Detection of Hepatitis G Virus (HGV) RNA: Clinical Characteristics of Acute HGV Infection

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The role of hepatitis G virus (HGV) infection in acute non-A–E hepatitis was investigated in adults with viral hepatitis. HGV RNA was present in 1 of 28 patients with non-A–E hepatitis but 9 of 22 with hepatitis C (\(P < .003\)). HGV RNA–positive patients (HGV-infected and HGV–hepatitis C virus [HCV]–coinfected) developed light-to-moderate jaundice. Clinical and biochemical features of HGV-positive and HCV-positive patients and patients with non-A, non-G hepatitis were similar. Three patients with HGV-HCV coinfection, tested within 18 months after disease onset, have remained HGV RNA–positive but have become HCV RNA–negative. Only 1 non-A–E hepatitis patient was confirmed as being infected with HGV alone, suggesting that HGV is not the main etiologic agent of non-A–E hepatitis. Although HGV RNA was significantly associated with hepatitis C, patients with mixed HCV-HGV infections did not demonstrate a more severe course of disease than did patients with HCV infection.

Despite the availability of diagnostic tests for detection of hepatitis C virus (HCV) [1, 2] and hepatitis E virus (HEV) [3–5], cases of acute and chronic hepatitis in which patients do not exhibit markers for any known hepatitis viruses still exist [6–8] and are referred to as non-A–E hepatitis. Some 4%–20% of acute hepatitis cases in Europe and the United States are associated with non-A–E hepatitis [9–12]. Although the polymerase chain reaction (PCR) has significantly enhanced the ability to detect viral infections, >40% of fulminant hepatitis cases are of unknown etiology [13].

Hepatitis G virus (HGV) and GB virus C (GBV-C) are parenterally transmitted agents potentially involved in the etiology of non-A–E hepatitis [14–16]. Molecular studies demonstrate the close relationship of these agents: The nucleotide sequences of the NS3 regions of HGV and GBV-C are 85% identical and the amino acid sequences are 100% identical, suggesting that HGV and GBV-C are independent isolates of the same virus. Together with HCV, HGV/GBV-C may belong to a distinct group of hepatitis viruses within the Flaviviridae family [14–16]. HGV RNA was detected in 1%–2% of blood donors in the United States [16] and is prevalent in patients with frequent parenteral exposure (intravenous drug users, hemodialysis patients, and hemophiliacs) [17, 18].

HGV can also be detected in some patients with acute non-A–E hepatitis, hepatitis C, and biopsy-documented chronic hepatitis in which hepatitis B virus (HBV) and HCV were excluded as etiologic factors [16]. However, the clinical impact of HGV infection remains to be fully elucidated. We sought to determine the role of HGV infection in the etiology of non-A–E hepatitis.

Materials and Methods

Patients. Adult viral hepatitis patients 15–75 years of age, all residents of Moscow, with acute HCV infection (\(n = 22\)) or non-A–E hepatitis (\(n = 28\)) were admitted to the First Infectious Disease Hospital in Moscow between June 1993 and December 1994. Diagnoses were based on retrospective and prospective data, including clinical symptoms and signs, biochemical liver function tests, characteristic laboratory findings, and serologic tests. Patients with non-A–E hepatitis did not demonstrate serologic markers of acute hepatitis A, B, C, D, or E and were HCV RNA–negative. Criteria for severity of viral hepatitis were previously reported [19]. Patients were interviewed for relevant clinical and epidemiologic data. Serum samples were available from all patients on admission and from some of the patients during follow-up. Serum samples for PCR were aliquoted and stored at –70°C until analysis.

The definition of maximum possible incubation period of the disease was the period from the first day of parenteral manipulation (within 12 months before onset) to onset of disease (appearance of dark urine or symptoms). The minimum possible incubation period was defined as the period from the last day of known parenteral exposure to the first appearance of hepatitis symptoms. The mean incubation period was calculated from the minimum and maximum incubation periods. The duration of the acute period of disease was defined as the period from onset to clear recovery, disappearance of jaundice and clinical symptoms, and significant decrease (to within 3 times normal) in concentrations of liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Asthenovegetative prodrome was defined as a combination of weakness, fatigue, and headache; dyspeptic prodrome was defined as the combination of anorexia and nausea or vomiting.
Serologic tests. Serum samples from all patients were screened for hepatitis B surface antigen, IgM antibodies to hepatitis B core antigen, and IgM antibodies to hepatitis A virus by use of commercially available EIAs (Abbott, Abbott Park, IL). IgM and IgG antibodies to hepatitis delta virus were detected by commercially available EIA (Diagnostic Systems, Novgorod, Russia). IgG antibodies to HEV were measured by an EIA based on a recombinant mosaic HEV protein [20]. HCV antibodies were detected by using a second-generation EIA (Abbott) and further characterized with a supplemental immunoblot system (MATRIX HCV; Abbott).

Biochemical tests. Serum samples were assayed weekly during the acute period of the disease for bilirubin, ALT, AST, γ-glutamyl aminotransferase, prothrombin time, protein, and protein fractions.

HCV RNA detection. HCV RNA was extracted from 100 μL of serum by the acid guanidine–phenol-chloroform method [21], reverse-transcribed, and amplified by use of the biotinylated primer pairs KY 78 (5′-biotin-CTCAAGCACCCTATCGGAGT-3′) and KY 80 (5′-biotin-GCAGAAGGCGTCAAGGCACG-3′) derived from the HCV 5′-untranslated region. The reverse transcription (RT)–PCR was performed according to previously described methods [22] with minor modifications by use of a thermal cycler (9600; Perkin-Elmer Cetus, Norwalk, CT). The amplification product was detected by a colorimetric microwell assay by use of an HCV-specific solid-phase capture probe. The denatured PCR product was hybridized to the immobilized capture probe nucleotide sequence was determined with an automated DNA sequencer (ABI Prism 377; Perkin-Elmer Cetus). The consensus sequence was aligned by use of the NALIGN module in the PGENE 6.85 software package (Intelligenetics, Palo Alto, CA) with the GenBank wild type HCV sequence.

Results

HGV RNA was detected in only 4% (1/28) of the non-A–E hepatitis patients, in contrast to 41% (9/22) of the acute HCV-infected patients (P < .003). Sequence analysis of both strands of amplification products from HGV-positive samples revealed 86%–89% homology in 370 nucleotides with the GenBank HGV sequence. Of the 9 patients who tested positive for both HGV RNA and HCV infection, 7 were positive for both HCV RNA and HCV antibodies, 1 was positive for HCV RNA but was HCV antibody–negative, and 1 had HCV antibodies only. All 10 HGV RNA–positive patients were also positive for at least one GBV-C primer pair. None of the 40 HGV RNA–negative patients was positive for GBV-C RNA (table 1).

Clinical and biochemical data from HGV-positive patients (including those with mixed HGV–HCV infections) were comparable with those from HCV and non-A, non-G hepatitis patients. Seventy to eighty-five percent of the patients in each group were exposed parenterally within 12 months before dis-
Table 1. Detection of GB virus C (GBV-C) RNA in HGV-positive and -negative samples.

<table>
<thead>
<tr>
<th>Group, patient no.</th>
<th>HGV RNA</th>
<th>GBV-C RNA*</th>
<th>GBV-C RNA²</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGV RNA—positive</td>
<td>100% (10/10)</td>
<td>80% (8/10)</td>
<td>70% (7/10)</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>2</td>
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<td>9</td>
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<td>+</td>
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</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>HGV RNA—negative</td>
<td>0/40</td>
<td>0/40</td>
<td>0/40</td>
</tr>
</tbody>
</table>

* Detected according to method of Yoshiba et al. [23].
² Detected by use of YK 1162–1165 primers.

ease onset (table 2). Most patients presented with asthenovegetative symptoms, although some patients in each group exhibited dyspeptic symptoms. Flu-like symptoms (fever) were observed in 8% (1/12) of the HCV patients and 29% (6/21) of the non-A, non-G hepatitis patients (P > .2). Arthralgia was not present in any of the patients examined in this study. Comparison of duration of incubation period and the duration of the acute period of the disease among groups is shown in table 2. All patients developed jaundice, but none developed severe forms of acute hepatitis.

The mean ALT and AST concentrations were similar (P > .1) for all patient groups (table 2); however, non-A, non-G hepatitis patients exhibited broader ranges and higher peaks (5170 U/L and 6110 U/L, respectively) than did HGV- or HCV-infected patients. There were no statistically significant differences in the concentrations of bilirubin, γ glutamyl aminotransferase, protein, or protein fractions or the prothrombin time among patient groups (data not shown).

Four HGV RNA—positive patients (3 with mixed HGV-HCV infection, 1 with HGV alone) were followed during 9–19 months of convalescence (figures 1–4). HGV RNA was detectable in the sera of all 3 patients with mixed HGV-HCV infection 9–18 months after onset of the disease; these patients were positive for HCV RNA during the acute phase of the disease and became negative for HCV RNA at 3–18 months of convalescence (figures 2–4). High HGV RNA titers were found 16–19 months after onset in 3 of 4 patients (figures 1–3). One patient had low-titer (10^1) HGV RNA during acute disease (figure 4), and 9 months later, HGV RNA could be detected only in undiluted serum.

Discussion

The finding that HGV RNA was detected in only 4% of non-A–E hepatitis patients as opposed to 41% of 22 HCV-infected patients suggests that HGV may be a possible, but is certainly not the main etiologic agent of non-A–E hepatitis. Indeed, 9 of the 10 HGV-positive patients also had markers of HCV infection. However, none of the HGV-positive patients exhibited symptoms or reported a history of hepatitis prior to their enrollment in this study; the acute hepatitis symptoms in these patients could be due to infection with HGV, HCV, or both viruses.

Both HCV and HGV patient groups had similar frequencies (70%–85%) of parenteral exposure within 12 months before disease onset. No specific data of possible nonparenteral exposure as a transmission factor were found. Additional studies on representative groups of patients with other parenterally transmitted hepatitis (hepatitis B or hepatitis D) and of patients in other high-risk groups, as well as a knowledge of the prevalence of HGV infection in the geographic region under investigation, are necessary to establish the HGV transmission risk factors with greater certainty.

All HGV RNA—positive patients were positive with at least one set of GBV-C primers derived from the NS-3 region of infection.
GBV-C, confirming the close relationship of HGV and GBV-C. The use of a more highly conserved region of the GBV-C genome for PCR might increase the sensitivity of GBV-C RNA detection in HGV RNA-positive samples.

Clinical and biochemical data were similar for the HCV- and HGV-positive patients; all patients developed acute hepatitis without complications. None of the HGV/GBV-C-positive patients in this study developed severe forms of hepatitis; we did not find evidence that GBV-C plays an important role in fulminant and subfulminant non-A–E hepatitis.

The mean period of acute infection (from beginning of disease onset to the disappearance of jaundice and other clinical symptoms and decrease in ALT concentrations) was the same for HCV and HGV patients. In contrast, patients with acute non-A, non-G hepatitis developed a moderately severe disease more frequently (79%), the mean duration of the disease was longer (19 ± 9 days), and peak ALT concentrations were higher than in patients with either HGV or HCV infections, suggesting that mixed HCV-HGV infection does not influence the severity of the disease. Prospective follow-up studies of 4 HGV-positive patients showed that these patients had persistent viremia (9–19 months), although none demonstrated symptoms of chronic liver disease and none had elevated serum aminotransferases during follow up. Long-term

![Figure 1](https://academic.oup.com/jid/article-abstract/175/6/1302/1023404)

**Figure 1.** Clinical course of HGV infection. Patient, 47-year-old man, denied any parenteral exposure within 12 months before onset of disease. ALT, alanine aminotransferase.

![Figure 2](https://academic.oup.com/jid/article-abstract/175/6/1302/1023404)

**Figure 2.** Clinical course of HGV and HCV coinfection. Patient was 17-year-old male intravenous drug user. ALT, alanine aminotransferase.
Figures 3 and 4 illustrate the clinical courses of HGV and HCV coinfection. The patient in Figure 3, a 33-year-old man, had multiple injections 5 weeks before onset of disease. ALT, alanine aminotransferase. The persistence of high-titer ($10^4$–$10^6$) viremia after clinical and biochemical recovery and the absence of reduction of HGV RNA titers during convalescence suggest that these patients might be chronic HGV carriers and that the acute hepatitis might be unrelated to HGV infection. An indirect indication of a leading role for HCV in the pathogenesis of acute hepatitis in cases of mixed HGV-HCV infection was the disappearance of HCV RNA during follow-up in patients positive for both HGV and HCV RNA. Prospective studies of longer duration and histologic examination of biopsy material are necessary to conclusively establish if prolonged HGV viremia is more likely to lead to chronic hepatitis.

The mechanism by which HGV infection persists remains unknown. The frequency of development of chronic hepatitis and which form(s) of chronic hepatitis is likely to develop after acute infection also remain unclear. Despite a growing list of known viral hepatitis agents, the agent(s) of acute non-A–E hepatitis remains to be elucidated.

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References