Distribution of Infecting Hepatitis C Virus Genotypes in End-Stage Liver Disease Patients at a Large American Transplantation Center

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Patients and Methods

We studied 202 anti-HCV-positive patients who were transplant candidates at the University of Pittsburgh Transplantation Institute and the Oakland VA Medical Center between October 1990 and May 1995. The mean age at the time of transplantation was 49.2 ± 9.8 years. Inclusion criteria were the availability of properly stored serum, liver samples, or both, hepatitis B surface antigen negativity, and the absence of other likely causes of liver disease. All samples were stored at -80°C until analysis.

HCV RNA was extracted from sera or liver tissue using the modified guanidinium thiocyanate–phenol–chloroform technique using a commercially available kit (RNAzol B; Biotecx Laboratories, Houston). The HCV RNA was then reverse-transcribed into cDNA and amplified by polymerase chain reaction (PCR) with appropriate measures to prevent PCR product contamination [9]. Oligonucleotides specific for the nonstructural 5 (NS5) region of the HCV genome were used as primers: 5'-GGCGGAATTCCTGGTCATAGCCTCCGTGAAG-3' and 5'-TGGGGAATCCGTATGATACCCGCTGCTTTGA-3'.

One hundred randomly chosen PCR-positive samples were sequenced directly by the Sanger dideoxynucleotide termination method with a modified T7 DNA polymerase (Sequenase version 2.0 kit; United States Biochemicals, Cleveland) using internal primers described by Simmonds et al. [2]. For some reactions the external primers were used for sequencing. A 222-bp fragment of the NS5 region was read and used for comparison as described by Simmonds et al. [2]. The remaining samples were genotyped using type-specific primers for the C region [10]. Statistical analyses were done using SPSS for Windows (SPSS, Chicago).

Results

Of the 202 anti-HCV-positive patients, 185 (92%) were HCV-positive by PCR using primers specific for the NS5 or C region. HCV strains from 100 patients were determined by direct sequencing; in the remaining 85 patients, they were genotyped using type-specific primers. Thirty samples were genotyped by both methods, revealing concordance of ≥95%. Samples from 13 patients (7.0%) could not be genotyped by either technique. Figure 1 shows the genotype distribution: type 1a,
Figure 1. Distribution of HCV genotypes in 185 liver transplant candidates before transplantation at Transplantation Institute, University of Pittsburgh.

85 patients (46.0%); type 1b, 52 patients (28.1%); type 2b, 14 (7.6%); type 4, 13 (7.0%); type 3a, 5 (2.7%); type 2b, 2 (1.1%); and type 2c, 1 (0.5%).

There were 29 foreigners among our patients. When the foreign patients were excluded from the analysis, the distribution of genotypes was as follows: type 1a, 82 patients (52.6%); type 1b, 41 (26.3%); type 2b, 12 (7.7%); type 4, 4 (2.6%); type 3a, 4 (2.6%); type 2a, 1 (0.6%); and not determined, 12 (7.7%). All but 3 of the 13 patients who were classified as infected with the genotype 4 strain were of Middle Eastern origin. These infecting strains (GenBank accession numbers U50766 and U50851-58) had only 78%-80% homology with previously described type 4 strains.

The mean age (in years) for the patients by genotype was as follows: type 1a, 47.8 ± 9.9; type 1b, 52.2 ± 9.9; type 2a, 51.7 ± 28.1; type 2b, 51.8 ± 8.5; type 2c, 53.0 ± 0.0; type 3a, 43.2 ± 6.1; and type 4, 49.2 ± 7.7. Statistical analysis (Bonferroni test) did not reveal any significant difference between individual groups; however, patients infected with type 1b strains were slightly older than other patients (51.8 ± 10.3 vs. 48.3 ± 9.4 years, \( P = .045 \), Student’s t test).

Using the definition of Desmet et al. [11], we noted moderate to severe recurrent hepatitis in 12 (8.8%) of 136 patients for whom there was appropriate clinical and histologic follow-up 1 year after transplantation. In the first year after transplantation, moderate to severe hepatitis recurred in 2.2% of the type 1b-infected group and 4.1% of the non-1b group (nonsignificant).

Analysis of records of 202 anti-HCV liver transplant candidates showed that 1a was the most common infecting genotype (46%) and 1b was a distant second (28.1%). The prevalence of 1b was even lower than reported for chronic hepatitis patients in this country: Lau et al. [12] and Mehaney et al. [13] reported types 1a and 1b as equally prevalent infecting genotypes in American patients, each accounting for ~25%–30%. Our findings show that, at least in the United States, HCV-1b is not overrepresented in HCV-related end-stage liver disease compared with numbers of patients with HCV-related chronic hepatitis. This speaks against the purported more aggressive behavior of the type 1b strains, since it stands to reason that a larger proportion of type 1b should be present in a patient population in which HCV-related liver disease has progressed to end-stage cirrhosis.

An alternative explanation for the low prevalence of HCV-1b among our patients, and one that does not exclude the genotype’s greater aggressiveness, would be relatively recent introduction of this genotype into the American population. Indeed, there is some evidence from European studies that various genotypes could have been introduced at different times [4]. However, there is currently no evidence that this might be the case in the United States. In addition, although the time of infection could not be reliably identified for the majority of our patients, we did not find any age differences between patients infected with various genotypes; in particular, patients infected with HCV-1b were not younger than others studied.

Discussion

Recently, Feray et al. [6] and Gane et al. [7] reported the distribution of HCV genotypes in their respective French and English HCV-related end-stage liver disease populations. Both groups reported a very high percentage of patients with 1b as the infecting strain (68% and 43%, respectively); however, this genotype is common in Europe. Our study is the most extensive to date on the prevalence of HCV genotypes in American end-stage liver disease patients.

References

High Prevalence of GB Virus C/Hepatitis G Virus in Healthy Persons in Ho Chi Minh City, Vietnam

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GB virus C or hepatitis G virus (GBV-C/HGV), a novel Flavivirus, is detected in 1.5% of US blood donors. The prevalence is higher in multiply transfused patients and in persons with liver disease. Because of the increased incidence of hepatitis in Asia, sera from healthy Vietnamese were tested for the presence of GBV-C/HGV RNA by the reverse transcription polymerase chain reaction. Viral RNA was detected in 5.7% of those tested; 6 of 81 volunteer blood donors had positive samples as did 5 of 97 army recruits and 2 of 50 postpartum women. When the 188-bp product from 6 subjects was sequenced, there was 75%-85% homology at the nucleotide level compared with published sequences, indicating a high degree of genotypic variation, even within a putatively well-conserved region of the viral genome. Viremia with this non–cell-associated novel virus appears to be common among normal persons in Vietnam.

Despite the identification of 2 new hepatitis viruses within the last 10 years, the etiology of ~10%-20% of hepatitis cases remains unknown [1]. Among these are cases of non-A non-E community-acquired hepatitis, fulminant hepatitis, and complicated hepatitis, specifically the hepatitis–aplastic anemia syndrome. Recently, two independent laboratories in pursuit of novel hepatitis viruses described the identification of a new RNA virus, provisionally designated either GB virus C (GBV-C) [2] or hepatitis G virus (HGV) [3]. One group isolated GBV-C by reverse transcription polymerase chain reaction (RT-PCR) from West African serum that was antibody-positive for 2 newly described tamarin viruses, GBV-A and -B [4]. The second group identified part of the HGV genome by screening an expression library made from cDNA from plasma of a patient with chronic hepatitis. Both groups subsequently obtained the full viral sequence, which was positive-stranded RNA of ~10 kb, with similarities at the genic level to members of the Flaviviridae, especially hepatitis C virus (HCV), as well as the simian viruses GBV-A and GBV-B [5]. The virus can be transmitted by blood transfusion [3] and GBV/HGV RNA has been detected in plasma pools and immunoglobulin preparations [6]. Viremia was found in 1.5% of US volunteer blood donors [3].

Although GBV-C/HGV has not been definitively associated with any disease [7], an increased prevalence of viremia was reported for persons with a history of parenteral exposures and in patients with liver disease [3, 8, 9]. In addition, GBV-C/HGV was detected in 2 cases of hepatitis associated with aplastic anemia (hepatitis–aplastic anemia syndrome), suggesting a possible association with this syndrome [2, 10].

The prevalence of both hepatitis–aplastic anemia and aplastic anemia are higher in the Far East, including Vietnam [11]. As part of our studies to ascertain the role of GBV-C/HGV in hepatitis–aplastic anemia, we surveyed the seroprevalence of this new virus in healthy persons from Ho Chi Minh City.