Hepatitis G Virus Infection in Human Immunodeficiency Virus Type 1—Infected Mothers and Their Children

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Hepatitis G virus (HGV), also called hepatitis virus GB-C or GB virus C, is a positive-stranded RNA agent. It presents a genomic organization common to that of the Flaviviridae family and conserved sequences but has <25% homology with hepatitis C virus (HCV). [1]. Serum HGV RNA indicates viremia, whereas the HGV envelope E2 glycoprotein—specific antibody (E2Ab) is associated with recovery [2]. HGV RNA and E2Ab are almost mutually exclusive and can be found respectively in <2% and 9% of healthy blood donors [1, 2]. Prevalence increases in association with HCV or hepatitis B virus (HBV) infection [1, 3]. The main infection route is parenteral exposure to blood through transfusions, hemodialysis, or sharing equipment in injecting drug use (IDU); indeed, >30% of transfusion recipients and up to 80% of IDUs are HGV marker—positive [1–5]. Sexual transmission is suspected [5–7]. HGV and human immunodeficiency virus type 1 (HIV-1) share the same infection routes, and a significant proportion of HIV-1—infected subjects are HGV—coinfected [7]. Mother-to-child infection occurs [8–10], but little is known about transmission in HIV-1—infected mothers [10].

Materials and Methods

Case definition. Children considered at risk of perinatal HIV-1 infection prospectively derived were defined as previously described [11]. HIV-1 infection was diagnosed according to the Centers for Disease Control and Prevention (CDC) criteria [12]. The CDC classification for HIV-1 infection in adults was used to define the mothers’ clinical condition at the time of delivery [13]. Positivity for HGV RNA defined an HGV infection [1, 2]. Exposure to HBV was defined by positivity for HBV surface antigen (HBsAg) or core antigen (HBcAg) antibody.

Data set. Fifty-eight HIV-1—positive mothers (median age, 28.5 years; range, 20–37) and their children were studied. All women had denied prior receipt of blood or blood products. Thirty-four women (58.6%; median age, 29.5 years; range, 20–37) had a history of IDU (median duration, 4.7 years; range, 2.0–9.4) and reported sharing equipment with injecting partners. Twenty-two IDU women (median age, 30.5 years; range, 23–37) had ceased injecting a median of 2.2 years (range, 0.9–12) before delivery, whereas 12 (median age, 29 years; range, 20–36) continued injecting during pregnancy. Risky sexual behavior (RSB) was the sole infection route for HIV-1 in 24 women (41.3%; median age, 26 years; range, 20–35); 14 of them reported RSB with IDU men. Fifty-six women (96.5%) were classified as clinical category A and 2 as category B. Twenty children (34.4%) were born by cesarean section (elective cesarean section in 14 cases [24.1%]). All children were exclusively formula-fed. No child received blood or blood products.

HGV markers. HGV RNA was extracted from 150 µL of serum or plasma by use of a monophasing solution of phenol and guanidine isothiocyanate (Trizol; Life Technologies Gibco, Grand Island, NY) and chloroform (Sigma-Aldrich, Milan, Italy) followed by isopropanol (Sigma-Aldrich) precipitation. Complementary DNA was obtained by reverse transcriptase–polymerase chain reaction (PCR) [3] using two primers from the noncoding region (5′-CGGCTAAAAGGTTGCGT-3′, position 101–120, and 5′-CGACGAGCTAGCTCAGG-3′, position 285–267) and two primers from the NS5 region (5′-CTGTGTTGTGAGTAGT-3′, position 77–101, and 5′-CGAATGGT-3′, position 211–188). The amplified nucleic acid was detected by an ELISA (Boehringer Mannheim, Mannheim, Germany). E2Ab was investigated by ELISA (mPLATE anti-HGenv; Boehringer Mannheim) [2].
HBV, hepatitis delta virus (HDV), and HCV markers. An RIA was used to investigate HBsAg and hepatitis B e antigen (HBeAg), antibodies to HBsAg, HBeAg, and HBeAg, and antibodies to HDV (Abbott Laboratories, North Chicago). Antibodies to HCV were determined by an adaptation of a third-generation ELISA (Ortho Diagnostic Systems, Raritan, NJ) and confirmed by an HCV recombinant line immunoassay (Immunogenetics, Antwerp, Belgium). Serum HCV RNA was determined by a homemade cDNA PCR procedure with nested primers (Sorin Biomedica, Saluggia, Italy) taken from the highly conserved nucleotide sequence of the HCV 5' untranslated region.

Liver enzymes. The levels of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and γ glutamyl transpeptidase were determined at 37°C in a multichannel analyzer.

CD4 T lymphocyte numbers. Heparinized peripheral blood was analyzed in a FACScan flow cytometer after double-staining with phycoerythrin- and fluorescein isothiocyanate-conjugated monoclonal antibodies to CD3 and CD4 molecules (Becton Dickinson, San Jose, CA).

Study design. HGV RNA and E2Ab were studied retrospectively in serum samples that had been stored at −70°C and had never been freeze-thawed before testing. Samples taken from all mothers and their children a median of 5 days (range, 1–27) after delivery were evaluated. Samples taken 2, 4, 6, and 8 months after delivery were evaluated in children born to HGV-infected mothers. Samples were subsequently taken from HGV-infected children every 2 months up to the date of the last follow-up. CD4 T lymphocyte numbers, HCV RNA and antibodies, HBV antigens and antibodies, and HDV antibodies were evaluated in women ~1 month before delivery and a few days after. CD4 T lymphocyte numbers and liver enzyme levels were determined in the first blood taken from the children after birth. Clinical data, liver enzyme values, HCV RNA and antibody (in children born to HCV antibody-positive mothers), and HBV markers (in children born to HBsAg-positive mothers) were obtained every 2 months.

Statistical analysis. Data were processed through SPSS for Windows (SPSS, Chicago) and Epi-Info version 5 (CDC, Atlanta; World Health Organization, Geneva) statistical packages. Ages were reported as median (range) and differences were evaluated by the Mann-Whitney U test. Differences in frequencies were evaluated by Fisher’s exact probabilities. Relative risk and 95% confidence limits (95% CL) were calculated, and their significance was evaluated through the Mantel-Haenszel calculation. P < .05 was considered significant.

Results

HGV infection in mothers. Exposure to HGV in HIV-1–positive women was 32 (55.1%) of 58: 20 women (34.4%) (median age, 28.5; range, 20–36) had E2Ab and 12 (20.6%) (median age, 29.5; range, 23–35) were HGV-infected. No woman was E2Ab- and RNA-positive at the same time. The E2Ab- and HGV RNA–positive subject ratio was 1.6. CD4 T lymphocyte numbers did not differ in those who were HGV-infected (613 ± 271 cells/μL), E2Ab-positive (711 ± 307 cells/μL), or HGV marker–negative (724 ± 314 cells/μL; Student’s t test). The gestation period (median of 39 [range, 37–41] vs. 39 [24–43] weeks; Mann-Whitney U test) and child’s birthweight (3143 ± 476 vs. 3110 ± 570 g; Student’s t test) were similar in HGV-infected and uninfected mothers. The risk of positive HGV markers was similar in the IDU women and RSB women, in IDU women who had ceased or who continued IDU, and in those with an injecting duration of ≥4.7 or <4.7 years (median injecting duration period in the IDU group). The risk was significantly higher in RSB women who acknowledged having had sexual intercourse with male IDUs (table 1).

HBV, HDV, and HCV infection in mothers. Eighteen women (31.03%) were HBV marker–positive: 4 were HBsAg-positive (all HBeAg-negative and one HDV-superinfected) and 14 had HBV antibodies. One HBsAg-positive woman was HCV-coinfected. Twenty-four women (41.3%) had HCV antibodies, and 18 of these were HCV RNA–positive. Five HCV RNA–positive women were HCV-coinfected. Eighteen (39.1%) IDUs versus 6 (25%) with RSB were HCV marker–positive (relative risk, 2.12; 95% CL, 0.99–4.54; P = 0.039, Mantel-Haenszel calculation), whereas positive HBV markers were not significantly associated with IDU.

HGV mother-to-child transmission. All HGV marker–negative mothers had negative children. Five (41.6%; 95% CL, 13.8%–69.4%) of 12 children born to HGV-infected mothers had HGV RNA–positive at their first blood test. Not one of the RNA-negative children born to HGV-infected women subsequently became positive. Maternal HIV-1 risk factors, age, CD4 T lymphocyte numbers, gestation period, birthweight, and mode of delivery were all similar in HGV-transmissive or –nontransmissive women. Transmission occurred in 3 cesarean (2 elective) deliveries.

HIV-1, HBV, and HCV mother-to-child transmission. Eleven children (18.9%) were HIV-1–infected. CD4 T lymphocyte numbers were significantly lower in mothers who were HIV-1–transmissive (465 ± 271 cells/μL) than in –nontransmissive mothers (728 ± 274 cells/μL; P = .006, Student’s t test). The frequency of birth by cesarean section was significantly lower among the HIV-1–infected children (1 [non-elective]/11; 9.09%) than among those who were uninfected (19 [13 elective]/47; 40.4%; P = .0468, Fisher’s exact probabilities). One child was HIV-1–infected and HGV-coinfected. One (5.5%) of 18 children born to HCV RNA–positive mothers had HCV RNA (HGV-coinfected but HIV-1–uninfected); the remaining 17 were consistently HCV RNA–negative and lost HCV antibodies during follow-up (median, 24.8 months; range, 11–64.2). No child born to an HBsAg-positive mother (median follow-up, 19.9 months; range, 14.1–41.2) was HBV-infected; an HBV recombinant vaccine and 0.5 mL/kg anti-HBsAg immunoglobulin (180 IU/mL) were administered within 8 h of birth.

Outcome of HGV infection in children. No HGV-infected child (follow-up, 16 months; range, 12–52) became icteric or showed serum liver enzymes below the upper limit of the normal range. Growth and development was normal in children who were HIV-1–uninfected. The age and infection status at...
Table 1. Seroprevalence of hepatitis G virus (HGV) anti-E2 glycoprotein antibody (E2Ab) and RNA in HIV-1–infected women with injecting drug use (IDU) or risky sexual behavior (RSB) and relative risk (95% confidence limits) of positive HGV markers.

<table>
<thead>
<tr>
<th>Group</th>
<th>E2Ab-positive</th>
<th>RNA-positive</th>
<th>Marker-negative</th>
<th>Relative risk (95% confidence limits)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDU (34)</td>
<td>13 (38.2)</td>
<td>7 (20.5)</td>
<td>14 (41.1)</td>
<td>1.09 (0.69–1.72)</td>
<td>NS</td>
</tr>
<tr>
<td>RSB (24)</td>
<td>7 (29.1)</td>
<td>5 (20.8)</td>
<td>12 (50.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDU continued (12)</td>
<td>4 (33.3)</td>
<td>2 (16.6)</td>
<td>6 (50.0)</td>
<td>0.79 (0.41–1.50)</td>
<td>NS</td>
</tr>
<tr>
<td>IDU ceased (22)</td>
<td>9 (45.4)</td>
<td>5 (22.7)</td>
<td>8 (36.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDU ≥ 4.7 years (17)</td>
<td>6 (35.2)</td>
<td>4 (23.5)</td>
<td>7 (31.8)</td>
<td>1.00 (0.57–1.75)</td>
<td>NS</td>
</tr>
<tr>
<td>IDU &lt; 4.7 years (17)</td>
<td>7 (31.8)</td>
<td>3 (17.6)</td>
<td>7 (31.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSB with IDU men (14)</td>
<td>6 (42.8)</td>
<td>4 (28.5)</td>
<td>4 (28.5)</td>
<td>3.57 (0.99–12.8)</td>
<td>.015</td>
</tr>
<tr>
<td>RSB with non-IDU men (10)</td>
<td>1 (10.0)</td>
<td>1 (10.0)</td>
<td>8 (80)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%). NS, not significant.
* Mantel-Haenszel calculation.

Discussion

HIV-1–infected women exposed to bloodborne and sexually transmitted infections show an HGV marker seroprevalence five times higher than in healthy blood donors [1, 2]. The effectiveness of the sexual route of infection is suggested by similar seroprevalence in IDU and RSB women and by the increased risk in RSB women whose sex partners were IDUs (frequently HGV-infected [2, 5]). Our findings comply with the results of seroprevalence studies in other groups at risk [5–8] and are also supported by the presence of HGV in other body fluids in addition to blood [8]. HCV, but not HBV and HGV, exposure is significantly associated with a history of IDU and supports the HGV sexual transmission mechanism. HCV (unlike HBV) is seldom sexually transmitted [5, 6], and a history of parenteral blood exposure is uncommon in HGV- and HCV-uninfected subjects [3]. As a synergic interrelationship may exist between HIV-1 and sexually transmitted infections [14], an HIV-1–related immune defect may favor an HGV sexual infection.

HIV-1–infected women may be at risk of a persistent HGV infection, similar to those immunocompromised patients receiving hemodialysis [4]. An E2Ab- and HGV RNA–positive ratio of 3.6 is reported in healthy blood donors [2], and the RNA seroprevalence decreases and the E2Ab seroprevalence increases with injecting duration in IDUs with low HIV-1 prevalence [2, 5]. By contrast, the ratio is about half in HIV-1–infected women, and marker seroprevalence is independent of injecting duration in HIV-1–infected IDU women. CD4 T lymphocyte numbers are similar in HGV-infected women and E2Ab-positive women, but more subtle immune defects are likely to hinder their recovery. HIV-1–infected subjects also have difficulty in HCV infection control, independent of CD4 T lymphocyte numbers [15].

Our study indicates that the HGV transmission rate in HIV-1–infected mothers is higher than that of HIV-1 or HCV. It is also presumably higher than that in normally immune-competent women [8]. Whether transmission relates to HGV maternal virus load or not will be the aim of further studies, and monitoring at-risk women during pregnancy could indicate whether primary infection favors transmission of the virus.

In the case of a bloodborne infection, HGV RNA becomes positive within 2 weeks of transfusion [3]. In HGV-infected

Table 2. Hepatitis G virus RNA and anti-E2 glycoprotein antibodies (E2Ab) in children with vertical infection.

<table>
<thead>
<tr>
<th>Child no.</th>
<th>Coinfection</th>
<th>Age at last follow-up (months)</th>
<th>RNA at last follow-up</th>
<th>E2Ab at last follow-up</th>
<th>Age at seroconversion (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>12</td>
<td>Positive</td>
<td>Negative</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>16</td>
<td>Negative</td>
<td>Positive</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>16</td>
<td>Negative</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>HCV</td>
<td>36</td>
<td>Positive</td>
<td>Negative</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>HIV-1</td>
<td>52</td>
<td>Negative</td>
<td>Positive</td>
<td>48</td>
</tr>
</tbody>
</table>

NOTE. HCV, hepatitis C virus.
children, RNA was found just a few days after delivery, and no other child at risk became positive thereafter. Three infected children were born by cesarean section, and no evidence of preferential HIV-1 (mostly acquired at delivery [11]) and HGV cotransmission was observed. This would suggest that HGV is more likely to be transmitted during pregnancy than at delivery, although it is impossible to determine definitively. A phylogenetic analysis of HGV in mother and child pairs [8, 9] would definitely prove any mother-to-child transmission. However, babies found to be infected at a median age of 5 days are unlikely to have acquired HGV (bloodborne or sexually transmitted) from any source other than their mothers. Conclusions about cotransmission of HGV and HBV or HCV cannot yet be drawn.

The clinical significance of a vertically acquired HGV infection remains obscure. We have not singled out signs of hepatitis, and studies in vertically or horizontally infected subjects failed in this sense too [1, 3, 8, 9]. Maybe HGV induces hepatitis only under certain circumstances, or the disease was pursued in the wrong place [1, 3].

References