Hepatic histology in hepatitis C virus carriers coinfected with hepatitis G virus

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Abstract

Background—A novel flavivirus has been described recently and designated hepatitis G virus (HGV). The virus is transmitted by the parenteral route but it is uncertain whether it is associated with chronic liver disease because liver biopsy is difficult to justify in this group.

Aims—To examine histological features of liver biopsy in patients infected with hepatitis C virus (HCV) according to the presence or absence of HCV and HGV RNA.

Methods—One hundred and thirty one consecutive HCV carriers undergoing staging liver biopsy were studied retrospectively. In each, HCV RNA and HGV RNA were detected by reverse transcription polymerase chain reaction on serum samples collected at the time of biopsy. The presence of each RNA was correlated with histological features blind to the RNA results; individual histological features of inflammation or fibrosis were scored separately.

Results—Nineteen patients were positive for both HGV and HCV RNA in serum, 91 were positive for HCV RNA alone, two were positive for HGV RNA alone, and 19 were negative for both RNA species. Neither age nor sex differed between the groups; a greater proportion of intravenous drug users were HGV RNA positive, but this was not statistically significant. There was no effect of HGV coinfection on the stage of fibrosis or any other histological parameter except steatosis; patients with HCV and HGV RNA had a higher mean score for fat than those patients with HCV RNA alone (p<0.05).

Conclusions—HGV coinfection has no important effects on histological features in chronic HCV carriers. It is unlikely that HGV infection causes chronic liver disease.

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Keywords: hepatitis C virus; hepatitis G virus; RNA; histology

Novel viruses, designated GBV-A and GBV-B have been identified using representational differential analysis from the serum of a surgeon (GB) with acute hepatitis whose serum induced non-AE hepatitis in tamarins. More recently, using a similar approach, related viruses have been identified in the serum of patients with idiopathic hepatitis; designated GBV-C and hepatitis G virus (HGV) they are considered independent isolates of the same virus since they have high homology for both nucleotide sequences and amino acid alignment. They are positive strand RNA viruses of approximately 9400 nucleotides encoding a single large polypeptide including helicase, protease, and replicase motifs. The genomic organisation is similar to that of the Flaviviridae with structural genes at the N terminal and non-structural genes at the C terminal but there is less genomic variability in the NS 5 region than with hepatitis C virus (HCV). There is 26% amino acid homology with HCV. In similar fashion GBV-A and GBV-B share 27% amino acid homology with each other and 28% with HCV.

Parenteral transmission appears to be the most important mode and has been confirmed by sequence analysis. Thus, intravenous drug users are at particular risk; in one series 16% of intravenous drug users were HGV RNA positive, increasing to 75% if they were anti-HBc positive and 99% where HCV antibody was also detected. HGV RNA has also been identified in patients with cryptogenic hepatitis and in 3.9% of blood donors with elevated serum alanine aminotransferase (ALT) and 0.8% of blood donors with a normal ALT. Other studies have shown a high prevalence in patients who have undergone haemodialysis, suggesting transmission through blood transfusion. The possibility of vertical transmission has also been raised.

An association with liver disease has been shown less consistently. In one early report a proportion of a small group of patients with acute liver failure were HGV RNA positive. However, in a series of patients with acute liver failure in which only patients who had never been transfused were studied, none was HGV RNA positive; this was supported by another study. Persistent infection has been reported in a significant proportion of patients but despite this, evidence of liver disease as a result of chronic infection is lacking. Thus, in patients infected chronically with HGV and undergoing haemodialysis no evidence of liver disease has been identified and only a very small proportion of those with cryptogenic cirrhosis are HGV RNA positive.

Doubts about the role of HGV as a cause of liver damage have arisen because histological studies have not been reported. Some authors have suggested that the absence of a notable biochemical abnormality precludes significant liver disease; however, biochemical indices are
not useful markers of histological progression in patients with chronic HCV infection.\textsuperscript{19} We therefore examined histological status in relation to HGV in a series of well characterised HCV carriers in whom histology has previously been reported.\textsuperscript{20} We believed that comparison of those HCV carriers who were HGV positive or negative would address the issue of whether HGV exacerbated liver disease. In addition, it would address the possibility that coinfec-tion with HGV might be one factor influencing the progression of chronic HCV infection.

**Methods**

**Patients**

One hundred and thirty one chronic HCV carriers undergoing routine liver biopsy were studied. They represent a consecutive series of patients with chronic HCV infection uncomplicated by other disorders of the liver or chronic hepatitis B virus infection. Our policy has been to perform liver biopsy on all chronic HCV carriers of more than five years duration irrespective of clinical findings or serum HCV RNA status. Many of these patients have been reported previously.\textsuperscript{20} Ninety two of the patients studied were male and the median age was 40 years (range 24–75). All patients were anti-HCV positive by second generation enzyme linked immunosorbent assay (ELISA) (Ortho) confirmed by RIBA 2 or 3. At the time of liver biopsy serum was stored below –30°C for subsequent analysis for viral RNA.

**Histology**

Each liver biopsy specimen was assessed as described previously.\textsuperscript{20} All specimens were fixed in 10% (vol/vol) neutral buffered formalin. Tissue sections, 3–4 mm thick, were cut and stained with haematoxylin and eosin, and silver for reticulin fibres. The histological sections were graded and staged semiquantitatively by a single, experienced pathologist based on a modified scoring system which was similar to other semiquantitative systems,\textsuperscript{21–23} and very close to the recent modification of Knodell.\textsuperscript{24} In addition, it takes into account the most commonly seen features in chronic HCV infection, namely lymphoid aggregates, bile duct damage, and parenchymal fat.

**Grading Features**

- **Portal or periportal interface hepatitis** (piecemeal necrosis): 0 = none, 1 = mild (focal, few portal areas), 2 = mild/moderate (focal, most portal tracts).
- **Lobular activity** (spotty necrosis, apoptosis, and focal inflammation): 0 = negligible inflammation, 1 = one focus in less than two-thirds of lobules or nodules, 2 = foci in more than two-thirds of lobules or nodules.
- **Portal inflammation**: 0 = none, 1 = mild, some or all portal areas, 2 = moderate, some or all portal areas.
- **Lymphoid aggregates**: 0 = none, 1 = occasional only, 2 = present in most inflamed portal tracts.
- **Bile duct damage**: 0 = absent, 1 = present.
- **Fat**: 0 = none or minimal, 1 = mild, 2 = moderate, 3 = diffuse.

**Confluent necrosis** was encountered so infrequently that it was not scored individually.

**Staging features**: the fibrosis score has been extended to take account of the intermediate degrees of fibrosis seen so frequently in these biopsy specimens.\textsuperscript{18}

- **Fibrosis**: 0 = none, 1 = confined to portal tracts, 2 = portal tracts plus spurs radiating into parenchyma, 3 = linkage of some portal tracts but intact architecture, 4 = linkage of most portal tracts with architectural distortion, 5 = cirrhosis.

**RNA Extraction and Reverse Transcription Polymerase Chain Reaction**

HCV RNA and HGV RNA were determined in serum. RNA was extracted, amplified, and detected as described previously.\textsuperscript{25} In brief, captured and eluted RNA was used directly in a combined 20 µl cDNA/polymerase chain reaction (PCR) (37°C for 30 minutes, followed by 95°C for three minutes, and 30–35 cycles of 48°C for 25 seconds, 72°C for 25 seconds, 95°C for 15 seconds, and a final extension at 72°C for seven minutes) in an oil free amplification system 9600 (Perkin-Elmer). Table 1 lists the primers used. An aliquot of 1 µl was used for the nested PCR using the same conditions, omitting the initial 37°C cDNA step. After agarose gel electrophoresis and Southern blotting the membrane was hybridised with 5′-digoxigenin (DIG) end labelled probes specific for either HCV or HGV (table 1) and a positive signal detected using a DIG chemiluminescent detection kit (Boehringer Mannheim) according to the manufacturer’s instructions. Two aliquots of each sample were assayed. In a case of ambiguous results HCV Amplicor (Roche) was used according to the manufacturer’s instructions.

**Statistical Analysis**

The unpaired Student’s t test was used to evaluate differences between groups of patients.

**Results**

Patients were classified into groups according to the presence or absence of HCV and HGV RNA in serum collected on the day of their liver biopsy. Nineteen were negative for both RNA types (group 1), 91 were HCV RNA positive but HGV RNA negative (group 2), and 19 were both HCV RNA and HGV RNA positive (group 3). The group of two patients who were negative for HCV RNA but positive for

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**Table 1 Oligonucleotides**

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Specificity</th>
<th>Function</th>
<th>Polarity</th>
<th>Position (nt)</th>
<th>Length (nt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF1</td>
<td>HCV</td>
<td>PCR</td>
<td>+</td>
<td>83–102</td>
<td>20</td>
</tr>
<tr>
<td>CB1</td>
<td>HCV</td>
<td>PCR</td>
<td>–</td>
<td>339–321</td>
<td>19</td>
</tr>
<tr>
<td>CF2</td>
<td>HCV</td>
<td>PCR</td>
<td>+</td>
<td>98–118</td>
<td>20</td>
</tr>
<tr>
<td>CB2</td>
<td>HCV</td>
<td>PCR</td>
<td>–</td>
<td>313–295</td>
<td>19</td>
</tr>
<tr>
<td>CDHP*</td>
<td>HGV</td>
<td>Hybridisation</td>
<td>+</td>
<td>132–151</td>
<td>20</td>
</tr>
<tr>
<td>GF1</td>
<td>HGV</td>
<td>PCR</td>
<td>+</td>
<td>106–126</td>
<td>21</td>
</tr>
<tr>
<td>GB1</td>
<td>HGV</td>
<td>PCR</td>
<td>–</td>
<td>371–347</td>
<td>25</td>
</tr>
<tr>
<td>GF2</td>
<td>HGV</td>
<td>PCR</td>
<td>+</td>
<td>125–149</td>
<td>25</td>
</tr>
<tr>
<td>GB2</td>
<td>HGV</td>
<td>PCR</td>
<td>–</td>
<td>306–285</td>
<td>22</td>
</tr>
<tr>
<td>GDHP*</td>
<td>HGV</td>
<td>Hybridisation</td>
<td>–</td>
<td>180–160</td>
<td>21</td>
</tr>
</tbody>
</table>

*Contains 5′ digoxigenin label.
Hepatitis C virus carriers coinfected with hepatitis G virus

HGV RNA was too small to be included in the analysis. There was no difference between the three remaining groups with respect to their age or the male to female ratio. Nineteen of 21 (90.5%) patients who were HGV RNA positive admitted to intravenous drug use in contrast to 78/110 (70.9%) who were HGV RNA negative (difference not significant). Table 2 shows the scores of histological features.

The proportion of patients with stage IV or V fibrosis was similar in group 2 (12/91; 13.2%) compared with group 3 (2/19; 10.5%) and exceeded that in patients with no HGV RNA detected (group 1: 1/19; 5.3%) but this was not statistically significant. The mean fibrosis scores in groups 2 and 3 were similar and exceeded those of group 1 (p<0.05). Portal tract inflammation was also increased in group 2 and was not significantly greater than in group 3. However, these values did exceed those of group 1 (p<0.05). With respect to piecemeal necrosis, lymphocyte aggregates, and bile duct damage, there were no significant differences between the groups. However, for the presence of steatosis there was a significant increase in the mean score in group 3 (HCV RNA and HGV RNA) compared with group 2 (HCV RNA) (p<0.05).

Discussion

This study shows that there are no major histological differences between patients who have both HCV RNA and HGV RNA in serum compared with those without HGV RNA. Coinfection with HGV was associated with a significantly higher mean score for steatosis although the proportion of patients who had no detectable fat in their liver biopsy specimen was similar in all groups. Fat has previously been shown to be one marker of an increased risk of fibrosis. In other respects there were no differences in the inflammatory scores or fibrosis stage that could be attributed to HGV coinfection. In a recent series of similar coinfected patients, 40/70 underwent a liver biopsy. No differences were found between patients with and without HGV coinfection. In addition, there was no obvious effect on long term graft function in 39 patients with non-A–C hepatitis despite an increased prevalence of HGV infection after liver transplantation.

The differences shown with respect to fibrosis could not be attributed to the presence of HCV RNA. This is in contrast to our previous finding which failed to find such a relation. However, it must be stressed that in this particular series of 19 patients who were HCV RNA negative at the time of their liver biopsy, six had no detectable fibrosis, only one had a normal biopsy, and one was precirrhotic. Thus, a single estimation of HCV RNA is not adequate to exclude the presence of underlying chronic liver disease.

The contrast between HGV and HCV with respect to liver damage in view of the persistent infection in both instances is striking. One possible explanation relates to the presence or absence of core protein. HGV lacks a typical core protein7 while the core of HCV may be absent of core protein. HGV lacks a typical core protein7 while the core of HCV may be important in the evolution of chronic liver disease via its link with programmed cell death.28

This paper has shown no serious consequences of HGV coinfection in this selected group of HCV carriers. In addition, it is clear that HGV coinfection cannot explain why some patients with chronic HCV infection proceed rapidly to fibrosis whereas others do not.

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Table 2  Relation between HCV RNA, HGV RNA and histological features

<table>
<thead>
<tr>
<th>Histological feature</th>
<th>Group 1 HGV RNA (n=19)</th>
<th>Group 2 HCV RNA + HGV RNA (n=91)</th>
<th>Group 3 HCV RNA + HGV RNA (n=19)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal tract inflammation</td>
<td>0.95* (0.85)</td>
<td>1.32* (0.61)</td>
<td>1.21 (0.54)</td>
<td>&lt;0.03*</td>
</tr>
<tr>
<td>Portal necrosis</td>
<td>0.42 (0.61)</td>
<td>0.53 (0.72)</td>
<td>0.53 (0.58)</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocytic aggregation</td>
<td>0.47 (0.61)</td>
<td>0.82 (0.77)</td>
<td>0.68 (0.58)</td>
<td>NS</td>
</tr>
<tr>
<td>Bile duct damage, present</td>
<td>15.8</td>
<td>15.4</td>
<td>15.8</td>
<td>NS</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>1.58* (1.22)</td>
<td>2.14* (1.36)</td>
<td>2.37* (1.12)</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Fat</td>
<td>0.63 (0.83)</td>
<td>0.51* (0.78)</td>
<td>0.95* (1.22)</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Lobular inflammation</td>
<td>0.63 (0.68)</td>
<td>0.95 (0.67)</td>
<td>0.74 (0.65)</td>
<td>NS</td>
</tr>
</tbody>
</table>

See text for scoring system. Two patients positive for HGV in serum but negative for HCV RNA are not included. Values are mean (SD) scores, except for bile duct damage, which was present or absent.