Use of Phylogenetic Analysis of Hepatitis C Virus (HCV) Hypervariable Region 1 Sequences To Trace an Outbreak of HCV in an Autodialysis Unit

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Received 20 July 2001/Returned for modification 11 November 2001/Accepted 24 January 2002

Hemodialysis patients are at high risk of infection by hepatitis C virus (HCV). The aim of this study was to investigate an HCV outbreak that occurred in an autodialysis unit by using epidemiological and molecular methods. Seroconversion to HCV antibody (anti-HCV) was observed in two patients over an 18-month period; two other patients had previously been recorded as anti-HCV positive. All four patients involved in the outbreak were tested for HCV RNA, and hepatitis C genotype determination was accomplished by a reverse hybridization assay. Furthermore, part of hypervariable region 1 (HVR1) of the hepatitis C genome was amplified and sequenced in samples from all HCV RNA-positive patients. Phylogenetic analysis of the nucleotide sequences obtained was carried out in order to investigate any possible epidemiological linkages among patients. The nucleotide sequences of the HVR1 regions of both newly infected patients were found to be identical to sequences of samples from previously recorded anti-HCV-positive original patients, suggesting that they were infected by the same isolate. Molecular and epidemiological analysis suggested that nosocomial patient-to-patient transmission was the most likely explanation for the virus spread in the autodialysis unit under study.

Hemodialysis is known to be a risk factor for Hepatitis C virus (HCV) transmission despite screening of blood products for HCV antibodies (anti-HCV) and the use of erythropoietin (1). Nosocomial transmission of the HCV has become the principal cause of HCV infection in hemodialysis units (10–12, 14, 20, 21). Such transmission occurs through direct patient-to-patient contact more often than through HCV-contaminated dialysis monitors (13). There remains a relatively high incidence of new infections in hemodialysis units, mainly through cross-contamination from HCV hemodialysis patients (8, 17). The prevalence of infection increases with the duration of dialysis treatment, independent of transfusion events (5, 6). The exact mechanism of the route of this contamination is rarely known, and the causal events that lead to the outbreak cannot generally be traced (15). These cases of nosocomial transmission seem to have occurred despite strict general hygiene precautions. The prevalence of HCV infections is not declining, and transmission in dialysis units is continuing. Adequate screening of HCV infections and strict enforcement of universal infection control practices are required (9). Home dialysis and autodialysis were developed as alternatives to hemodialysis centers, and there have been fewer reported HCV infections in the former than in the latter (17).

HCV, like other RNA viruses, exhibits enormous genomic diversity. HCV isolates show four levels of genetic variability: types, subtypes, isolates, and quasispecies. This heterogeneity is a consequence of high error rates in RNA replication. HCV circulates as a heterogeneous population of genetically different but closely related genomes known as the quasispecies (2). Hypervariable region 1 (HVR1) of the genome is highly variable among and within patients and can be used to identify individual HCV isolates, which is of particular interest for epidemiological studies (3, 4). The simultaneous seroconversion of two patients in a dialysis unit in the same room at the same time strongly suggests nosocomial contamination. In this study, using HVR1 sequence analysis, we investigated two new cases of HCV infection that were contracted during maintenance autodialysis. In order to trace this contamination, we investigated possible epidemiological linkages among patients by using phylogenetic analysis of the nucleotide sequences. Patients and dialysis procedure. A total of 20 hemodialyzed patients with chronic renal failure were monitored in an autodialysis unit. Two of them (10%) were chronically HCV infected and genotyped 1a by InnoLipa assay (Innogenetics, Ghent, Belgium). They had been on hemodialysis for more than 20 years and were known to be HCV positive since the opening of the unit (putative source patients; patient 1 and patient 2). They had been infected with HCV via transfusion. All dialysis patients of the unit were tested for serum alanine aminotransferase (ALT) activities every month and for anti-HCV on admission and then every 6 months. If HCV infection was clinically suspected, additional samples for determination of anti-HCV and HCV RNA were drawn. According to general practice in autodialysis units, dialysis machines were assigned individually and dialyzers were not reused. Standard universal infection control practices were applied.
In June 1998 and May 2000, two new cases of HCV infection occurred in the dialysis center. The first infected patient (patient 3), a 54-year-old woman with polycystic kidney since age 24, was admitted with an ALT level of 100 IU/liter (normal, <31). In November 1998, a liver biopsy showed chronic active hepatitis and the patient’s ALT level was 130 IU/liter. During this period, a monthly fluctuation of ALT levels ranging from 50 to 150 UI was observed. She had started dialysis maintenance at the center (with no transfusion or other invasive procedure) 4 months prior to her illness. She was negative for HCV and HBV infection, and she had normal ALT values during this period.

FIG. 1. Phylogenetic analysis of HCV isolates from the pairs of hemodialysis patients, patients 1 and 3 (A) and patients 2 and 4 (B), based on the nucleotide sequence of part of the HVR-1 nucleotide sequence of each patient. The phylogenetic tree was constructed by the NJ method program in the PHYLIP package (version 3.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples.
The second new infected patient (patient 4), a 52-year-old man with nephroangiosclerosis, presented an increase in ALT level at 600 IU/liter (normal, \(<31\) which went up to 750 and 800 UI in June and July 2000, respectively. The patient had started the dialysis maintenance at the center with no transfusion or other invasive procedure 10 months before his illness. He was negative for HCV and HBV infection and he had normal ALT values during this period.

For each of these two new infected patients, an enzyme immunosorbent assay for HCV (Ortho Diagnostics, Raritan, N.J.) was positive, and viremia was detected by PCR (Amplicor HCV; Roche Molecular Systems, Neuilly, France) 1 month before or at the same time as the positive results for the anti-HCV test. The genotype of these patients was determined by the InnoLipa assay and indicated genotype 1a. Other causes of acute viral hepatitis were excluded, autoantibody screening was negative, and the patients were negative for HIV infection.

The second new infected patient (patient 4) began an interferon treatment regimen of 3 MU twice a week in July 2000. Results negative for HCV RNA were observed in the first month and during the 6 months after the start of therapy.

The temporal relationship between HCV positivity and the maintenance dialysis regimen led us to suspect a nosocomial infection.

**Sequencing and phylogenetic analysis.** In order to find out if the virus had been transmitted between the HCV genotype 1a patients, we performed sequence analysis of part of the HVR1 regions of the genomes of these four patients (the two previously infected and the two new cases). The HVR1 fragment (nucleotide positions 1156 to 1234) was chosen for sequence analysis because this domain exhibits a sufficiently high degree of variability to allow analyses to distinguish between HCV isolates of the same subtype. The HVR1 fragments isolated from the source patients (patients 1 and 2) and new infected
patients (patients 3 and 4) were aligned by using the SeaView program (20). Representative HVR1 sequences of each subtype of genotype 1a were used as control sequences for the HVR1 fragments. A set of control sequences was built with a BLAST (blastn) search of the nonredundant databases of the National Center for Biotechnology Information, with the consensus sequence of the family as a control.

The trees were inferred through the neighbor-joining (NJ) method by using Kimura’s two-parameter distance (7). The NJ tree was generated from the distance matrix on the basis of all pairwise comparisons of sequences. The trees obtained were unrooted. In order to assess the confidence placed in the tree topology, we used the bootstrap method.

The nucleotide sequences of the HVR1 regions of both newly infected patients were found to be identical to sequences of previously recorded anti-HCV-positive index patients, suggesting that they had been infected by the same isolate. The trees obtained with the NJ method are shown in Fig. 1A (patient 1 and 3) and Fig. 1B (patient 2 and 4). In these trees, the representative 33 and 52 nonredundant sequences of genotype 1a and the HVR1 fragments of the newly infected patients and the source patients are respectively compared. Those of the two infected patients are in the same branch and distinct from those of the other branches of the tree. The HVR1 patient subtree was supported by bootstrap values of 95% (NJ).

Phylogenetic analysis of the HVR1 sequence of HCV from the serum of one of the two source patients found it to be clustered with one of the sequences of the newly infected patients (patients 1 and 3, respectively). The same was true for those of the other two patients (patients 2 and 4). The sequences of the two pairs of patients were therefore closely related. The results of epidemiological analyses were also consistent with patient-to-patient transmission; those of the source and new infected patients were dialedy either in the same shift (Fig. 1A) or in the next closely related shift (Fig. 1B).

The timing of the events and the molecular characterization of the two HCV isolates provide strong evidence that HCV was transmitted during hemodialysis maintenance. The similarity in the sequences in the HVR1 regions from the isolates demonstrates that a cluster of strains exists in both pairs of patients. It should be noted that this region showed sufficient variability to discriminate isolates and is therefore the most appropriate for the documentation of person-to-person transmission from samples closely related in time. The results from molecular biology investigations and epidemiological evaluation are complementary pieces of evidence for possible nosocomial transmission of HCV (16, 18, 19). This case emphasizes the risk of transmission and the importance of infection control procedures in hemodialysis units (22). It also highlights the usefulness of molecular epidemiological techniques for the investigation of outbreaks of HCV infection. In this setting, molecular analysis of viral isolates indicates that the patient-to-patient mode is the most frequent mode of HCV transmission (23).

Dialysis machine-related transmission could be excluded, as machines are assigned individually and are not shared by patients. The spread of HCV is mainly related to a lack of strict observance of appropriate precautionary measures, which are an efficient and possibly sufficient means of prevention (24). The exact reasons for contamination in this unit could not be documented, but lack of strict observance of precautionary measures would appear to be likely. Epidemics or single instances of patient-to-patient transmission have only occasionally been reported in hospital settings other than that of hemodialysis units, and once again, nonobservance of precautionary measures (i.e., inadequate cleaning or disinfecting of medical instruments) was involved.

REFERENCES