Hepatitis G virus infection in primary Sjögren’s syndrome: analysis in a series of 100 patients

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Abstract
Objective—To determine the prevalence and clinical significance of hepatitis G virus (HGV) infection in a large cohort of patients with primary Sjögren’s syndrome (SS).

Patients and methods—The study included 100 consecutive patients (92 female and eight male), with a mean age of 62 years (range 31–80) that were prospectively visited in our unit. All patients fulfilled the European Community criteria for SS and underwent a complete history, physical examination, as well as biochemical and immunological evaluation for liver disease. Two hundred volunteer blood donors were also studied. The prevalence of HGV-RNA was investigated in the serum of all patients and donors. Additionally, HBsAg and antibodies to hepatitis C virus were determined.

Results—Four patients (4%) and six volunteer blood donors (3%) presented HGV-RNA sequences in serum. HGV infection was associated with biochemical signs of liver involvement in two (50%) patients. When compared with primary SS patients without HGV infection, no significant differences were found in terms of clinical or immunological features. HCV coinfection occurs in one (25%) of the four patients with HGV infection.

Conclusion—The prevalence of HGV infection in patients with primary SS is low in the geographical area of the study and HCV coinfection is very uncommon. HGV infection alone does not seem to be an important cause of chronic liver injury in the patients with primary SS in this area.

Sjögren’s syndrome (SS) is a systemic autoimmune disease that mainly affects exocrine glands and that usually presents as a persistent dryness of the mouth and the eyes because of functional impairment of the salivary and lacrimal glands. The histological hallmark is a focal lymphocytic infiltration of the exocrine glands. In the absence of an associated connective tissue disease, patients with this condition are classified as having primary SS. The factors that might trigger this autoimmune disorder remain unknown, but viral infections have repeatedly been suggested as a possible cause. Furthermore, a possible relation between hepatitis C virus (HCV), a member of the Flaviviridae family, which is a virus that can be excreted in saliva, and SS has recently been postulated. Using polymerase chain reaction (PCR) assay, some studies showed a prevalence of HCV infection in primary SS significantly high when compared with the prevalence of HCV infection that has been found in general population and raises the possibility of a link between HCV infection and SS.

The hepatitis G virus (HGV) is a recently discovered member of the Flaviviridae family that may cause acute and chronic infection in humans. HGV is similar in sequence to the hepatitis GB-C (HGBV-C) virus that may be responsible of cases of non-A, non-B hepatitis. HGV and HGBV-C are probably different isolates of the same agent. Preliminary data indicate that HGV/HGBV-C infection can be detected in a comparatively high proportion of apparently healthy volunteer blood donors, in recipients of blood transfusions, patients on haemodialysis, intravenous drug abusers, and in patients with acute and chronic liver disease. Although it may occur in 3% to 8% of patients with cryptogenic disease, HGV sequences are also found in 10% to nearly 20% of chronic liver disease patients who are infected by the hepatitis B virus (HBV) or by the hepatitis C virus (HCV),

To the best of our knowledge, there have been no previous studies about the implication of HGV infection in extrahepatic syndromes associated with HCV infection, such as cryoglobulinaemia, glomerulonephritis or SS. To determine if there is a possible link between SS and HGV infection, and the possible relation with other hepatitis virus infections, we have investigated the presence of HGV-RNA sequences in a large series of patients with primary SS as well as their clinical significance.

Methods
We included 100 consecutive patients (92 female and eight male; mean age 62 years; range 31–80) who attended our Systemic Autoimmune Diseases Unit from March 1993 until August 1996. All patients were white and fulfilled four or more of the diagnostic criteria for SS proposed by the European Community Study Group in 1993. None of these patients presented clinical or immunological evidence of other systemic autoimmune disease and no patient had been previously diagnosed as having primary biliary cirrhosis or autoimmune hepatitis.

Two hundred consecutive, first donation, volunteer blood donors were also studied. One hundred and fourteen were men and 86 women. Their mean age was 39 years, ranging from 18 to 65.
Table 1. Clinical and immunological manifestations of patients with primary SS (SS) and HGV infection compared with patients without HGV infection

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>SS with HGV infection (n=4) (%)</th>
<th>SS without HGV infection (n=96) (%)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry mouth</td>
<td>3 (75)</td>
<td>95 (99)</td>
<td>NS</td>
</tr>
<tr>
<td>Dry eyes</td>
<td>3 (75)</td>
<td>92 (96)</td>
<td>NS</td>
</tr>
<tr>
<td>Parotidomegaly</td>
<td>3 (75)</td>
<td>34 (35)</td>
<td>NS</td>
</tr>
<tr>
<td>Esplenomegaly</td>
<td>1 (25)</td>
<td>7 (7)</td>
<td>NS</td>
</tr>
<tr>
<td>Articular involvement</td>
<td>2 (50)</td>
<td>44 (46)</td>
<td>NS</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>0 (0)</td>
<td>14 (15)</td>
<td>NS</td>
</tr>
<tr>
<td>Cutaneous vasculitis</td>
<td>0 (0)</td>
<td>13 (14)</td>
<td>NS</td>
</tr>
<tr>
<td>Pulmonary involvement</td>
<td>2 (50)</td>
<td>10 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>Liver involvement</td>
<td>2 (50)</td>
<td>13 (14)</td>
<td>NS</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>0 (0)</td>
<td>11 (11)</td>
<td>NS</td>
</tr>
<tr>
<td>Autoimmune thyroiditis</td>
<td>0 (0)</td>
<td>16 (17)</td>
<td>NS</td>
</tr>
<tr>
<td>ANA (+)</td>
<td>3 (75)</td>
<td>63 (66)</td>
<td>NS</td>
</tr>
<tr>
<td>RF (+)</td>
<td>2 (50)</td>
<td>42 (44)</td>
<td>NS</td>
</tr>
<tr>
<td>Ro-SSA (+)</td>
<td>1 (25)</td>
<td>25 (26)</td>
<td>NS</td>
</tr>
<tr>
<td>La-SSB (+)</td>
<td>0 (0)</td>
<td>13 (14)</td>
<td>NS</td>
</tr>
</tbody>
</table>

ANA=antinuclear antibodies. RF=rheumatoid factor. NS=not significant difference.

For HGV-RNA analysis, RNA was extracted from 140 µl of serum using silica gel based membrane columns (QIAamp HCV, Qiagen, Hilden, Germany) and eluted in a final volume of 50 µl of Rnase free water. Complementary DNA was synthesised from 10 µl of RNA by 45 minutes incubation at 42°C with 25 U of Expand reverse transcriptase (Boehringer Mannheim, Mannheim, Germany), 50 nM of random primers, 1 U of ribonuclease inhibitor, and 200 µM of each deoxynucleotide in a final volume of 20 µl. Specific amplification of the 5'non-translated region (5'NCR) of HGV genome was carried out using the following primers: 5'-CGG CCA AAA GGT GGT GGA TG-3', sense primer, position 101–120 and 5'-CGA CGC TGA CGT GGG G-3' antisense primer, position 285–267 (Hepatitis G virus Primer and Capture Probe Set, Boehringer Mannheim, Mannheim, Germany). PCR was performed in a Techne Progene thermal cycler (Techne, Cambridge, UK) by 40 cycles of 94°C for 45 seconds, 55°C for one minute, 72°C for one minute, and a final extension cycle of five minutes at 72°C. Digoxigenin labelled PCR products were detected by solution hybridisation to a biotin labelled 5'NCR specific capture probe that is complementary to the inner part of the amplicon. Hybrids were immobilised to a streptavidin coated microtitre plate surface. The bound hybrid was detected by an anti-digoxigenin peroxidase conjugate (PCR ELISA DIG detection, Roche, Mannheim, Germany). Appropriate positive and negative controls were used for extraction, retrotranscription, amplification, and detection steps. Positive controls included serum samples known to contain HGV RNA in high and low concentration, and amplification and detection of a control human gene (tissue plasminogen activator gene). Negative controls included serum samples negative for HGV RNA, as well as reagents without template. Absorbance was measured at 405 nm. The mean optical density (OD) was 0.046 (range: 0.038–0.057) in negative controls and 1.204 (range 1.021–1.427) in positive controls. Samples giving a signal five times above the mean OD of negative controls were considered positive. Positive results were accepted upon agreement on repeated testing.

Serum samples from all patients were tested for hepatitis B surface antigen (HBsAg) by ELISA. Serum from all patients were analysed for HCV antibodies by a third generation ELISA (Ortho 3.0 Diagnostic Systems, Neckargemund, Germany). In anti-HCV positive serum samples, presence of HCV-RNA was analysed by PCR (Promega, Madison, WI, USA).

**STATISTICAL ANALYSIS**

For analysing qualitative differences we used a χ² test or the Fisher’s exact test when appropriate. For comparison of quantitative parameters, Student’s t-test was used in large samples of similar variance, and non-parametric Mann-Whitney U test for small samples. p Values < 0.05 were considered to indicate statistical significance and odd ratio (OR) were calculated with 95% confidence intervals (CI).

**Results**

HGV-RNA was detected in four (4%) patients with primary SS and in six (3%) of the volunteer blood donors studied (the difference was not statistically significant). All patients were women. Mean age at the onset of clinical manifestations of dry syndrome was 40 years (range 29–48) and at the time of protocol was 49 years (range 31–69). When primary SS patients with and without HGV infection were compared, no significant differences were found (table 1).

One patient showed HCV-HGV coinfection, and presented hepatomegaly and increased serum aspartate transaminase (AST) and serum alanine transaminase (ALT). Another patient, with HGV infection alone, had an intermittent four year history of mildly raised transaminase serum activities without evidence of other causes of hepatic involvement. No clinical signs of liver disease were present in this patient. In the two remaining HGV positive patients, no clinical or biochemical evidence of liver involvement was found.

Antibodies to HCV were present in 14 (14%) patients with primary SS and in two (1%) of the blood donors studied (p < 0.001, OR 16.1, CI 3.4, 105.0). HCV-RNA was positive in all of them. HBsAg was detected in two (2%) patients with primary SS, both with HCV coinfection and liver involvement, whereas HBsAg was detected in only one (0.5%) of the control group.

**Discussion**

In this study, we analysed the frequency and consequences of HGV infection in patients with primary SS. HGV-RNA sequences were found in only 4% of patients with primary SS, similar to the prevalence found in the healthy volunteer blood donors studied (3%). In contrast, HCV infection was detected in 14% of patients with primary SS, whereas the proportion in the blood donors was only 1%.
The clinical consequences of chronic HGV infection have not yet been clearly defined. HGV related disease is generally mild, with normal levels of aminotransferases. Clinical data show that many people infected with HGV alone do not show evidence of liver disease. In fact, abnormal ALT serum activities in subjects with HGV infection can often be ascribed to a coexisting infection with HCV or HBV.\(^\text{59}\) Virtually all increases of ALT in patients on maintenance haemodialysis infected by HGBV-C were ascribable to coinfection with HCV or HBV.\(^\text{13}\)

Three clinical outcomes have been described for the HGV infection\(^\text{14}\): rapid recovery with resolution of raised transaminase activity and eventual clearance of viraemia, delayed recovery with intermittent increased transaminases, and chronic hepatitis.\(^\text{6}\) Certainly, there are no prospective studies that report histologically the progression from acute HGV/HGBV-C infection through various stages of chronic hepatitis to the development of cirrhosis, hepatocellular carcinoma, or end stage liver disease.

In our patients with primary SS, HGV infection was associated with biochemical signs of liver involvement in two of four patients, but in one of these, HCV infection was also detected. In this patient with HCV-HGV dual infection transaminase activities were higher than in the patient with HGV infection alone. Thus, HGV infection alone does not seem to be an important cause of chronic liver injury in our patients with primary SS, and is associated with a quite small increase in aminotransferases in only one (1%) of our patients with primary SS.

In conclusion, HGV seems to play a small part in the aetiology of liver involvement among patients with primary SS, but HGV-RNA detection is recommended for patients with primary SS and unexplained liver disease, especially in those patients without evidence of HBV or HCV infections.

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