Molecular characterization of viruses associated with gastrointestinal infection in HIV-positive patients

ABSTRACT

Background: Diarrhea is a major cause of morbidity and mortality among HIV-infected patients worldwide. **Objective**: We sought to determine the frequency of viral gastrointestinal infections among Brazilian HIV-infected patients with diarrhea. **Methods**: A collection of 90 fecal specimens from HIV-infected individuals with diarrhea, previously tested for the presence of bacteria and parasite was analyzed by polymerase chain reaction and sequence analysis for the presence of enteric viruses such as astrovirus, norovirus, rotavirus groups A, B and C, adenovirus, herpes simplex virus, Epstein-Barr virus, cytomegalovirus, and human bocavirus. **Results**: Twenty patients (22.2%; n = 90) were infected with parasites (11 single infections and nine coinfected with virus). Enteropathogenic bacteria were not found. Virus infections were detected in 28.9% (26/90) of the specimens. Cytomegalovirus was the most common virus detected (24.4%; 22/90). Coinfections with viruses and/or parasite were observed in 10 (11.1%) samples. **Conclusion**: Gastrointestinal virus infections were more frequent than parasitic or bacterial infections in this patient population.

Keywords: HIV infection; gastrointestinal disorders; cytomegalovirus; human bocavirus.

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INTRODUCTION

Gastrointestinal (GI) disorders are among the most common and debilitating conditions that affect individuals with AIDS/HIV[1] and diarrhea is a major cause of morbidity and mortality worldwide.[2] Although the introduction of HAART (Highly Active Antiretroviral Therapy) had reduced the prevalence of GI infections,[3] opportunistic viruses, parasites and bacteria pathogens can still be associated with GI dysfunctions in patients infected with HIV. Viral enteritis is an important GI manifestation among HIV-infected patients. Several viruses have been implicated of producing enteric disorders in those patients such as cytomegalovirus (CMV), herpes simplex virus (HSV), adenovirus (AdV), norovirus (NoV), astrovirus (AstV) and rotavirus (RV).[2,4-7] However, except for CMV, which is an established etiological agent of gastrointestinal disease in patients with HIV the role of other viruses remains unclear. We sought to determine the frequency of viral gastrointestinal infections among Brazilian HIV-infected patients with diarrhea.

A collection of 90 fecal specimens from HIV-infected individuals with diarrhea, between 11 and 71 years of age, obtained from August 2006 to December 2007 was screened by polymerase chain reaction (PCR) for the presence of viruses. Of these, 58 (64.4%) were collected from hospitalized patients and 32 (35.6%) from non-hospitalized patients. All patients were on antiretroviral therapy; nevertheless, no information regarding time on HAART and viral load were obtained. Only one fecal specimen was available per patient. Diarrhea was defined as the occurrence of three or more daily episodes of loose stool. The age distribution of the subjects was as follows: five (5.5%) were < 15 years of age; 16 (17.8%) were between 15-30 years; 52 (57.8%) were between 30-50 years; and 17 (18.9%) > 50 years. Relevant clinical information was collected by means of a standardized questionnaire, including hospitalization status, age, sex, and clinical symptoms. The specimens were collected at a University Hospital in the city of Rio de Janeiro, Brazil. The specimens were previously tested, as part of the hospital

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routine procedures, for parasites using Lutz, Baermann-Moraes methods and modified Ziehl-Neelsen stain for detection of coccidia, and by culture isolation and automated VITEK identification method for bacteria. The study protocol was approved by the Ethics Committee of the *Hospital Universitário Clementino Fraga Filho* of the *Federal University of Rio de Janeiro*, Rio de Janeiro, Brazil.

Stool suspensions were prepared as 10% (wt/vol) in phosphate-buffered saline (pH 7.2), clarified by centrifugation at 2,500 x g for 5 min. Nucleic acid was extracted from 200 µL of the sample by using Wizard® Genomic DNA Purification KIT (Promega, Madison, WI, USA) or RNAgents® kit (Promega) according to the manufacturer's instructions. The extracted nucleic acid was stored at -700ºC prior to PCR assay. Specimens were tested by conventional PCR assays, as previously described, for the presence of AstV;[8] NoV;[9] RV group A, B and C;[10-12] AdV;[13] HSV, Epstein-Barr virus (EBV), CMV;[14] and human bocavirus (HBoV).[15] PCR products were detected by agarose gel electrophoresis and staining with ethidium bromide. To confirm the specificity of the PCR products, the amplified DNAs of 15 CMV positive samples and all HSV, AdV, and HBoV positive samples were purified using the Wizard SV gel and PCR Clean-Up system kit (Promega), and the sequences determined using the BigDye® Terminator Cycle Sequencing Kit and the ABI PRISM® 3100 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). DNA sequences were edited using the software Chromas (Technelysium Pty Ltd, Australia) and compared to sequences available in the GenBankÒ (www.ncbi.nlm.nih.gov) using the BLAST tool (www.ncbi.nlm.gov/BLAST).

A total of 26 (28.9%, n = 90) samples were positive for viral pathogens. Fourteen (15.6%) were single infection with CMV and 2 (2.2%) with HSV. Coinfections with other viruses and/or parasites were observed in 10 (11.1%) samples. AdV and HBoV were only detected as mixed infections (Table 1). The specificity of the PCR assays was demonstrated by sequencing the amplified products, which confirmed the PCR results. EBV and RNA viruses such as RV, NoV and AstV were not detected. Twenty patients (22.2%) were infected with parasites (data not shown): 11 single infections and nine coinfected with virus. Enteropathogenic bacteria were not found. Age of the virus infected patients ranged from 16 to 59 years. Gender distribution was 16 (61.5%) female and 10 (38.5%) male. Among the positive patients for viral infection, 18 (69.2%) were hospitalized at the time of sample collection.

Although we were unable to obtain detailed information on the patients, such as time on HAART and viral load, information on the $TCD4^+/TCD8^+$ ratio was available for 51 out of 90 patients. Only four patients presented a $TCD4^+/TCD8^+$ ratio ≥ 1; two of them were positive for CMV infection. The remaining 47 patients presented a $TCD4^+/TCD8^+$ ratio < 1, and 23 of these were negative for all pathogens tested and six were positive for parasites only (Table 1). Generally, HIV-infected patients develop GI opportunistic infections when the $TCD4^+$ cells count is low inverting the $TCD4^+/TCD8^+$ ratio to < 1.[1,16] In this study, 16 (61.5%; n = 26) positive samples for viral infection came from individuals with $TCD4^+/TCD8^+$ ratio < 1; 11 were only positive for viruses (nine CMV, one HSV-2, one CMV + AdV); five were a mixture of virus and a parasite (Table 1).

Since the introduction of HAART, there was a reduction in the incidence of many opportunistic GI disorders among HIV/AIDS patients.[4,5] Yet, diarrhea remains a major cause of morbidity and mortality in this patient population.[1,2] CMV is the most common opportunistic agent leading to diarrhea among HIV-infected patients.[1,2,17] We found that 22 out of the 26 virus-positive patients were infected with CMV; 11 single infections; two coinfections with AdV or HSV-2; and five coinfections with parasites.

HSV infections are frequent among HIV-patients.[2,18] Intestinal HSV infection in AIDS patients usually presents with abdominal pain, tenesmus and bloody or non-bloody rectal discharge; high volume diarrhea rarely occurs.[4] We found two patients infected with HSV (1 HSV-1 and 1 HSV-2). Both patients presented diarrhea at the time of stool collection.

For some authors AdV is the second most frequent virus associated with diarrhea among HIV-positive patients.[19] We only found AdV as coinfection with CMV and/or parasite.

HBoV has been described as a respiratory pathogen. Yet, increasingly, HBoV has become recognized as a possible putative agent of acute gastroenteritis.[20] Both patients infected with HBoV were coinfected with the parasites *Isospora beli* or *Strongyloides stercoralis*, and one of the patients was also infected with CMV (Table 1). Despite the fact that both patients were coinfected with other pathogens associated with diarrheal illness in HIV-patients the role of HBoV as an enteric pathogen in these cases could not be complete ruled out. It is still possible that the presence of HBoV could aggravate the illness. A semi-quantitative PCR measure of HBoV in stool specimens was performed using dilution series of the extracted DNA from stools. We were able to detect DNA until the 10^{-3} and 10^{-5} dilution respectively in the two samples. High titers of DNA in the specimens suggest that the virus replicated in the gut. Moreover, at the time of stool collection, both patients were hospitalized in a critical state of immunosuppression which favors opportunistic infections.

In conclusion, gastrointestinal infections (viral and/or parasitic) were detected in 41.1% (37/90) of the samples, although all patients enrolled in the study were on HAART therapy. Viral infections were more frequent parasitic or bacterial infections among HIV-infected patients with diarrhea than.

Table 1. Characteristic of the patients infected with viruses

Patient	Age (years)	Gender	Status	Virus detected	Parasite detected	TCD4+/TCD8+ ratio
RC02	58	Female	Hospitalized	CMV	Negative	8.0
RC10	49	Female	Hospitalized	AdV	*Isospora beli* and *Strongyloides stercoralis*	ND1
RC11	52	Male	Non-hospitalized	CMV	Complex *Entamoeba histolytica / E. dispar*	0.9
RC25	37	Male	Hospitalized	CMV	Negative	0.1
RC30	30	Female	Hospitalized	CMV	Negative	ND
RC33	40	Male	Non-hospitalized	CMV	Negative	0.3
RC34	41	Male	Non-hospitalized	CMV	Negative	0.5
RC36	35	Female	Hospitalized	CMV	*Isospora beli*	0.3
RC37	51	Female	Non-hospitalized	CMV	Negative	2.0
RC39	43	Female	Hospitalized	CMV	Negative	0.1
RC41	59	Female	Non-hospitalized	CMV	Negative	0.3
RC43	16	Female	Hospitalized	CMV	Negative	0.2
RC44	43	Male	Hospitalized	CMV + AdV	Negative	0.0
RC46	34	Female	Hospitalized	CMV	*Giardia lamblia*	0.5
RC49	41	Male	Hospitalized	CMV	*Enterobis vermicularis*	0.1
RC63	38	Female	Non-hospitalized	CMV	Negative	0.0
RC64	32	Male	Hospitalized	CMV + AdV	*Giardia lamblia*	ND
RC65	29	Female	Hospitalized	CMV	Negative	ND
RC66	45	Female	Hospitalized	CMV	Negative	ND
RC70	29	Female	Non-hospitalized	CMV	Negative	0.4
RC86	57	Female	Hospitalized	HBoV	*Isospora beli*	ND
RC102	44	Male	Non-hospitalized	HSV-2	Negative	0.46
RC114	32	Male	Hospitalized	HSV-1	Negative	ND
RC124	34	Male	Hospitalized	CMV	Negative	0.2
RC138	37	Female	Hospitalized	CMV + HBoV	*Strongyloides stercoralis*	0.1
RC145	36	Female	Hospitalized	CMV	*Isospora beli*	ND

[1]ND, not done.

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Ethical approval

The study protocol was approved by the Ethics Committee of the *Hospital Universitário Clementino Fraga Filho of the Universidade Federal do Rio de Janeiro*, Rio de Janeiro, Brazil, and is in accordance with the Brazilian Ministry of Health Regulation 196/96.

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