Detection of Hepatitis G Virus RNA in Persons with and without Known Risk Factors for Blood-Borne Viral Infections in Sweden and Honduras

CLAUDIA LARA,1,2 ROBERT HALASZ,1 ANDERS SÖNNERBORG,1 AND MATTI SÄLLBERG1,3*

Divisions of Clinical Virology, F 68, and Oral Microbiology, F 88,3 Department of Immunology, Microbiology, Pathology, and Infectious Diseases, Karolinska Institute, Huddinge University Hospital, S-141 86 Huddinge, Sweden,1 and Universidad Nacional Autonoma de Honduras, Honduras2

Received 15 April 1997/Returned for modification 4 August 1997/Accepted 1 October 1997

We analyzed 224 and 163 serum samples from individuals in Sweden and Honduras, respectively, for the presence of the hepatitis G virus (HGV or GB virus-C) RNA. HGV infection in both Sweden and Honduras was related to common risk factors for blood-borne infections, despite a surprisingly high frequency in groups without known risk factors.

The recently identified hepatitis G virus (HGV or GB virus-C [GBV-C]) (11, 19) has been found worldwide by detection of HGV RNA by a reverse transcription-PCR (RT-PCR) (3, 4, 9, 18). Similar to hepatitis B virus (HBV) and hepatitis C virus (HCV), HGV seems to be transmitted by contact with contaminated blood (11, 19) and vertically from mother to child at partus (5). HGV RNA has been detected in serum, saliva, and liver (2, 11, 13, 19). Interestingly, HGV coinfections do not seem to enhance the pathology of chronic HCV infection (1). A suggested relation between HGV and fulminant hepatitis has been debated (6, 7, 10, 12, 17). Thus, knowledge of HGV infection is still unclear with respect to both transmission routes and any possible pathology.

We studied the prevalence and risk group correlations of HGV infection in Sweden and Honduras using a total of 387 serum samples. Of 224 serum samples from individuals residing in Sweden, 100 were from healthy volunteers, 50 were from intravenous drug users (IVDUs), and 30 were from homosexual men. Of 163 samples from individuals residing in Honduras, 50 were from healthy university students, 10 were from female sex workers, 18 were from homosexual men, 10 were from polytransfused patients, and 17 were from hemophiliacs. Neither the Swedish nor the Honduran healthy subjects had subjective or clinical signs of disease at the time of sampling.

A total of 44 and 58 samples from patients with suspected viral hepatitis of unknown origin were obtained from Sweden and Honduras, respectively. The samples were all negative for immunoglobulin M (IgM) antibodies to hepatitis A virus (Abbott Laboratories, Chicago, Ill.), negative for HBV surface antigen (HBsAg; Abbott), and negative for antibodies to HCV (Abbott). The Swedish samples were also serologically negative for active cytomegalovirus and Epstein-Barr virus infections. For all of these subjects, the clinical diagnosis excluded hepatitis of other nonviral origin.

Total RNA was extracted from 100 µl of serum by guanidinium extraction as described previously (2). cDNA synthesis was initiated by using reverse transcriptase (Boehringer Mannheim, Mannheim, Germany). HGV DNA was amplified by PCR of the 5′ noncoding (5′-NC) region by using either of two different outer primer pairs followed by nesting with the same inner primer pair. The two outer pairs were as follows (locations are according to reference 11; sense HGV-1X (nucleotides [nts] 205 to 222; 5′-TTGTCCTGCGGCGACAGC-3′) and antisense HGV-2X (nts 533 to 516; 5′-AATGCCACCCGCCTCACC-3′) or sense HGV-1V (nts 147 to 166; 5′-ATTCCC GGTTCACTTCTGGTAGC-3′) and antisense HGV-2V (nts 595 to 579; 5′-ATGGCCCGGGGCTCCAC-3′). The inner primer pair has been described previously (2, 5). The nested PCR amplifies a product of approximately 245 bp. All samples positive by any of the 5′-NC region PCRs were subjected to a

<table>
<thead>
<tr>
<th>Population and risk group</th>
<th>No. (%) of sera with the indicated marker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV-antibody</td>
</tr>
<tr>
<td>Swedish</td>
<td></td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>100</td>
</tr>
<tr>
<td>IVDUs</td>
<td>50</td>
</tr>
<tr>
<td>Homosexual men</td>
<td>30 NAa</td>
</tr>
<tr>
<td>Patients with suspected</td>
<td>44</td>
</tr>
<tr>
<td>non-A, non-C hepatitis</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>224</td>
</tr>
<tr>
<td>Honduran</td>
<td></td>
</tr>
<tr>
<td>Healthy university students</td>
<td>50 NA</td>
</tr>
<tr>
<td>Female sex workers</td>
<td>10 7 (70)</td>
</tr>
<tr>
<td>Homosexual men</td>
<td>18 5 (28)</td>
</tr>
<tr>
<td>Polytransfused patients</td>
<td>10 8 (80)</td>
</tr>
<tr>
<td>Hemophiliacs</td>
<td>17 NA</td>
</tr>
<tr>
<td>Patients with suspected</td>
<td>58</td>
</tr>
<tr>
<td>non-A, non-C hepatitis</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>163</td>
</tr>
</tbody>
</table>

a HIV, human immunodeficiency virus.

b All HBsAg-positive subjects were also included among the anti-HBV core antigen (anti-HBc)-positive subjects.

c Of these, two were positive for HBV alone, seven were positive for both HBV and HCV, two were positive for HCV alone, and one was negative for both HBV and HCV.
d NA, not available.
new RNA extraction and were run with primers for the NS3 region for HGV as described previously (2, 5, 22). The expected size of the product of this PCR was 140 bp. For sequence analysis the product from the first-round PCR (5 μl) was further amplified with biotinylated primers from the NS3 region (22). The sequencing reaction was carried out by using the Cy5 AutoRead Sequencing kit and an ALFexpress DNA Sequencer (Pharmacia Biotech, Uppsala, Sweden). Alignment and analysis of the NS3 region sequences was performed by using the GeneWorks, version 2.3, software package (Intelli- genetics, Mountain View, Calif.), and simple dendrograms were constructed by the unweighted pair group method with arithmetic averages (UPGMA) (15) included in the GeneWorks software package.

Two sets of outer PCR primers from the 5′-NC region of the HGV genome were used to analyze the prevalence of HGV viremia in serum samples from Sweden and Honduras. Of a total of 387 samples, 27 (7%) samples were positive for HGV RNA. HGV RNA was identified in one of the Honduran samples with the 5′-NC region HGV-1V–HGV-2V primer set but not the 5′-NC region HGV-1X–HGV-2X or NS3 primer set. Of the 26 (96%) samples in which HGV RNA was detected with the 5′-NC region primer set HGV-1X–HGV-2X, HGV RNA was also identified in 20 (74%) samples with the NS3 primer set, indicating a lower sensitivity of the NS3-based PCR (8, 14, 23).

HGV RNA was detected in 3 (3%) of 100 healthy Swedish volunteers and 1 (2%) of 50 healthy Honduran university students (Table 1). These frequencies are similar to those from other studies of healthy subjects (11, 19). The frequency of samples with HGV RNA was higher among the Swedish IVDS than among the Swedish healthy volunteers (P < 0.0001; Fisher’s exact test). No significant differences in the frequencies of detection of HGV RNA were found between the healthy volunteers and the homosexual men or patients with suspected cases of non-A, non-C hepatitis. The finding may be influenced by the low number of samples analyzed. Overall, our findings are in line with those from other reports.
from European countries (4, 18) and support a relation between HGV infection and known risks for blood-borne infections.

Among the Honduran samples no difference in the frequency of HGV viremia was found among the different groups (Table 1). There was a trend of an elevated frequency of HGV viremia among the polytransfused patients compared to that among the healthy university students (P = 0.0693; Fisher’s exact test).

Among the 27 HGV-RNA positive samples (21 Swedish and 6 Honduran), 20 were partially sequenced for the NS3 region and a limited analysis confirmed an HGV origin for all samples (Fig. 1). Almost all the Swedish HGV strains grouped with the two HGV prototype strains which are distinct from the GBV-C prototype strain (20). Interestingly, HGV from two Swedish HGV-infected IVDUs had NS3 sequences that contained five of the six nucleotides of the motif which was found in HGV from German patients with fulminant hepatitis (6).

In the Swedish material we observed an elevated prevalence of HGV RNA in blood from groups at risk for blood-borne infections, such as IVDUs. The high prevalence of HGV RNA in the healthy population and homosexual men emphasizes the question of whether chronic HGV infection may be transmitted by sexual contacts (16, 21). Thus, chronic HGV infection might also be transmitted by social contacts or other routes yet to be defined.

We thank Rene Stefan and Lilian Garcia for kindly providing samples.

The study was supported by grants from the Swedish Medical Research Council, the Cancer Foundation, and Karolinska Institute Research and Training Programme for Central American Countries.

REFERENCES


