Genetic diversity of hepatitis B virus and hepatitis C virus in human immunodeficiency virus type 1-co-infected patients from Venezuela

Rossana C. Jaspe,1 Yoneira F. Sulbarán,1 Carmen L. Loureiro,1 Nahir Martínez,2 Marisol Devesa,1 Yesseima Rodríguez,1 Jaime R. Torres,2 Héctor R. Rangel1 and Flor H. Pujol1

Correspondence Flor H. Pujol fhpujol@gmail.com

Received 28 August 2013
Accepted 19 May 2014

The aim of this study was to evaluate the prevalence and genetic diversity of hepatitis B virus (HBV) and hepatitis C virus (HCV) in human immunodeficiency virus type 1 (HIV-1)-co-infected Venezuelan patients. The prevalence of HBV and HCV markers of infection in HIV-1 patients was 14 % for anti-hepatitis B core antigen, 3 % for hepatitis B surface antigen and 0.7 % for anti-HCV, respectively. HBV prevalence was higher than HCV, as expected for a country where sexual intercourse, not intravenous drug use, is the main mode of HIV-1 transmission. The HCV genotype distribution in HIV-1-co-infected patients was similar to that obtained in HCV-mono-infected patients, but genotype 1a was more frequent in HIV-1-infected patients. The HBV genotype distribution exhibited differences between mono-infected and HIV-1-co-infected individuals. HBV F3 was the most common subgenotype in both groups, followed by F1b in HIV-1 co-infection and F2 in HBV mono-infection. In addition, genotype G (single infection) was found in an HIV-1-co-infected individual. A high prevalence of occult HBV infection was detected in HIV-1-co-infected naïve patients (18 %), with F2 being the most common genotype (75 %). To the best of our knowledge, these results correspond to the first description of frequency and molecular characterization of HBV and HCV in HIV-1 Venezuelan patients.

INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are usually more frequent in human immunodeficiency virus type 1 (HIV-1)-infected patients than in the general population as these viruses share the same routes of transmission. There is evidence of a more rapid progression of chronic hepatitis to cirrhosis and hepatocellular carcinoma in HIV-1-co-infected individuals (Chung, 2006). Worldwide, ~380 million people are chronic carriers of HBV infection (6 % of the global population), whereas HCV affects ~170–180 million people (2–3 % of the global population). Furthermore, among the >30 million people infected with HIV worldwide, an estimated 2–4 million (5–25 %) and 4–5 million (5–15 %) are chronically infected with HBV and HCV, respectively (Lemoine et al., 2013). In Venezuela, the prevalence of HBV active infection in non-Amerindian populations is 2–7 % (Martínez et al., 2007), HCV prevalence is 0.7–2.1 % (Aguilar et al., 2001) and estimated HIV prevalence is ~0.5 % (0.3–1.3 %) (http://www.unaids.org/en/dataanalysis/datatools/aidsinfo/).

Up to 10 HBV genotypes (A–J) and several subgenotypes have been reported based on the differences in full-length genomes. In Venezuela, HBV genotype F is the most frequent in the general population (Devesa et al., 2008; Machado et al., 2010). Seven HCV genotypes and a large number of subtypes have been described. Moreover, genotype 1 is the most frequently detected in Venezuela, followed by genotypes 2 and 3, although a significant reduction of HCV G1b circulation has been observed over the last decade, with an increase in the circulation of genotype G2) – a subtype quite rare in other countries, including the Americas (Pujol & Loureiro, 2007; Sulbarán et al., 2010). However, the genotype distribution in patients co-infected with HIV-1 remains unknown. The aim of this study was to evaluate the prevalence and genetic diversity of HBV and HCV in HIV-1-co-infected patients living in Venezuela.
METHODS

Blood samples. This study was approved by the Bioethical Committee of Instituto Venezolano de Investigaciones Científicas. In total, 512 samples were analysed, all of them collected after written informed consent from HIV-1-positive patients living in Venezuela, and stored at −30 °C until use. The presence of HBV and HCV serological markers was determined in a cohort of 418 serum samples (268 male and 150 female) collected between 2002 and 2011. In addition, 44 serum samples (77% male, median age 34 years) from HIV-1-naive patients were collected between 2005 and 2008, and used to determine occult HBV infection. The genotypic distribution of HBV and HCV in HIV-1-infected patients was determined in 50 HIV-1-infected patients (22 HBV: 82% male, all but one adults; 28 HCV: 91% male, all but one adults), previously diagnosed for HBV and/or HCV, and collected between 2005 and 2011. Most of the HIV-1-infected patients analysed were from the Capital District.

HBV and HCV serological markers in HIV-1-co-infected patients. Serum samples were tested by enzyme immunoassay for hepatitis B surface antigen, HBsAg (Hepatitis B Surface Antigen kit; DRG International/Murex HBsAg V 3; Abbott), and for antibodies against core antigen, anti-HBc (ETI-AB-COREK PLUS; DiaSorin/Murex anti-Hbc total; Abbott), and against HCV, anti-HCV (Antibody to Hepatitis C Virus kit, 3rd generation; DRG International).

PCR and sequencing. In a total of 22 HCV–HIV-1-co-infected samples, the HBV genotype was determined by direct sequencing and phylogenetic analysis of a PCR-amplified product from the S’ non-coding region (Pujol & Loureiro, 2007) and/or NS5B (Subbaran et al., 2010); 28 HIV–HIV-1-co-infected and 44 HBsAg-negative HIV-1-infected patients were analysed by nested PCR of the S region (Devesa et al., 2008) and/or core region (Gutiérrez et al., 2004). All PCR products were purified by using the QIAquick PCR Purification kit (Qiagen) and then subjected to direct nucleotide sequencing. In all cases, both sense and antisense inner primers were used for sequencing (performed by Macrogen Service Center, Seoul, Korea). The sequences of HBV or HCV in the HIV co-infected patients were deposited in GenBank under the accession numbers KF414631–KF414679, HM777078, HM777086, HM777094, HM777102, HM777121, HM777254, HM777269, HM777342 and HM777354.

HBV genotype G cloning. To investigate the presence of genotype G co-infection with other strains of HBV, 41 HBV clones were produced and sequenced. Cloning was performed using TOPO TA Cloning (Invitrogen). Ten HBV clones corresponding to the complete genome sequences were amplified and sequenced as described previously (Devesa et al., 2008). Thirty-one additional clones with the S gene fragment (nt 58–1101) were amplified and sequenced using primer set 58P–1101N (Hu et al., 2000).

Phylogenetic and sequence analysis. Sequence alignment and amino acid sequences were deduced using DNAMan 5.2.2 (Lynnon Bio Soft). Phylogenetic analysis was performed by the neighbour-joining method (1000 bootstrap replicates, with genetic distances estimated with Kimura two-parameter correction). The HBV and HCV genotype distribution found in HIV-1-co-infected patients was compared with that found in HBV and HCV mono-infected Venezuelan patients (352 HCV and 80 HBV). The sequences of viruses circulating in mono-infected patients were obtained previously (Jaspe et al., 2012; Subbaran et al., 2010; M. Devesa and others, unpublished results). The prevalence of precore mutations in HBV genotype G isolates was analysed in sequences available in GenBank (accession numbers AB064310–AB064314, AB056513–AB056516, AB375168–AB375170, AF160501, AF405706, AP007264, DQ078791, DQ207798, EF464097–EF464099, EF634480, EF634481, GU563556, GU563559, GU565217, HE981171–HE981176, HQ231885, JQ707451, JQ707457, JQ707472, JQ707474, JQ707657 and JQ707660).

Statistical analysis. Statistical differences were evaluated by the χ² test with Yates’ correction or Fisher’s exact test, according to Epi Info version 3.5.3 (http://www.cdc.gov/epiinfo/).

RESULTS

Prevalence of HBV and HCV in Venezuelan HIV-1-positive patients

Serological markers for HBV and HCV were determined in samples from 418 HIV-1-infected patients, including 150 females and 268 males (Table 1). HBsAg was positive in 3.1%, with a higher prevalence in young men (18–30 years old), and anti-HBc was positive in 14%, with a higher prevalence in men of middle age (41–50 years old). HBV exposure was higher in males compared with females (P<0.0001). Anti-HCV was positive in 0.7% of individuals, without any gender difference in prevalence. There was no significant difference in age between the group infected with HBV or with HCV. The prevalence of HBV active infection (HBsAg) and exposure (anti-HBc) was significantly higher than that of HCV (P=0.02 and P<0.0001, respectively).

HCV and HBV genotype distribution in Venezuelan patients co-infected with HIV-1

The genotype distribution in 22 HCV isolates co-infected with HIV-1 was compared with that found in 352 HCV mono-infected patients. The genotype distribution in the mono-infected patients was 32.1% subtype 1a, 32.4% subtype 1b, 32.2% genotype 2 and 4.3% other genotypes. The HCV genotype distribution in HIV-1-co-infected patients (Table 2) was similar to that obtained in HCV mono-infected patients, with genotype 1 being the most prevalent, followed by genotype 2. HCV subtype 1b was less prevalent in HIV-1-infected patients compared with mono-infected patients (9.1 versus 32.4%, Fisher exact test P=0.03), with a tendency for the subgenotype 1a frequency to increase (50 versus 32.1%).

The core and/or complete S regions of HBV were analysed in 28 HBV isolates co-infected with HIV-1, and compared with the genotype distribution found in 80 HBV mono-infected patients (M. Devesa and others, unpublished results). The HBV genotype distribution exhibited some differences between mono-infected and HIV co-infected patients (Table 2). Whilst F3 was the most common subgenotype in both groups [12/28 (43%) in HIV–HIV-1-infected versus 48/80 (60%) in HBV mono-infected], F1b was the second most common in HIV co-infected patients [10/28 (36%) versus 5/80 (6%), P<0.001] compared with F2 in HIV mono-infected patients [2/28 (7%) compared with 13/80 (16%)]. F1b Venezuelan isolates displayed 98.5–100% identity between them. Two of these sequences displayed stop codons in the S protein: B2503 and B2245 (the latter from a triple co-infected patient, i.e. HIV–HBV–HCV). No
genetic relatedness of Venezuelan HBV F1b isolates was found with isolates from other Latin American countries, such as Argentina or Chile, where this subgenotype is more common (Fig. 1). One HIV-1-infected patient was found infected with HBV genotype G, which to our knowledge has not been reported previously in Venezuela.

Characterization of HBV genotype G isolate

HBV genotype G was found in a 41-year-old Venezuelan homosexual man, co-infected with HIV-1, with no history of sexual intercourse outside Venezuela. In order to assess if this patient was co-infected with another genotype, the S gene was cloned and sequenced. Forty-one clones were analysed; all of them were genotype G. The complete genome sequence of this strain showed 99% identity with sequences of the HBV genotype G strain from patients from different countries around the world, some of them also reported as co-infected with HIV (e.g. GenBank accession numbers GU563556 from Belgium and EF464098 from Brazil).

Similar to other genotype G isolates, the Venezuelan HBV genotype G genome harboured an insertion of 36 bp in the core gene, but only contained one stop codon in the precore region, at position 28 (G1896A). Repeated sequencing showed that the other stop codon observed frequently in genotype G isolates indeed coded a glutamine. The absence of a stop codon in the second codon of the precore sequence was also found in 14/38 (37%) HBV genotype G sequences analysed from GenBank (data not shown). Ten of the 14 sequences (71%) came from other American countries (two from Brazil and eight from the USA) and also had a glutamine in this position.

Occult HBV infection

Occult HBV infection is characterized by the presence of HBV infection without detectable HBsAg, generally with low viraemia, with the diagnosis being dependent on sensitive HBV-DNA PCR assays. Occult infection was analysed in 44 Venezuelan HIV-1-positive naïve patients and classified as residual (with anti-HBc positivity) or silent (without either HBsAg or anti-HBc positivity) (Gutiérrez et al., 2004). Residual infection was detected in 3/19 (16%) samples and silent infection in 5/25 (20%) samples (Fig. 2). Phylogenetic analysis of the partial HBV S region showed genotype F2a as the most common genotype (75%) (Table 2).

DISCUSSION

Due to shared modes of transmission, co-infection between HBV, HCV and HIV is common. In our study, the prevalence of both HBV active infection and exposure appeared to be higher than HCV in HIV-1-infected patients. Additionally, HBV infection in HIV-1-infected patients was more frequent than in the general and blood donor population (Martínez et al., 2007; Pozo et al., 2007).

Table 1. Prevalence [n (%)] of HCV and HBV in HIV-1 positive patients

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Positive HBV serological markers</th>
<th>Positive HCV serological marker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBsAg</td>
<td>Anti-HBc</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>18–30</td>
<td>0/38 (0)</td>
<td>3/35 (8.6)</td>
</tr>
<tr>
<td>31–40</td>
<td>0/59 (0)</td>
<td>3/92 (3.3)</td>
</tr>
<tr>
<td>41–50</td>
<td>0/33 (0)</td>
<td>5/94 (5.3)</td>
</tr>
<tr>
<td>51–60</td>
<td>1/17 (5.9)</td>
<td>1/36 (2.8)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>0/3 (0)</td>
<td>0/11 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>1/150 (0.7)</td>
<td>12/268 (4.5)</td>
</tr>
<tr>
<td>Total F + M</td>
<td>13/418 (3.1)</td>
<td>59/418 (14)</td>
</tr>
</tbody>
</table>

F, female; M, male.

Table 2. HCV and HBV genotype distribution and prevalence (%) in Venezuelan patients co-infected with HIV-1

<table>
<thead>
<tr>
<th>HCV genotype (n=22)</th>
<th>1a (50)</th>
<th>1b (9.1)</th>
<th>2a (0)</th>
<th>2b (9.1)</th>
<th>2c (4.5)</th>
<th>2j (14)</th>
<th>2x* (0)</th>
<th>3a (9.1)</th>
<th>4d (4.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV genotype (HBsAg-positive) (n=28)</td>
<td>A (3.6)</td>
<td>C (0)</td>
<td>D (7.1)</td>
<td>F1a (0)</td>
<td>F1b (36)</td>
<td>F2 (7.1)</td>
<td>F3 (43)</td>
<td>F4 (0)</td>
<td>G (3.6)</td>
</tr>
<tr>
<td>HBV genotype (HBsAg-negative) (n=8)</td>
<td>A (0)</td>
<td>C (13)</td>
<td>D (0)</td>
<td>F1a (0)</td>
<td>F1b (0)</td>
<td>F2 (75)</td>
<td>F3 (13)</td>
<td>F4 (0)</td>
<td>G (0)</td>
</tr>
</tbody>
</table>

Numbers in bold indicate the most frequent genotype in each group.
*HCV genotype 2s or 2r.
Fig. 1. Phylogenetic analysis of the S-coding region (660 bp: nt 165–825) of HBV strains from Venezuelan HIV-1-co-infected patients. Genetic distance was estimated by Kimura two-parameter correction and the phylogenetic tree was reconstructed with the neighbour-joining method. Numbers at each node correspond to bootstrap values (>70%) obtained with 1000 replicates. Isolates are designated by their GenBank accession number, followed by their country of origin, except for Venezuelan isolates from this study which are shown in bold. The sequences of occult HBV infection are underlined. VNZ, Venezuela; BRA, Brazil; COL, Colombia; CHI, Chile; ARG, Argentina; SAL, El Salvador; JAP, Japan; NET, Netherlands; FRA, France; MEX, Mexico; GER, Germany; BAN, Bangladesh.
HBV prevalence in HIV-1-infected patients is similar to that found in Brazil (Araujo et al., 2008). However, no difference was observed for HCV infection between patients co-infected with HIV-1 and the Venezuelan general population (Aguilar et al., 2001). In contrast, HCV infection appears to be more frequent in HIV-1-infected patients in Brazil (Pereira et al., 2006). Sexual intercourse is known to be more efficient for HBV transmission than for HCV (Cainelli, 2013). In Venezuela, sexual transmission is also more frequent than intravenous drug use, which is rare, as a risk factor for HIV-1 infection (Rangel et al., 2009). This may explain in part the fact that HCV prevalence was similar in HIV-1-infected patients and in mono-infected patients.

The HCV genotype distribution in HIV-1-co-infected patients was similar to that observed in HCV mono-infected patients. However, subgenotype 1b was less prevalent in co-infected patients, with an increase in subgenotype 1a. In contrast, the HBV genotype distribution exhibited some differences between mono-infected and HIV-1-co-infected patients, with F3 being the most common subgenotype in both groups, following by F1b in HIV-1 co-infection and F2 in HBV mono-infection.

Clonal analysis revealed a case of HBV genotype G mono-infection in a homosexual HIV-1-co-infected patient. To the best of our knowledge, this is the first report of such a genotype in Venezuela. Mono-infection by HBV genotype G, although unexpected, due to the peculiarities of the HBV genotype G genome (Kato et al., 2002), has been documented previously (Chudy et al., 2006; Zaaijer et al., 2011). The sequence identified in our patient is closely related to a sample from a Dutch donor harbouring an HBV genotype G mono-infection (Zaaijer et al., 2011). Both HBV genotype G genomes possess an insertion of 36 bp in the core gene, but contain only one stop codon in the precore region, at position 28 (G1896A). Nonsense mutations in the precore region of HBV genotype G prevent hepatitis B e antigen (HBeAg) expression and can partially explain its requirement for co-infection with another genotype (Gutelius et al., 2011). Contrary to the Dutch donor (Zaaijer et al., 2011), our patient had tested positive for HBsAg and HBeAg in a sampling 3 years previous. Hence, the presence of a co-infection of HBV genotype G with other HBV genotypes in the past cannot be excluded.

Occult HBV infection was found with a high frequency in our HIV-1-infected naive patients. This prevalence is similar to that previously reported in other Latin American countries (14–19 %) (Araujo et al., 2008; Sucupira et al., 2006). However, the prevalence of occult HBV observed was higher than that found previously in blood donors from Caracas (4.3 %) (Gutiérrez et al., 2004), but lower than in Venezuelan Amerindians (34 %) (Cardona et al., 2011). Phylogenetic analysis showed genotype F2a as the most common (75 %), and such HBV isolates closely resembled those circulating in Venezuelan HIV-1-co-infected individuals (Fig. 1). In a previous study of Venezuelan blood donors, occult infection was associated with genotypes A and D (Gutiérrez et al., 2004), whilst the majority of occult cases in Venezuelan Amerindians were due to HBV genotype F3 (Cardona et al., 2011). All these studies show that HBV occult infection can be caused by any of the HBV genotypes circulating in Venezuela, but the frequency of genotypes found in occult cases may vary from one epidemiological setting to another. Substitutions related to occult HBV phenotypes (Biswas et al., 2013), with vaccine escape mutants (Carman et al., 1990; El Chaar et al., 2010; Huang et al., 2012), impaired virion and/or S protein secretion in vitro (Huang et al., 2012), or YMDD variants in polymerase (Allen et al., 1998), were not detected (data not shown).

In conclusion, to the best of our knowledge, these results correspond to the first description of the frequency and molecular characterization of HBV and HCV in HIV-1 patients living in Venezuela. As expected, the prevalence of HBV was higher than HCV. Whereas little difference was found in HCV genotype distribution between mono-infected and HIV-1-co-infected patients, some differences were indeed observed among the HBV genotypes found in HIV co-infected patients. Finally, a high prevalence of occult HBV infection was detected in HIV-1-co-infected patients.

ACKNOWLEDGEMENTS

This work was supported by Grant LOCTI from Venezuela.

REFERENCES

Aguilar, M. S., Cosson, C., Loureiro, C. L., Devesa, M., Martínez, J., Villegas, L., Flores, J., Ludert, J. E., Alarcón de Noya, B. & other authors (2001). Prevalence of infection with hepatitis C virus in...


