this parasite, it appears only in the trophozoite form, which is easily destroyed by the conservation and flotation methods. Therefore, these techniques are not used for this pathogen[6].

Verification of the possible presence of *D. fragilis* as one of the pathogens that cause diarrhea in AIDS patients is essential, even with respect to their treatment. A simplified iron hematoxylin (SIH) stain was found to be a rapid and effective technique for the diagnosis of *D. fragilis*, considering its sensitivity to and specificity for the parasite.

This study evaluated the efficacy of the SIH stain to identify *D. fragilis* and assessed the prevalence of this parasite in HIV/AIDS patients with diarrhea.

METHODS

This was an observational, prospective, and prevalence study. We collected fecal samples from 82 patients from the *Instituto de Infectologia Emílio Ribas* (IIER), who had a positive serological diagnosis for HIV infection and clinical suspicion of diarrhea.

All the feces samples were smeared for SIH staining (2 slides) and 1 slide each was prepared for the techniques of sedimentation[7], centrifugation-sedimentation by the formalin-ether method[8], and Kinyoun staining[9]. Among the 82 patients for whom clinical data indicated diarrhea, the results for 1 patient were positive for *D. fragilis*.

The patients were informed that they were participating in a clinical study and provided consent.

The following methods were used to detect generic fecal parasites: spontaneous sedimentation, formalin-ether sedimentation, and the Kinyoun staining. SIH staining was used as a specific test for *D. fragilis*.

For statistical analysis of the descriptive variables, we used the following summary measures. For the continuous variables (i.e., age, viral load, and CD4 count), we calculated the average, standard deviation, median, range (minimum and maximum values), and number of patients. For the qualitative variables, their frequency and percentage occurrence in each case are presented. We calculated the 95% confidence interval (CI) for the proportion of cases of *D. fragilis* found by the SIH method. We calculated the sample size in order to obtain a CI for the proportion of *D. fragilis* parasites found.

For descriptive analysis, the Statistical Package for Social Sciences (SPSS) version 15 software for Windows was used, while the Number Cruncher Statistical System (NCSS) 2004 and Power Analysis and Sample Size (PASS) 2000 for Windows were used to calculate sample size.

Ethical considerations

The study was approved by the Ethics Committee of IIER (CEP-IIER) (Research Protocol No. 58/05; approved on June 22, 2006).

RESULTS

The average age of the 82 patients with HIV/AIDS who participated in this study was 37 years. Twenty (24.4%) patients were female and 62 (75.6%) were male. From the total population, 16 (19.5%) patients were heterosexual and 12 (14.6%) were homosexual.

Forty-one (50%) patients contracted HIV infection through unprotected sex, 12 (14.6%) through injected drugs, and 5 (6.1%) through vertical transmission.

CD4+ T cell lymphocyte counts ranged from 2–1,306 cells/mm^3 (median: 51 cells/mm^3), while the viral load had a variation of 49–750,000 copies/mL (median: 26,762 copies/mL).

Twenty different symptoms were reported by the study participants. The most common symptoms were diarrhea (95.1%), fever (48.8%), and vomiting (47.6%), followed by cough (15.9%), weakness, and weight loss (11%) (**Figure 1** and **Table 1**).

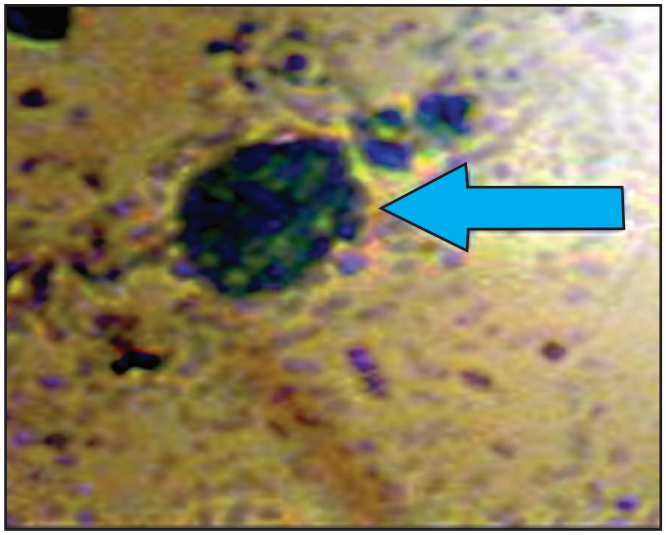

FIGURE 1 - Light microscopy of *Dientamoeba fragilis* (100× magnification) using the simplified iron hematoxylin stain.

TABLE 1 - Frequency of parasite detection, considering the number of occurrences (total number of patients), for the Hoffman, Ritchie, and Kinyoun methods.

	Simplified iron hematoxylin		Hoffman		Ritchie		Kinyounn*	
Parasites/techniques	**n***	**%**	**n***	**%**	**n***	**%**	**n***	**%**
Blastocystis hominis	1	1.2	1	1.2	1	1.2	1	1.2
*Dientamoeba fragilis***	1	1.2	0	0.0	0	0.0	0	0.0
Endolimax nana	2	2.4	3	3.7	3	3.7	0	0.0
Entamoeba coli	3	3.7	3	3.7	3	3.7	0	0.0
Entamoeba coli/Endolimax nana	1	1.2	1	1.2	1	1.2	0	0.0
Giardia lamblia	0	0.0	0	0.0	3	3.7	0	0.0
Schistosoma mansoni	0	0.0	1	1.2	0	0.0	0	0.0
Strongyloides stercoralis	0	0.0	2	2.4	2	2.4	0	0.0
Strongyloides stercoralis/Schistosoma mansoni	0	0.0	3	3.7	3	3.7	0	0.0
Isospora belli	0	0.0	0	0.0	0	0.0	6	7.3
Cryptosporidium sp.	0	0.0	0	0.0	0	0.0	9	11.0

*n: number of patients = 82; **p < 0.001 (generalization of Fisher's exact test and chi-square test for tables with expected frequencies < 5 units).

DISCUSSION

The population surveyed in this study corresponds to the classic epidemiological pattern of AIDS, as seen in the data reported by the Ministry of Health of Brazil in 2008. Most patients are young adults with an average age of 37 years, and 75.6% are male and 24.4% are female. The most common route of HIV infection in this study was from unprotected sex (46.3%). This study adopted a method involving the use of the Schaudinn fixative with mercuric chloride, which was reported by Horen[10], in which the core of *D. fragilis* stains better and water is not heated during the preparation of mercuric chloride, as recommended for the traditional SIH technique, because of the toxicity of mercury vapor.

In this study, we found no statistically significant difference between the Hoffman and SIH methods ($p = 0.216$) or between the SIH and Ritchie methods ($p = 0.244$), although some parasites (i.e., *Giardia lamblia, Schistosoma mansoni, Strongyloides stercoralis, and Strongyloides stercoralis/Schistosoma mansoni*) are most frequently detected with the Hoffman method. *D. fragilis* was detected in 1.2% of the 82 patients by using the SIH method and was the only parasite not detected by the Hoffman[7] and Ritchie[8] methods, due to the fact that SIH is a more suitable stain for the detection of trophozoites, as reported by Garcia[11].

In several studies reporting the detection of this parasite, there was disagreement about the methodology used and divergence among the number of patients studied[12–14], indicating that in many cases, laboratories are unaware of the staining techniques, leaving the patient without an accurate diagnosis.

Recent molecular studies, such as the one performed by Starcks et al.[15], have reported a prevalence of 5.2% for *D. fragilis*, which is higher than that reported in other studies because it used the reverse transcription polymerase chain reaction to detect this parasite. This finding emphasizes the fact that as this test is more sensitive and has less interference than microscopic diagnosis, it could provide a more accurate diagnosis for this parasite.

Lainson and Silva[16] used the Giemsa technique for 34 HIV-positive patients and detected *D. fragilis* in 3% of subjects, stating, however, the need for a technique specific for amoebae.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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