

The New England Journal of Medicine

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Volume 334

JUNE 6, 1996

Number 23

INFECTION WITH HEPATITIS GB VIRUS C IN PATIENTS ON MAINTENANCE HEMODIALYSIS

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Abstract Background. A recently discovered non-A–E hepatitis virus has been designated hepatitis GB virus C (HGBV-C), but little is known about its mode of transmission and its clinical manifestations. We studied 519 patients on maintenance hemodialysis to determine whether they were infected with HGBV-C.

Methods. HGBV-C RNA was identified in serum by a reverse-transcription–polymerase-chain-reaction assay with nested primers deduced from a nonstructural region. A nucleotide sequence of 100 bp in the nonstructural region was determined on HGBV-C clones.

Results. HGBV-C RNA was detected in 3.1 percent of the patients on hemodialysis (16 of 519), as compared with 0.9 percent of healthy blood donors (4 of 448, $P < 0.03$). None of the 16 patients had evidence of active liver disease, although 7 were also infected with hepatitis C virus.

Eight patients with HGBV-C infection were followed for 7 to 16 years. In two patients the virus was present at the start of hemodialysis. One had a history of transfusion, and HGBV-C RNA persisted over a period of 16 years; the other became free of HGBV-C after 10 years. In five patients, HGBV-C RNA was first detected 3 to 20 weeks after blood transfusion and persisted for up to 13 years. One patient with no history of transfusion was infected with an HGBV-C variant with the same sequence as in two of the patients with post-transfusion HGBV-C infections.

Conclusions. Patients on maintenance hemodialysis are at increased risk for HGBV-C infection. This virus produces persistent infections, which may be transmitted by transfusions but may also be transmitted by other means. (N Engl J Med 1996;334:1485-90.)

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THE discovery of hepatitis C virus (HCV)¹ was a major step toward the control of non-A, non-B hepatitis.² HCV is responsible for the majority of acute and chronic cases of non-A, non-B hepatitis,³ and the screening of blood for antibodies against HCV (anti-HCV) prevents post-transfusion non-A, non-B hepatitis.⁴

However, it has become increasingly evident that there are patients with acute or chronic non-A, non-B hepatitis who are not infected with HCV and that there remains a residual risk of post-transfusion hepatitis in recipients of blood that tests negative for anti-HCV or hepatitis B surface antigen (HBsAg).⁵ These patients also have no evidence of infection with hepatitis D or E virus (hence the designation of non-A–E hepatitis). A GB hepatitis agent isolated in 1967 from a surgeon (whose initials were G.B.) with acute hepatitis has been propagated in tamarins.^{6,7} It has turned out to be two novel RNA viruses of the Flaviviridae family, which are known as hepatitis GB virus A (HGBV-A) and hep-

atitis GB virus B (HGBV-B).⁸ Another closely related virus, called hepatitis GB virus C (HGBV-C), has been recovered from patients with non-A–E hepatitis in Africa, Canada, and the United States.⁹ HGBV-C is a single-stranded RNA virus that can be categorized as belonging to the Flaviviridae family on the basis of its structure. HGBV-C resembles HCV, but its nucleotide sequence is too divergent to be classified as a genotype of HCV.¹⁰ Recently, yet another non-A–E hepatitis virus has been reported and designated hepatitis G virus.¹¹ It is similar in sequence to HGBV-C and may actually be the same virus with a different genotype.

We used a reverse-transcription–polymerase-chain-reaction (RT-PCR) assay with nested primers deduced from the nonstructural region of HGBV-C to determine whether 519 patients on maintenance hemodialysis had HGBV-C RNA.^{9,12} To shed light on the transmission, persistence, and evolution of this non-A–E hepatitis virus, we studied eight patients with HGBV-C infection for 7 to 16 years after the start of hemodialysis, and the nucleotide sequences of HGBV-C isolated from them were determined.

METHODS

Patients

We studied 519 patients with chronic renal failure who were undergoing maintenance hemodialysis at four dialysis centers (267 men and 252 women; mean [±SD] age of both groups, 56±12 years). The pa-

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Supported in part by the Ministry of Education, Science, and Culture of Japan and the Ministry of Health and Welfare of Japan.

tients had been on hemodialysis for a mean of 6.0 ± 4.7 years (range, 0 to 22). Serum from the patients was tested for markers of hepatitis virus infection, and serum levels of alanine aminotransferase were measured. The study was approved by the ethics committees of the institutions, and all patients gave informed consent.

Determination of HGBV-C RNA by RT-PCR Assay

Nucleic acids were extracted from 100 μ l of serum as described previously¹³ and were converted to complementary DNA (cDNA) with an antisense primer (G9, 5'TCYTTGATGATDGAAGTGC3', in which Y=T or C and D=A, G, or T).¹² Reverse-transcribed cDNA was subjected to the first round of PCR with primers G9 and G8 (5'TATGGGCATGGHATHCCYCT3', in which H=A, C, or T). PCR was performed with TaKaRa Ex Taq (TaKaRa Biochemicals, Kyoto, Japan) for 35 cycles (consisting of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 55°C, and extension for 60 seconds at 72°C) followed by an extension cycle at 72°C for 8 minutes. The second round of PCR was carried out for 30 cycles (consisting of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 55°C, and extension for 45 seconds at 72°C) with nested primers G10 (sense, 5'CATTCVAAGGCGGAGTGYGA3', in which V=A, C, or G) and G11 (antisense, 5'TCYTTACCCCTRTAATAGGC3', in which R=A or G). All primers were deduced from the nucleotide sequence of a nonstructural region of HGBV-C described by Simons et al.⁹ The expected sizes of the products of the first and second rounds of PCR were 158 and 83 bp, respectively; the finding of products of these sizes would confirm the presence of HGBV-C RNA in test serum. To avoid cross-contamination, PCR was performed under stringent conditions recommended by Kwok and Higuchi,¹⁴ with 1 positive control and 2 negative controls for every 20 samples.

For the semiquantitation of HGBV-C RNA, serial dilutions of extracted nucleic acids were tested by RT-PCR assay. The relative concentration of HGBV-C was expressed as the reciprocal of the highest dilutions of extracted nucleic acids in which HGBV-C RNA was detectable; values were expressed as the titer per milliliter of serum.

Nucleotide Sequences of HGBV-C Isolates

The products of PCR with primers G8 and G9 were amplified by a heminested PCR with primers G8 and G11 and cloned into M13 phage vectors. A sequence of 100 bp was determined on three HGBV-C clones from each serum sample as described elsewhere,¹² and the consensus sequence was adopted.

Markers of Hepatitis B and C Infections

Serum samples were analyzed for anti-HCV with a second-generation enzyme-linked immunosorbent assay (ELISA) (ELISAI, Ortho Diagnostic Systems, Tokyo, Japan). Serum samples were tested for HCV RNA with an RT-PCR assay,^{13,15} and the relative concentration of RNA was expressed as the reciprocal of the highest dilution of extracted nucleic acids in which HCV RNA was detectable; values were expressed as the titer per milliliter of serum. Serum samples were tested for HBsAg and its corresponding antibody (anti-HBs) by passive hemagglutination (MyCell, Institute of Immunology Co., Tokyo, Japan) and for antibody against hepatitis B virus (HBV) core antigen (anti-HBc) by inhibition of hemagglutination.¹⁶

Statistical Analysis

Differences in the frequency with which HGBV-C RNA was found in the study groups were analyzed with the chi-square test and Fisher's exact test. Group means were compared with Student's t-test.

RESULTS

Prevalence of HGBV-C, HCV, and HBV Infections in Patients on Hemodialysis

Among the 519 patients on maintenance hemodialysis, HGBV-C RNA was detected in 16 (3.1 percent), HCV RNA in 107 (20.6 percent), and HBsAg in 15 (2.9 percent). Of the 16 patients with HGBV-C RNA, 7 were also infected with HCV and 1 was infected with HBV. Thus, 50 percent of the patients with HGBV-C

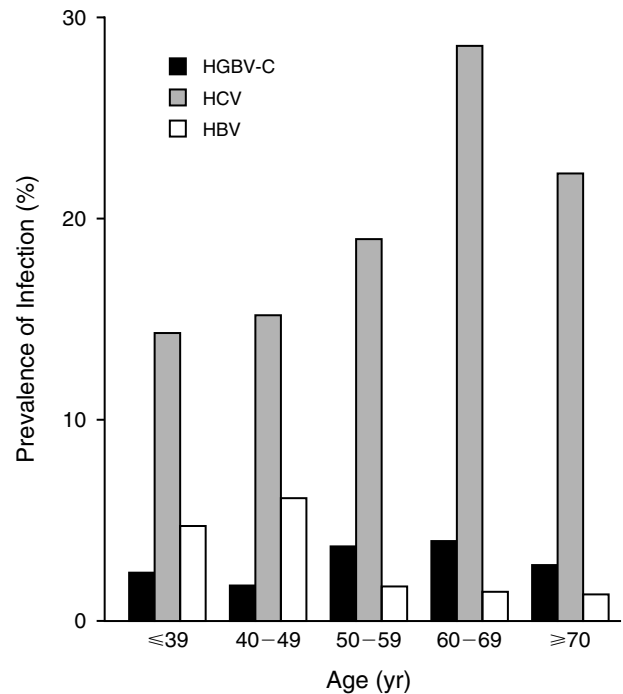


Figure 1. Age-Specific Prevalence of HGBV-C, HCV, and HBV among 519 Patients on Maintenance Hemodialysis.

The detection of HCV RNA on RT-PCR assay was considered to indicate infection with HCV, and a positive test for HBsAg was considered to indicate infection with HBV.

infection were also infected with other hepatitis viruses. Anti-HCV was detected in 127 (24.5 percent) patients, including 103 with HCV RNA in serum; 4 patients with HCV RNA were negative for anti-HCV. Anti-HBs, anti-HBc, or both were detected in the absence of HBsAg in 293 (56.5 percent) patients, representing a resolved HBV infection.

HGBV-C RNA was detected in 4 of 448 apparently healthy blood donors (0.9 percent) in Japan who were older than 30 years (303 men and 145 women; mean [\pm SD] age, 43 ± 9 years). The frequency of HGBV-C was significantly lower in control subjects than in the patients on maintenance hemodialysis (0.9 percent vs. 3.1 percent, $P < 0.03$).

Figure 1 shows the age-specific prevalence of the three hepatitis viruses among the patients. The pattern of infection with HGBV-C was similar to that of HCV infection: it tended to increase with age and peaked in patients in their 60s. It differed from HBV infection, which was most frequent in the 40s.

Characteristics of 16 Patients Infected with HGBV-C

Table 1 shows clinical and virologic features of the seven patients coinfecting with HGBV-C and HCV and the nine patients infected with HGBV-C alone. None of the patients had elevated levels of serum alanine aminotransferase, and there was no significant difference between the mean alanine aminotransferase levels in the two groups (18 ± 5 vs. 11 ± 5 U per liter). None of the patients had clinical evidence of liver disease.

The 519 patients were divided into four groups ac-

Table 1. Characteristics of 16 Patients on Maintenance Hemodialysis Who Were Infected with HGBV-C, According to HCV Status.

PATIENT NO. AND HCV STATUS	AGE (YR)/ SEX	DIALYSIS yr	TRANS- FUSION	ALT* U/liter	HGBV-C RNA† titer/ml	HCV RNA‡ titer/ml	ANTI- HCV§ U/liter	ANTI-HBs, ANTI-HBc, OR BOTH‡	
								HBsAg‡	ANTI-HBc‡
HCV-positive									
1	50/M	22	Yes	21	10 ³	10 ⁷	≥2.00	-	+
2	65/M	2	Yes	16	10 ³	10 ⁷	≥2.00	-	+
3	67/M	2	No	14	10 ³	10 ⁸	≥2.00	-	+
4	68/F	4	Yes	23	10 ²	10 ⁶	≥2.00	-	+
5	54/M	5	Yes	24	10 ²	10 ³	≥2.00	-	-
6	42/M	15	Yes	22	10 ³	10 ⁷	≥2.00	-	-
7	67/F	9	Yes	8	≥10 ⁴	10 ⁷	≥2.00	-	-
HCV-negative									
8	76/F	9	Yes	7	10 ³	-	0.06	+	+
9	41/F	8	No	17	10 ²	-	0.12	-	+
10	55/F	11	Yes	8	10 ¹	-	0.13	-	-
11	54/M	10	No	8	10 ³	-	0.20	-	+
12	63/M	11	Yes	7	10 ³	-	0.15	-	+
13	30/F	6	No	9	10 ⁴	-	0.07	-	+
14	51/F	6	Yes	8	≥10 ⁴	-	0.07	-	+
15	56/M	5	Yes	22	≥10 ⁴	-	0.06	-	-
16	73/F	1	Yes	16	≥10 ⁴	-	0.08	-	+

*ALT denotes alanine aminotransferase; normal values were ≤40 U per liter.

†The titer was determined by RT-PCR assay with nested primers deduced from the nonstructural region of HGBV-C (see the Methods section).

‡A plus sign denotes a positive test, and a minus sign a negative test.

§Values indicate absorbance at 492 nm on enzyme-linked immunosorbent assay.

according to the presence or absence of HGBV-C and HCV infection (Table 2). The 9 patients infected with HGBV-C alone had alanine aminotransferase levels (11±5 U per liter) that were similar to those of the 403 patients without HGBV-C or HCV infection (12±13 U per liter) and significantly lower than those of the 100 patients infected with HCV only (19±12 U per liter, P<0.01). Aside from this, there were no significant differences among the four groups of patients. Overall, a history of transfusion was reported by 75 percent of the patients with HGBV-C (12 of 16 patients), a frequency similar to that in the patients with HCV (81 percent; 87 of 107 patients). The combined frequency of transfusion in these two groups was significantly greater than that in the group without HGBV-C or HCV infection (62 percent; 251 of 403 patients; P<0.001).

Follow-up of the Eight Patients with HGBV-C Infection Monitored from the Start of Hemodialysis

The clinical courses of the eight patients who had been followed in the same dialysis unit since they began hemodialysis are shown in Figure 2. Six were negative for HGBV-C RNA at the induction of hemodialysis (Patients 4, 7, 8, 11, 14, and 16), of whom five received transfusions after they began hemodialysis. The interval between the last transfusion and the detection of HGBV-C RNA was four weeks for Patient 16 and seven weeks for Patient 4, who was

negative at three weeks, and three weeks for Patient 14. The interval between transfusion and the appearance of HGBV-C RNA in the circulation was much longer in the other two patients. Patient 8 was negative for HGBV-C RNA 13 weeks after transfusion and positive at 17 weeks. Patient 7 received only a single transfusion and was negative for HGBV-C RNA 17 weeks later but positive at 20 weeks. The titer of HGBV-C RNA was initially low in all these patients and increased gradually. In Patient 7, serum levels of HGBV-C RNA peaked and then decreased soon after they became detectable, but started to increase again eight years later.

Patient 4 was already HCV-positive when she began hemodialysis and became positive for HGBV-C RNA after multiple transfusions; her alanine aminotransferase level was elevated (83 U per liter) about a year after she became positive for serum HGBV-C RNA. Patient 14 had a single elevation of the alanine aminotransferase level four weeks after she became positive for serum HCV RNA. Patient 8 had an increased alanine aminotransferase level (125 U per liter) nine months after HGBV-C appeared in serum. She became positive for HBsAg concurrently, however, suggesting that HBV infection was responsible for the elevated alanine aminotransferase levels. In Patient 7, the serum alanine aminotransferase level rose four weeks after HCV RNA was first detected in serum, and the increase coincided with that of HGBV-C RNA. Thus, the HCV infection was responsible for the elevation. HBV persisted in Patient 8 throughout

Table 2. Characteristics of 519 Patients on Maintenance Hemodialysis, According to Their HGBV-C and HCV Status.*

CHARACTERISTIC	HGBV-C- POSITIVE, HCV-NEGATIVE (N = 9)	HGBV-C- POSITIVE, HCV-POSITIVE (N = 7)	HGBV-C- NEGATIVE, HCV-POSITIVE (N = 100)	HGBV-C- NEGATIVE, HCV-NEGATIVE (N = 403)
	Age — yr	55±14	59±10	57±12
Male sex — no. of patients (%)	3 (33)	5 (71)	52 (52)	207 (51)
Duration of dialysis — yr				
Mean	7.4±3.1	8.4±7.0	7.3±5.3	5.6±4.5
Range	1-11	2-22	0-20	0-19
History of transfusion — no. of patients (%)	6 (67)	6 (86)	81 (81)	251 (62)
Alanine aminotransferase				
Elevated levels — no. of patients (%)†	0	0	6 (6)	7 (2)
Mean level — U/liter	11±5	18±5	19±12	12±13
HCV RNA — titer/ml	—	10 ^{6.4} ±10 ^{1.5}	10 ^{4.6} ±10 ^{2.4}	—
Anti-HCV — no. of patients (%)	0	7 (100)	96 (96)	24 (6)
HBsAg — no. of patients (%)	1 (11)	0	1 (1)	13 (3)
Anti-HBs, anti-HBc, or both — no. of patients (%)	5 (56)	5 (71)	65 (65)	218 (54)

*Plus-minus values are means ±SD.

†Normal values were ≤40 U per liter.

the observation period, as HCV did in Patients 4, 7, and 14.

Patients 9 and 10 were already positive for HGBV-C RNA when they began hemodialysis. They had initial HGBV-C RNA titers of $\geq 10^4$ and 10^3 , respectively — titers that were higher than those in the early stages of HGBV-C infection in the other six patients. In Patient 9, serum levels of HGBV-C RNA became undetectable 10 years after the start of hemodialysis and was accompanied by an increase in serum alanine aminotransferase. She had had two previous episodes of elevated alanine aminotransferase levels. HGBV-C RNA remained detectable in Patient 10, but the titers fluctuated during the 16 years in which she was on hemodialysis. She had received transfusions five to six months before she was referred to the dialysis unit.

Nucleotide Sequences of HGBV-C from the Eight Patients with HGBV-C Infection Monitored from the Start of Hemodialysis

Figure 3 shows the nucleotide sequences of HGBV-C cDNA spanning 100 bp in a nonstructural region of the genome, cloned from serum from the eight patients at various times, as well as the sequences reported by Simons et al.⁹ In three patients (Patients 4, 11, and 14) the sequence remained the same over a period of two to nine years. Only 1 to 3 percent of the 100 bp changed in the other five patients over a period of 6 to 16 years. The sequences identified in the eight patients differed from one another by 0 to 21 percent and from those reported by Simons et al.⁹ by 11 to 23 percent.

The nucleotide sequences of the HGBV-C cDNA from Patients 7, 8, and 11 were very similar, with at least one clone showing the identical sequence, suggesting that these patients were infected with closely related strains of HGBV-C. Patient 11 had no history of transfusion, whereas Patients 7 and 8 had received transfusions.

DISCUSSION

Little is known about the transmission, epidemiology, and disease-inducing capacity of HGBV-C, except that it sometimes produces a fulminant non-A–E hepatitis.¹² Since HGBV-C may be transmitted parenterally,⁵ patients on maintenance hemodialysis should be at increased risk for such infection, as they are for HCV and HBV infections.^{17–20} In support of this view,

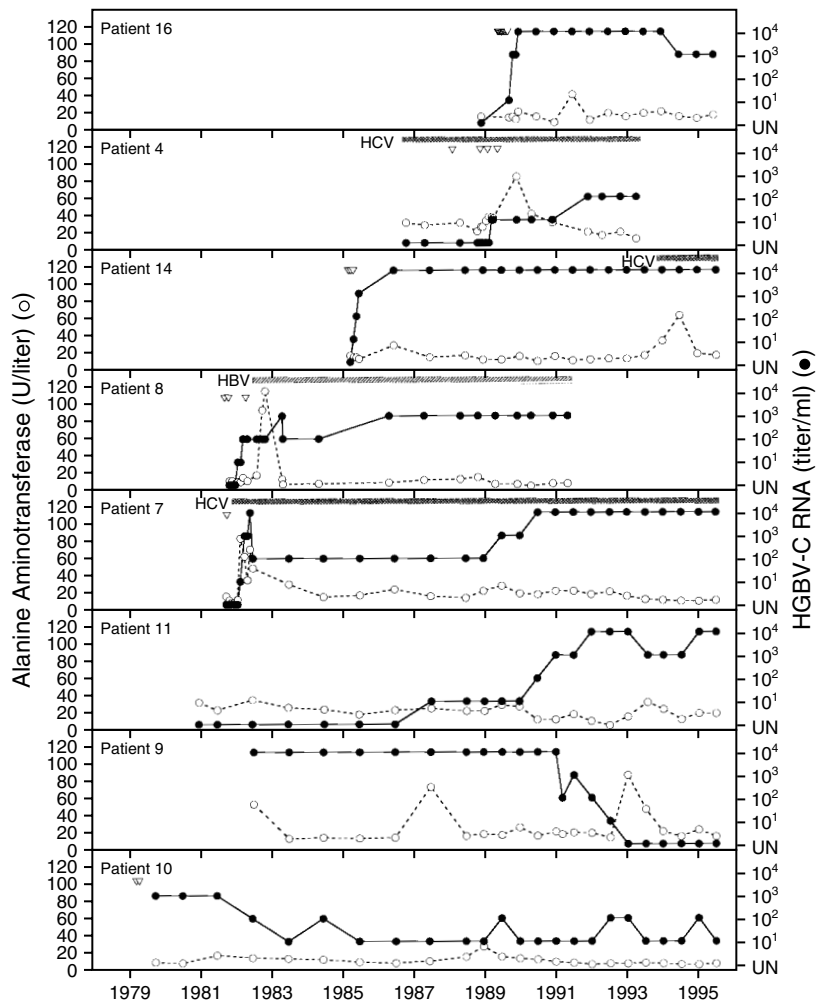


Figure 2. Serum Alanine Aminotransferase Levels and Titers of HGBV-C RNA in Eight Patients on Maintenance Hemodialysis.

The patients had been followed since the start of dialysis. Each inverted triangle represents a transfusion. Patients 4, 7, 8, and 14 were also infected with HCV or HBV. UN denotes undetectable.

we detected HGBV-C RNA in 3.1 percent of 519 patients on hemodialysis as compared with 0.9 percent of 448 apparently healthy blood donors ($P < 0.03$). Infection with HCV was more common and was detected in 107 patients (20.6 percent), including 7 who were also infected with HGBV-C. Thus, patients on maintenance hemodialysis are at increased risk for both HGBV-C and HCV, and there may be a common route of transmission of the two viruses in these patients.

In view of the wide divergence noted in a sequence of the nonstructural region serving as the target of the RT-PCR assay (Fig. 3), the primers we used to synthesize cDNA and for PCR might not be compatible with all HGBV-C isolates. Hence, we may have underestimated the rate of HGBV-C infection among patients on hemodialysis.

Some characteristics of HGBV-C infection have been identified by the present study. First, once acquired, infection with HGBV-C tends to persist. In only one of

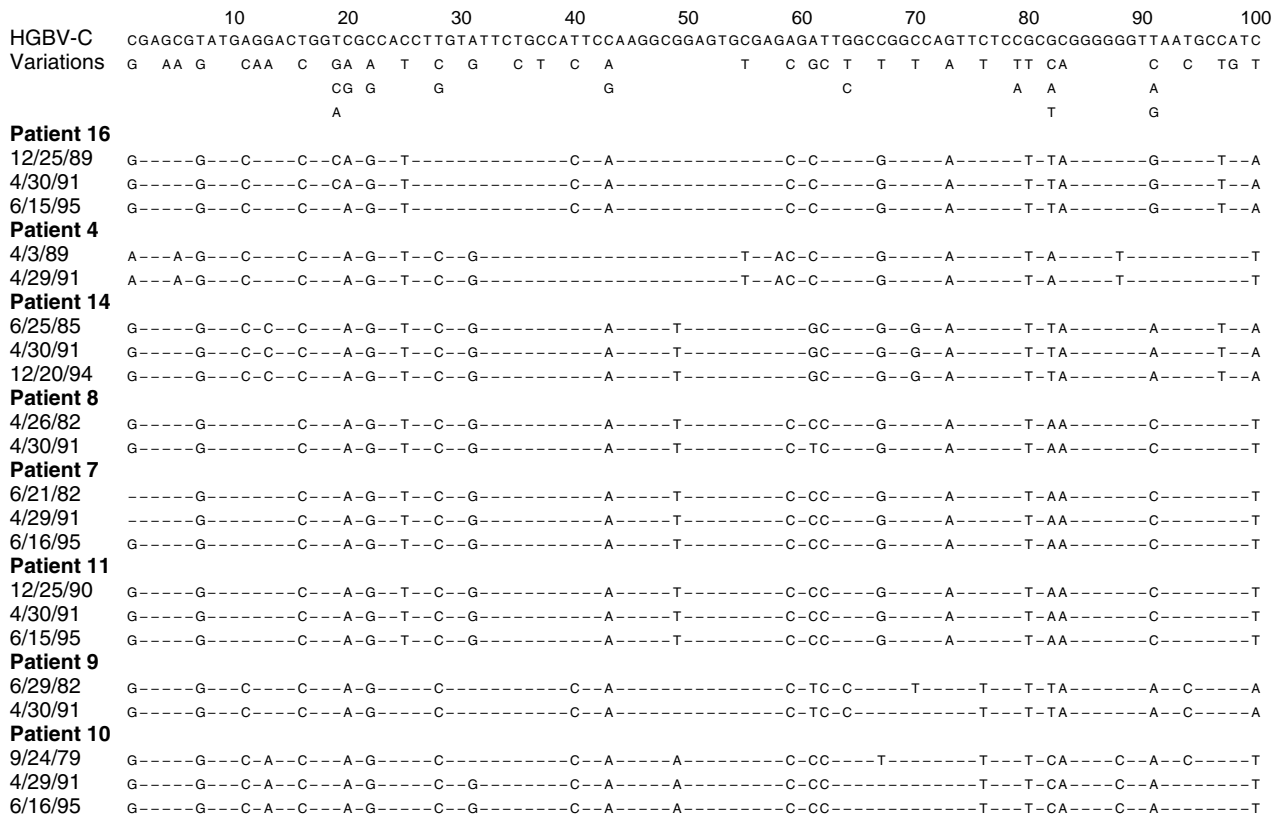


Figure 3. Nucleotide Sequence of a 100-bp Portion of HGBV-C Complementary DNA Obtained from Eight Patients on Maintenance Hemodialysis Who Were Followed for 2 to 16 Years.

The original sequence of HGBV-C and variations found in seven other isolates described by Simons et al.⁹ are indicated at the top (the numbers denote base pairs). The consensus sequence of three clones from a serum sample obtained on the indicated dates is shown for the eight patients. For the patients, only deviations from the sequence identified by Simons et al.⁹ are shown.

the eight patients followed for 7 to 16 years did HGBV-C RNA disappear from the circulation, and this patient had been positive for HGBV-C RNA for at least 10 years (Fig. 2). Some point mutations emerged in a 100-bp sequence in the nonstructural region of HGBV-C in five of the eight patients (Fig. 3). On the basis of these mutations, we estimated that the virus has a mutation rate of 0.8×10^{-3} to 1.9×10^{-3} nucleotide substitutions per site per year, which is close to the rate of 1.4×10^{-3} to 1.9×10^{-3} reported for HCV.^{21,22}

Second, HGBV-C is transmitted by transfusions. A history of transfusion was reported by 12 of the 16 patients with HGBV-C RNA (75 percent) — a rate similar to that for our patients with HCV RNA (81 percent) and higher than that for those with neither virus (62 percent). Five of the eight patients received transfusions while they were on hemodialysis, and they became positive for serum HGBV-C RNA 3 to 20 weeks after the last transfusion. Another patient had already received transfusions before she began hemodialysis.

The virus may also be transmitted by intravenous drug use, since it has been detected more frequently in patients with chronic HCV infection who were drug abusers than in those who were not (12 of 49, or 24 percent, vs. 9 of 128, or 7 percent; $P < 0.01$).²³

Transmission mechanisms other than transfusion

were suspected for some of the patients. One patient with no history of transfusion had HGBV-C RNA whose nucleotide sequence was nearly identical to that of two patients who had received transfusions, thereby indicating that the three were infected with closely related strains (Fig. 3). The interval between transfusion and the appearance of HGBV-C in serum was quite long in these two patients: 13 to 17 weeks in one case and 17 to 20 weeks in the other. In contrast, in the other three HGBV-C-positive patients who received transfusions HGBV-C RNA appeared in serum from three to seven weeks after the last transfusion. In addition, Linnen et al. described two patients in whom hepatitis G virus RNA became detectable a few weeks after transfusion.¹¹ Taken together, these results suggest patient-to-patient transmission, despite the fact that the patients also received transfusions.

Third, the infection with HGBV-C has not been found to cause inflammation of the liver, because the patients who were infected with HGBV-C did not have elevated serum alanine aminotransferase levels. Furthermore, virtually all elevations of alanine aminotransferase in these patients were ascribable to coinfection with HCV or HBV. Patients on maintenance hemodialysis are known to have compromised immune responses.²⁴ It is therefore not certain what ef-

fect persistent HGBV-C may have in persons with normal immune responses.

Infection with non-A–E hepatitis viruses has two distinct clinical profiles. In general, non-A–E hepatitis occurring after transfusion or community-acquired infection has a benign course.⁵ At the other end of the spectrum, fulminant hepatitis due to non-A–E hepatitis virus is so severe that it is hard to believe it is caused by the same virus. There might be a fulminant HGBV-C strain with some crucial mutations in a particular region of the genome. Such a possibility is supported by the finding of HBV variants with mutations in the pre-core region or core promoter, which interfere with the synthesis and secretion of hepatitis B e antigen and are found in the circulation of essentially all patients with fulminant hepatitis B.²⁵⁻²⁷

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To obtain information about continuing medical education courses in the New England area, call between 9 a.m. and 12 noon, Monday through Friday, (617) 893-4610, or in Massachusetts, 1-800-322-2303, ext. 1342.