



# Hepatitis delta in HIV/HBV co-infected patients in Brazil: is it important?<sup>☆</sup>

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## SUMMARY

**Objectives:** This study was carried out to evaluate the prevalence of hepatitis delta virus (HDV) among human immunodeficiency virus (HIV)/hepatitis B virus (HBV) co-infected patients from São Paulo, in the Southeast Region of Brazil.

**Methods:** A total of 3259 HIV patients with serological markers for HBV were initially enrolled in the study. Among these patients, 154 (4.7%) were hepatitis B surface antigen (HBsAg)-reactive. Serum samples were obtained from 86 HBsAg-positive patients and were submitted to anti-HDV serological assay.

**Results:** One (1.2%) HIV/HBV patient was found to be anti-HDV-positive, and the HDV infection was confirmed by PCR. Phylogenetic analysis showed that this HDV sequence grouped with other HDV genotype 1 sequences from Mediterranean European countries, suggesting that this virus has a common ancestor with HDV from that region. This patient was probably infected by sexual transmission, as he reported unprotected sexual intercourse with multiple partners over the course of many years but denied intravenous drug use or any travel to the Brazilian Amazon, an area known to have a high HDV prevalence.

**Conclusions:** HDV infection is infrequent in the Southeast Region of Brazil, however there have been a few cases in this region. HIV/HBV patients are at potential risk for HDV infection, therefore investigations for the presence of HDV infection must be carried out in these patients.

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## 1. Introduction

Fifteen million people are infected with hepatitis delta virus (HDV) worldwide, and thus it remains a relevant cause of liver-related illness and death.<sup>1</sup>

The disease associated with HDV infection is typically more severe than that due to hepatitis B virus (HBV) infection alone, but its clinical spectrum ranges from viral asymptomatic carrier to extremely severe disease.<sup>2</sup>

Genetic heterogeneity of HDV may influence the course of disease in different geographic areas.<sup>3,4</sup> The molecular characterization of HDV prevalent in different regions of the world has revealed eight groups, designated genotypes 1 to 8 (HDV-1 to HDV-

8). HDV-1 is the most widespread worldwide, but is predominant in Europe, North America, North Africa, and the Middle East; HDV-2 and HDV-4 are found in East Asia, HDV-3 in the Amazon Basin, and HDV-5 to HDV-8 were recently identified in individuals from Africa.<sup>3,5–14</sup>

The prevalence of HDV varies greatly depending on the geographic area. In Brazil, HDV has only been reported in the Amazon Region, where severe cases of acute and chronic HDV hepatitis have frequently been described.<sup>6,7,15–18</sup>

Human immunodeficiency virus (HIV), HBV, and HDV share the same routes of transmission. Among HIV/HBV co-infected patients, the presence of HDV infection has been associated with an unfavorable effect on the severity of liver disease by various authors.<sup>19–21</sup> It has been suggested that patients co-infected with HIV and HBV should be tested for anti-HDV antibodies (IgG and IgM), particularly if they have lived in an area where HDV is endemic, and especially in countries where routine immunization against HBV is not common. Ideally, HDV RNA should be detected and the viral load level should be determined in patients who are positive for HDV antibody (anti-HDV).<sup>22</sup>

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The prevalence of anti-HD antibodies in HIV-positive patients with hepatitis B surface antigen (HBsAg) ranges from 1.9% to 50%, depending on the geographic region and the risk group category.<sup>23–25</sup> Among HIV-infected patients with liver cirrhosis in Spain, 4.6% presented an HDV infection.<sup>26</sup>

Limited information is available about the seroprevalence of HDV among HIV/HBV co-infected patients in Brazil.<sup>27,28</sup> The aim of this study was to evaluate the prevalence of HDV in a group of HIV/HBV co-infected patients in São Paulo, in the Southeast Region of Brazil.

## 2. Materials and methods

### 2.1. Study design

This cross-sectional study was conducted at two institutions located in São Paulo City Metropolitan Area: one at the AIDS Outpatient Clinic of the Hospital das Clínicas, University of São Paulo School of Medicine and one at the Infectious Diseases Research Unit, ABC Foundation Medical School São Bernardo do Campo.

### 2.2. Patient population

The medical records of all patients followed at the study institutions from May 2006 to November 2007 were reviewed to identify those who were HIV-infected and HBsAg-reactive. Patients were selected if they had anti-HIV antibodies detected by ELISA and confirmed by Western blot together with detectable serum HBsAg during follow-up. These patients were then invited to a medical interview, and a blood sample was collected for further virological analysis. All patients participating in the study signed an informed consent and the research protocols were approved by the institutional ethics committees.

Medical records were reviewed to analyze clinical, demographic, and serological characteristics. Data collected included age, sex, CD4+ T cell count (current and nadir), use of antiretroviral therapy, hepatitis B e antigen (HBeAg) reactivity, and HBV viral load (determined by Cobas Amplicor HBV Monitor Test assay; Roche Molecular Systems, USA). The use of antiretroviral therapy (including HBV DNA polymerase inhibitors, such as tenofovir or lamivudine) was defined as the use of any antiretroviral medication for >6 months any time prior to baseline.

### 2.3. Serology

A microparticle enzyme immunoassay (MEIA, AxSYM, Abbott Diagnostic, Germany) was used to detect hepatitis B serological markers (HBsAg, total antibodies to hepatitis B core antigen (anti-HBc), antibodies to hepatitis B surface antigen (anti-HBs), HBeAg, and antibodies to hepatitis B e antigen (anti-HBe)). Anti-HD was detected by an ELISA (ETI-AB-DELTAK-2, DiaSorin, Italy).

### 2.4. HDV RNA and HBV DNA detection

RNA was extracted from 140 µl of serum using a QIAamp RNA Viral Mini Kit (Qiagen, Germany) in accordance with the manufacturer's instructions, and HDV RNA was amplified by nested polymerase chain reaction (PCR).<sup>6</sup> For HBV detection, DNA was extracted using a QIAamp DNA Mini Kit (Qiagen), and absolute quantification of HBV DNA was performed with an in-house real-time PCR assay (sensitivity = 50 IU/ml).<sup>29</sup>

### 2.5. HDV genotyping

The nested PCR products were purified using a ChargeSwitch PCR Clean-Up Kit (Life Technologies, USA). Cycle sequencing

reactions of purified PCR products were performed using a BigDye Terminator Kit v3.1 (Life Technologies, USA) and the same primers utilized in the second-round PCR for HDV. The sequences were read in an automated ABI Prism 377 DNA Sequencer (Applied Biosystems, USA). The HDV sequence was genotyped by phylogenetic reconstructions using reference sequences from each HDV genotype obtained from GenBank ( $n = 176$ ). Sequences were aligned using CLUSTAL\_X<sup>30</sup> and edited using BioEdit software.<sup>31</sup>

Bayesian phylogenetic analyses were conducted using the Markov Chain Monte Carlo (MCMC) simulation implemented in BEAST v.1.6.1,<sup>32</sup> and 10 million generations were sufficient to obtain the convergence of parameters. The maximum clade credibility (MCC) tree was obtained from summarizing the 10 000 substitution trees and it was then modified using a 10% burn-in using Tree Annotator v.1.6.1.<sup>32</sup>

## 3. Results

### 3.1. Patients

A total of 3259 patients with a confirmed HIV infection and serological markers for hepatitis B (anti-HBc and HBsAg) were initially enrolled in the study: 2412 patients from the AIDS Outpatient Clinic of the Hospital das Clínicas and 847 patients from São Bernardo do Campo. Among these patients, 154 (4.7%) were HBsAg-reactive. Serum samples were obtained from 86 patients; these patients comprised the study population and underwent further virological testing for hepatitis delta infection. Table 1 summarizes the main characteristics of the 86 HBsAg-reactive patients in whom anti-HD testing was performed.

Among 86 HBsAg-reactive patients, only one (1.2%) anti-HD-positive patient was identified. HBV DNA was not detected by real-time PCR in this patient, but the current HDV infection was confirmed by HDV RNA detection. Phylogenetic analyses showed that the HDV genotype in this case was HDV-1 and its sequence was most closely related to some HDV-1 sequences from Mediterranean European countries (Figure 1).

### 3.2. Case report

The patient was a 37-year-old male, active homosexual, originating from Ceará (northeast of Brazil) but who had lived

**Table 1**  
Main baseline characteristics of 86 HIV-infected patients with concomitant HBV infection

Variables	
Male gender, $n$ (%)	83 (96.5)
Age (years), mean $\pm$ SD	42 $\pm$ 7.2
Risk factors for HIV transmission	
Men who have sex with men, $n$ (%)	64 (74.4)
Intravenous drug use, $n$ (%)	16 (18.6)
Clinical variables	
Clinical evidence of advanced liver disease, $n$ (%)	17 (19.8)
Antiretroviral therapy history, $n$ (%)	81 (94.2)
CD4 cell count (cells/ $\mu$ l), mean $\pm$ SD	545 $\pm$ 250
Nadir CD4 cell count (cells/ $\mu$ l), mean $\pm$ SD	310 $\pm$ 140
ALT level, normal, $n$ (%)	54 (62.8)
ALT level $>1.5 \times$ ULN	32 (37.2)
Anti-HDV	1 (1.2)
Characteristics of HBV	
HBeAg-reactive, $n$ (%)	42 (48.8)
HBV DNA $<60$ IU/ml, $n$ (%)	50 (58.1)
HBV DNA $>60$ IU/ml, $n$ (%)	34 (39.5)
HBV-DNA unknown, $n$ (%)	02 (2.32)

HIV, human immunodeficiency virus; HBV, hepatitis B virus; SD, standard deviation; ALT, alanine aminotransferase; ULN, upper limit of normal; HDV, hepatitis delta virus; HBeAg, hepatitis B e antigen.



**Figure 1.** Maximum clade credibility (MCC) tree estimated by Bayesian analysis of 177 partial sequences of delta antigen gene. The sample obtained from the HIV/HBV/HDV co-infected Brazilian patient is marked with a black triangle. HDV-1 sequences ( $n = 93$ ) obtained from GenBank are identified by their accession number followed by geographic origin. The collapsed clades correspond to the non-HDV-1 genotypes: HDV-2 ( $n = 9$ ), HDV-3 ( $n = 36$ ), HDV-4 ( $n = 13$ ), HDV-5 ( $n = 6$ ), HDV-6 ( $n = 3$ ), HDV-7 ( $n = 6$ ), HDV-8 ( $n = 10$ ). The values of posterior probability are shown above their respective key nodes.

in São Paulo for the last 20 years. In 1999, he was diagnosed with an asymptomatic HIV infection and with a hepatitis B virus infection (HBsAg-positive). At that time, alanine aminotransferase (ALT) levels were normal. The patient started antiretroviral therapy with zidovudine, lamivudine, and indinavir in 1999. In 2004, his HIV viral load was undetectable and CD4 T-cell count was 808/ $\mu$ l, and he started to present persistently high ALT levels (3–4-fold the upper limit of normal (ULN)). He was HBeAg-reactive at this time, but HBV DNA could not be determined, as this test was not available in the service at that time. He was then submitted to a liver biopsy, which showed a METAVIR score of F1 A2. After the liver biopsy, his treatment was changed to tenofovir, atazanavir, ritonavir, and lamivudine. There was no improvement in ALT levels, which continued to be elevated (3–10 $\times$  ULN). HBeAg continued to be reactive and HBV DNA, which was occasionally determined from 2007 onwards, was persistently undetectable by Cobas Amplicor HBV Monitor Test. In 2009, anti-HD serology was carried out and a reactive result was obtained; the patient was finally identified as HDV-infected.

#### 4. Discussion

HDV infection does not appear to be frequent among patients co-infected with HIV and HBV in the southeast of Brazil. According to our data, its presence was found in only one (1.2%) patient among 86 analyzed.

In Brazil, HDV has been described only in the Amazon Region, where it is endemic and associated with severe forms of the disease. In fact, Brazilian studies of HDV prevalence outside endemic areas are scarce, but these few studies have shown a very low prevalence: anti-HD was positive in only one out of 81 patients from São Paulo State in a study carried out at the end of the 1980s.<sup>33–35</sup>

There have been only two studies on the prevalence of HDV among HIV/HBV patients in Brazil: one of these studies was carried out in the Western Amazon Region and showed that the HDV prevalence was 9.4% among HBsAg/anti-HIV-positive patients from this region, yielding a minimum prevalence of 1.9% overall.<sup>27</sup> This prevalence was lower than that found in the general population from the same region, where anti-HD antibodies reached 34.4% in HBsAg-positive patients.<sup>18,36</sup> Another study evaluated HDV prevalence among HIV/HBV patients from Mato Grosso State, in the Central Region of Brazil, and found only one (2.7%) anti-HD-positive case among 37 HBsAg-positive patients. HDV infection among HIV/HBV patients in Brazil seems to be less frequent, even in endemic regions, than in other countries, where its frequency may reach 50%.<sup>37</sup>

HDV transmission among HIV/HBV patients has been more related to a history of intravenous drug use (IVDU) than to high-risk sexual behavior.<sup>37</sup> In the studied population, 18.6% of patients were IVDU, but no patient in this group was anti-HD-positive. The HIV/HBV/HDV co-infected patient identified in this study denied IVDU, but referred to unprotected sexual intercourse with multiple partners over the course of many years, indicating that HDV transmission occurred through sexual contact in this case.

HDV is presently classified into eight genotypes – HDV-1 to HDV-8.<sup>9,11</sup> Studies on genotype distribution in Brazil have been restricted to the Amazon Region (Western and Eastern), where HDV genotype 3 has been found to be prevalent.<sup>6,7,38</sup> Nevertheless, in one Brazilian study, HDV genotype 1 was found in 55% of the chronically infected patients from the Western Brazilian Amazon.<sup>39</sup>

Phylogenetic analysis showed that the HDV isolated from the HIV patient in our study was genotype 1 (HDV-1) and it grouped with HDV-1 sequences from Europe. It was not possible to determine the phylogenetic relationships with the other HDV-1

identified in the Amazon Region because the sequences were not characterized in that study; the genotypes were characterized by PCR followed by hybridization with a specific <sup>32</sup>P-labeled oligonucleotide probe.

Our patient denied any travel to the Amazon Region. As outside of the Amazon Region HDV prevalence is extremely low or even absent in Brazil, it is probable that the HDV infection in this case occurred through contact with someone who had lived in a region endemic for HDV genotype 1. The sequence from the patient clustered with other sequences from Mediterranean European countries, suggesting that this virus has a common ancestor with HDV from that region. The prevalence of HDV markers in some cohorts of chronic HBsAg carriers with HIV co-infection in Europe has varied from 4% to 44%, with HDV genotype 1 being the most frequent.<sup>13,40–44</sup>

In the HIV/HBV/HDV co-infected patient identified in this study, HBV DNA was undetectable but HDV RNA was positive; this is in accordance with previous studies, which have shown that HDV suppresses HBV replication.<sup>45</sup> Other studies have shown that most HIV/HBV/HDV co-infected patients are HDV RNA-positive, which represents a serious problem as the liver disease is more aggressive in these patients and the treatment is rarely effective.<sup>37</sup> Furthermore, these patients represent an important source for HDV transmission.

The distribution of infectious agents around the world may be a dynamic phenomenon. Changes in habits and customs and mobility between groups may permanently alter the distribution and occurrence of infections usually restricted to certain geographic regions. Based on this picture and on the present results, we recommend that investigations for the presence of HDV infection be carried out among HIV/HBV co-infected patients, particularly those with advanced liver disease, even in areas of low HDV risk. Furthermore, prevention measures should be implemented among HIV patients to avoid HBV and HDV infections, such as HBV vaccination and the avoidance of high-risk injecting practices and unprotected sex.

In conclusion, our results demonstrate the presence of HDV infection among HIV/HBV co-infected patients from a non-endemic area of Brazil. These findings indicate the need to investigate for the presence of HDV infection in HIV/HBV co-infected patients, particularly those with advanced liver disease, even in areas of low HDV risk. Furthermore, prevention measures should be implemented among HIV patients to avoid HBV and HDV infections, such as HBV vaccination and avoidance of high-risk injecting practices and unprotected sex.

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**Conflict of interest:** The authors declare no conflicts of interest.

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