

Brief Report

TRANSMISSION OF HEPATITIS C VIRUS FROM A PATIENT TO AN ANESTHESIOLOGY ASSISTANT TO FIVE PATIENTS

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PREVENTION and treatment of infections with hepatitis C virus (HCV) remain a major challenge.¹ The main source of HCV infection in developed countries was formerly transfusion of contaminated blood and blood products but is now injection-drug use.²⁻⁴ In general, a potential risk factor can be established for about 90 percent of all cases of HCV infection.³ One way of contracting HCV may be transmission from infected medical personnel to susceptible patients during medical care. Provider-to-patient transmission of HCV is rare, and in most cases HCV-positive surgeons are the probable source.⁵⁻⁷ We studied an outbreak of HCV in a municipal hospital. Our findings suggest that anesthesiology assistant contracted HCV from a chronically infected patient and subsequently transmitted the virus to five other patients.

METHODS

Patients

The municipal hospital in which the HCV outbreak occurred provides general as well as specialty medical and surgical services. Between July 1 and October 13, 1998, HCV infection was diagnosed in four patients (Patients 2, 3, 4, and 6 in this report) on the basis of clinical symptoms, a rise in the serum alanine aminotransferase concentration, or detection of serum HCV antibodies and HCV RNA. All of these patients had undergone orthopedic or general surgery in the same hospital 6 to 18 weeks earlier. A comprehensive investigation was initiated by the public health authorities, and we were asked to determine the circumstances of the suspected nosocomial HCV infections. An institutional review board of Essen University Hospital approved the study protocol, and the patients provided written informed consent.

Epidemiologic Studies

The charts of all patients with HCV infection were reviewed in detail. Interviews were conducted to obtain further information about prior medical interventions, prior hepatitis infections, and

risk factors for the acquisition of HCV. To search for other potential cases of HCV transmission, we performed a retrospective seroepidemiologic study of all patients who had undergone surgery in the hospital between January and July 1998. Fifty-eight of these patients had died, and 904 were still alive; serum was obtained for antibody testing from 833 of these 904 patients. Hospital personnel were interviewed with special attention to compliance with infection-control practices and were tested for HCV antibodies. The hospital — in particular, the surgical facilities — was inspected by experts in hygiene and occupational health.

Virologic and Molecular Studies

The presence of HCV antibodies was determined by enzyme-linked immunosorbent assay (ELISA; Sanofi Diagnostics Pasteur, Freiburg, Germany). Reactivity was confirmed by immunoblot assay (Mikrogen, Munich, Germany). HCV RNA was detected qualitatively and was also quantified with polymerase-chain-reaction (PCR) kits (Roche Diagnostics, Mannheim, Germany). HCV isolates were identified by genotyping, and HCV hypervariable region 1 (nucleotides 1491 to 1572, numbered as reported by Choo et al.⁸) was amplified as described elsewhere.^{9,10} Products of the second PCR were purified from the agarose gel (QIAquick, Hilden, Germany) and cloned into a plasmid vector (TOPO TA cloning kit, Invitrogen, Groningen, the Netherlands). Four to six clones from each subject were sequenced in both directions (with the Dye Terminator DNA sequencing kit, Perkin-Elmer, Norwalk, Conn.).

As area controls, the PCR products of HCV isolates that were obtained from chronically infected patients (located within a radius of approximately 200 km from the hospital) were subjected to direct sequencing.¹¹ To prevent possible cross-contamination of the samples, stringent procedures were used for nucleic acid extraction and amplification,¹² and the analyses were performed several weeks apart. Sequences of HCV hypervariable region 1 that were obtained from the samples have been submitted to GenBank (accession numbers AF227763 through AF227786).

Statistical Analysis

A matrix of nucleotide distances was calculated by Kimura's two-parameter method.¹³ The statistical significance of the differences was assessed by a two-sided Wilcoxon rank-sum test with the use of SPSS statistical software (SPSS, Chicago). Phylogenetic trees were constructed with use of the neighbor-joining algorithm on the previous sets of pairwise distances (PHYLIP, version 3.5¹⁴). The significance of the grouping was evaluated by the bootstrap method (1000 replicates).

RESULTS

Epidemiologic Findings

Six patients were found to have hepatitis C viremia. They are listed according to the dates of surgery in Table 1. The HCV infection of Patient 1 was first diagnosed in 1996; she probably contracted the virus through the transfusion of contaminated blood or clotting-factor concentrates at the time of surgery for heart-valve replacement in 1980. Patients 2, 3, and 6 had acute icteric hepatitis C 6 to 18 weeks after orthopedic or general surgery, whereas Patients 4 and 5 were asymptomatic after surgery. Only Patient 1 had ever received blood or blood products. None of the other patients had a history of hepatitis, nor were they aware of any history of hepatitis in their families. All reported no other risk factors for HCV infection, including tattooing or body piercing, intravenous drug use, or high-risk sexual behavior. The operations were performed in two rooms. There was no known

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TABLE 1. CHARACTERISTICS OF THE PATIENTS AND THE ANESTHESIOLOGY ASSISTANT WHO WERE INFECTED DURING THE OUTBREAK OF NOSOCOMIAL HEPATITIS C.

SUBJECT	AGE (YR)/ SEX	DATE OF SURGERY	TYPE OF SURGERY	INCUBATION PERIOD (WK)	DATE OF BLOOD SAMPLING*
Patient 1	75/F	4/28/98	Freeing of adhesions	—†	6/3/99
Assistant	29/M	—	—	6	8/11/98
Patient 2	67/M	5/19/98	Osteotomy of tibia	9	7/13/98
Patient 3	72/F	5/20/98	Osteotomy of tibia	6	7/2/98
Patient 4	65/F	5/20/98	Insertion of hip prosthesis	16‡	2/3/99
Patient 5	39/F	6/2/98	Vaginal hysterectomy	—§	4/21/99
Patient 6	45/M	6/9/98	Herniorrhaphy	18	10/14/98

*The samples were used for determining the HCV RNA level in Patient 1 and the assistant and for sequencing HCV hypervariable region 1 of all isolates.

†Patient 1 had had a known chronic infection with HCV since 1996.

‡Patient 4 had a clinically inapparent course of HCV infection. The estimation of the incubation period was based on a rise in serum alanine aminotransferase activity.

§Patient 5 had a clinically inapparent course of HCV infection that was first detected during the retrospective investigation.

contact between the patients either before or during their hospitalizations, which were in different parts of the hospital.

Testing of staff members revealed the presence of HCV antibodies in one anesthesiology assistant. He had taken part in all six operations. At the end of February 1998, the assistant was HCV-negative, but he had acute icteric hepatitis C in June 1998. Besides occupational exposure, the anesthesiology assistant had no known risk factors for HCV infection. Intravenous drug abuse was ruled out by extensive drug screening and numerous interviews. The assistant was almost entirely responsible for the administration of general anesthesia, including the preparation of narcotic drugs, the placement of venous and arterial catheters, the intubation of the patients, and the subsequent artificial respiration. He usually did not wear gloves, because he claimed that they diminished his sense of touch and therefore impaired his work.

On questioning, he reported that during the time under investigation he had a wound on the medial side of the third finger of his right hand, sustained in the middle of April 1998 when he opened a box containing infusion solutions. The wound was initially the size of a thumbnail and bled repeatedly. He used a bandage for three or four days but not thereafter, although the wound was still weeping. The assistant admitted that this was negligent behavior, but at the time he already considered the open wound to be an old injury and was not aware that such an attitude might be risky for him as well as for his patients. Between April 28, 1998, the day of surgery in Patient 1, and June 9, 1998, the day of surgery in Patient 6, the assistant participated in 39 operations. Between

the time he went on sick leave because of acute hepatitis C and July 1998, another 118 operations were performed in the hospital, and no further HCV infections occurred.

Hygiene and occupational health inspections as well as interviews with staff members indicated that numerous breaches of general infection-control practices had taken place. For instance, needles were frequently recapped after use, and gloves were not always worn in settings in which exposure was likely. Multidose vials for flushing solutions, saline, local anesthetic drugs, and heparin were often used in the operating rooms, although the solutions were changed every second day. As a disinfectant for surfaces, the hospital used a product based on a peroxide compound (Dismozon pur, Bode Chemie, Hamburg, Germany) that is not recommended for areas grossly contaminated with blood. The central sterilization facility worked properly, as indicated by the relevant technical protocols.

Virologic and Molecular Findings

All six patients and the anesthesiology assistant were positive for serum HCV antibodies and HCV RNA. At the time of the investigation, Patient 1 and the assistant had high plasma levels of HCV RNA (2.6×10^7 copies of HCV RNA per milliliter and at least 1×10^6 copies of HCV RNA per milliliter, respectively). Genotyping revealed HCV type 1a infection in all cases. The alignment of sequences of HCV hypervariable region I demonstrated a very high degree of homology between the isolates obtained from the patients and those obtained from the anesthesiology assistant (Fig. 1). Among the subjects, nucleotide dis-

Patient 1a	CATACCCACACCACCGGGGGGACTGCCGCTCAGACCACGTACGGAATCGC	(50)
Patient 1b	(50)
Assistant a	(50)
Assistant b	(50)
Patient 2a	(50)
Patient 2b T	(50)
Patient 3a	(50)
Patient 3b	(50)
Patient 4a	(50)
Patient 4b	(50)
Patient 5a	(50)
Patient 5b	(50)
Patient 6a	(50)
Patient 6b	(50)
C1	ACA GT A GC . C . AT . . . A . TA . TT . T . T	(50)
D10749	G . A . . . AT . GT . T ACAA C . GCG . . . T . . CT . . . C . T . T	(50)
Patient 1a	CAGTCTTTTTACTCCGGGTGCCAAGCAGAAT	
Patient 1b	
Assistant a	
Assistant b	. G	
Patient 2a	
Patient 2b	
Patient 3a	
Patient 3b T A	
Patient 4a	. . . CT A	
Patient 4b A A	
Patient 5a	
Patient 5b T	
Patient 6a	
Patient 6b	
C1	T . . G . . C . . A . . G . TC . . C . T . . C C	
D10749	T C . . C . . A . . A . . C . . T C	

Figure 1. Alignment of the Sequences of HCV Hypervariable Region 1 from the Patients, the Anesthesiology Assistant, and Selected Controls.

The two most divergent clonal sequences from each person (designated a and b) have been included. C1 and D10749 denote the selected controls.

tances varied only from 0 to 0.05, whereas a comparison of the subjects' sequences with those of the 10 area controls with type 1a HCV and the 13 genotype-1a sequences drawn from GenBank showed nucleotide distances that ranged from 0.25 to 0.59 ($P < 0.001$) (data not shown).

The relatedness of the sequences of hypervariable region 1 from all six patients and the anesthesiology assistant was further demonstrated by phylogenetic analysis. All sequences of HCV hypervariable region 1 from the patients and the anesthesiology assistant segregated into a cluster, which was clearly separated from all other sequences of HCV genotype 1a (data not shown). These results indicate that all six patients and the assistant were infected with the same HCV isolate.

The available epidemiologic and molecular biologic evidence suggested that Patient 1, who had chronic HCV infection, was the index patient in the chain of transmission. The anesthesiology assistant contracted HCV from Patient 1 and subsequently transmitted the virus, during the incubation stage of his disease, to at least five patients.

DISCUSSION

Our findings provide evidence that a nonsurgical staff member infected with HCV transmitted the virus to at least five patients. To our knowledge, such events have been occasionally recorded only for non-surgical personnel infected with hepatitis B virus (HBV).¹⁵⁻¹⁸ Our conclusion is supported by both epidemiologic and molecular evidence. The five patients had no known risk factors for HCV infection, and there were no evident contacts among them — a fact that excludes the possibility of patient-to-patient transmission. On the other hand, the anesthesiology assistant was the only staff member infected with HCV and could be identified as the sole common denominator in all six cases. He tested negative for serum HCV antibodies approximately eight weeks before Patient 1 underwent surgery on April 28, 1998, and had symptoms of acute hepatitis C six weeks after this operation. Since viral RNA usually appears in the blood within the first week after the transfusion of contaminated blood,¹⁹ HCV was probably present in the blood of the anesthesiology assistant and transmissible starting in the first week of May 1998. Fur-

thermore, the incubation periods of HCV recorded in the patients, which ranged from 6 to 18 weeks after surgery, are in agreement with the incubation times reported for HCV after post-transfusional infection (2 to 26 weeks²⁰).

Our molecular investigations were based on sequence analyses of HCV hypervariable region 1, which is commonly used to distinguish between related and unrelated isolates of the same subtype.²¹ The evolutionary distances between the sequences of hypervariable region 1 obtained from our subjects were similar to those reported previously for epidemiologically linked strains of HCV in infections caused by needlestick injuries²² or transmitted from mothers to their babies.^{23,24} Phylogenetic analysis confirmed that the assistant and all six patients were infected with the same HCV isolate.

We could identify an index case as well as the direction of the spread of HCV. However, we were not able to pinpoint the precise mechanisms leading to the infections. The only identifiable condition that might have caused the spread of the virus was the wound on the assistant's right hand. Given the high plasma levels of HCV RNA in both Patient 1 and the assistant, and given that the assistant usually did not wear gloves in the operating room, it is possible that a fraction of a microliter of blood or wound secretions might have transmitted HCV from Patient 1 to the assistant and subsequently from him to the five other patients. Wound secretions due to exudative dermatitis have previously been implicated in provider-to-patient transmission of HBV,¹⁵ and the high prevalence of HCV in patients with chronic skin diseases like psoriasis is most likely attributable to infection through minute skin abrasions.²⁵

In our study, patients could have been exposed to minimal and invisible amounts of the assistant's blood or wound secretions directly through mucosal lesions caused by intubation or through indwelling venous and arterial cannulas. Other possible but less likely routes of transmission include inadvertent contamination of instruments or multidose vials with blood or wound secretions from the assistant. Multidose vials have been implicated previously in nosocomial HCV,²⁶ HBV,^{27,28} and human immunodeficiency virus²⁹ infections.

Whatever the precise mechanisms of HCV transmission in this outbreak, the spread of the virus could probably have been prevented if so-called universal precautions for infection control had been taken.³⁰ Breaches of infection control are therefore associated with a high risk of the transmission of blood-borne pathogens.

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