Transmission of Hepatitis C Virus in a Gynecological Surgery Setting

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A cluster of hepatitis C virus (HCV) infections among gynecological patients who underwent surgical intervention in the same setting is described. An epidemiological investigation was conducted to identify the cases, the likely source of infection, and the route of transmission. Four recent HCV infections were identified. Based on molecular fingerprinting analysis and epidemiological investigation, transmission between the putative source patient (an HCV-positive woman who was the first patient of the surgical session) and outbreak patients was highly suggestive. All patients, including the source patient, were infected with HCV type 1b. Molecular characterization of HCV clones by sequence analysis of both structural envelope regions (20 clones from the source patient and 58 from the outbreak patients) and the nonstructural NS5 region of the viral genome (12 clones from the source patient and 32 from the outbreak patients) showed close homology between the viral isolates from the source and those from the outbreak patients that was higher than that observed between the viral isolates from the source and those from four unrelated, HCV type 1b-infected patients from the same geographical area (in the latter case, 33 clones were sequenced for the envelope regions and 30 were sequenced for the NS5 region). The mean percent divergence between clones was 4.69 for the envelope and 3.71 for the NS5 region in the source patient and the outbreak patients compared with 6.76 (P = 0.001) and 5.22 (P = 0.01) in the source patient and control patients, respectively. Among the risk factors investigated, only that of having undergone surgery in the morning session of the same day reached statistical significance (P = 0.003). The investigation showed that the source patient and outbreak patients shared only the administration of propofol in multidose vials. The study documents the risk of nosocomial transmission of HCV and the importance of infection control procedures in the operating room and highlights the crucial role of molecular strategies, especially sequence-based phylogenetic analysis of cloned viral isolates, in the investigation of HCV outbreaks.

Hepatitis C virus (HCV) infection is a major health problem worldwide. Approximately 80% of the individuals infected with HCV progress to chronic infection (4), and 0.4 to 2.5% of these develop hepatocellular carcinoma (11). In the past, blood transfusion and administration of blood products were important sources of HCV transmission, but currently, high-risk drug and sexual exposures account for most cases of HCV transmission. However, for approximately 10% of patients the source of transmission is unknown (2).

Nosocomial HCV infection, which is mostly due to patient-to-patient transmission, can be identified by genotyping of HCV strains and through sequence-based molecular fingerprinting (1, 2, 4). In some hospital settings commonly using intravenous lines (i.e., dialysis and hematology wards), blood-borne pathogens are more easily transmitted. However, owing to the peculiar characteristics of HCV (high proportion of asymptomatic cases, long incubation period, and the fact that patients may never return to the same care provider), the actual risk of nosocomial infection with HCV has rarely been measured.

Risk factors for nosocomial HCV infection include transmission through blood components (3) (currently very rare), organ transplantation (12), patient-to-patient transmission through shared dialysis equipment (23) or devices such as colonoscopes and breathing circuits (8, 9), and multidose vials (24). Unfortunately, however, in many cases it is nearly impossible to establish or even surmise the source of infection. Moreover, since most cases of HCV infection are asymptomatic, the spread of HCV among hospitalized patients may often go unnoticed.

In March 1998, two women with recent HCV infection who had both undergone gynecological surgery on 9 January 1998 in the same operating room were admitted to the Infectious Diseases Unit of Reggio Emilia Hospital. An investigation was conducted to identify further cases, the likely source of infection, and the route of transmission. Molecular characterization of HCV genomes conducted through genotype analysis and sequencing of the structural envelope regions 1 and 2 (E1 and E2), including the hypervariable region 1 (HVR-1) and the nonstructural NS5 of the viral genome, revealed close homology between the HCV genome of an HCV-positive woman, who was the first patient of the day’s session, and those of four outbreak patients, who underwent surgery later in the same morning.

MATERIALS AND METHODS

Epidemiological investigation. At the end of March 1998, the medical records of the 16 patients who had undergone gynecological surgery on 8 January (8 patients), 9 January (6 in the morning and 1 in the afternoon), and 10 January (1
patient) were reviewed. The patients were traced to obtain information on demographic characteristics, HCV serological status, hair removal before the operation, preanesthesia medications, antibiotic prophylaxis, anesthetics administered, whole blood or other blood component transfusions, length of hospital stay, and clinical condition.

To determine the risk factors for infection, a retrospective cohort study was conducted. All patients were asked for a blood sample for HCV serology testing. HCV RNA detection, and genotyping and for determination of serum aminotransferase concentrations. Antibodies to HCV and HCV RNA were offered to the sexual partners of the anti-HCV-positive patients. The operating room staff members were also asked to provide blood samples for HCV serology testing.

Finally, the regional records of acute HCV infections reported in 1997 and up to March 1998 were reviewed for similar cases among inpatients of the gynecological surgery unit.

The gynecological operating room was fitted with one operating bed. Infection control practices were assessed by reviewing daily operation schedules, charts, and sterilization records; by examining equipment; and by interviewing and observing personnel.

**Virological study.** Anti-HCV antibodies were detected by a third-generation enzyme-linked immunosorbent assay (Abbott Laboratories, Chicago, Ill.); serological confirmation of antibody reactivity was performed with immunoblot assays (Matrix 2.0; Abbott).

Detection of HCV RNA was performed for all patients and for the sexual partners of patients with a positive anti-HCV test (reverse transcriptase PCR [RT-PCR] Amplicor assay; Roche, Basel, Switzerland).

HCV genotyping was performed by nested PCR of the HCV core region according to the method of Okamoto et al. (19), with minor modifications (22). For HCV RNA quantitation, RNA was extracted from 100 μl of plasma using the guanidinium thiocyanate method (10); 10 μl of RNA dissolved in diethyl pyrocarbonate-treated water was quantified using competitive RT-PCR (16). For sequence analysis, a total of 435 nucleotides encompassing partial fragments of the E1 and E2 regions, the entire HVR-1, and the nonstructural NS5 region of the HCV genome were amplified by RT-PCR from plasma samples of all patients and from four control patients infected with HCV type 1b, unrelated to this cluster (admitted to the Hospital of Ancona, Ancona, Italy, for primary HCV infection); cloned into a plasmid vector; and transformed to competent cells. The plasmid DNA extracted from transformant colonies was sequenced in both forward and reverse directions by fluorescence-labeled dideoxynucleotides with an automated sequencer (model 373A; Perkin-Elmer, Norwalk, Conn.), according to methods described previously (15).

Nucleotide sequences were edited and assembled using the Sequence Navigator program included in the ABI373A software package. Multiple nucleotide and amino acid sequences were aligned using the Megalign (DNASTAR Inc., Madison, Wis.) and the CLUSTAL W 1.7 programs. A pairwise matrix of evolutionary distances of nucleotide sequences was generated using DNADIST (Kimura’s two-parameter method) included in version 3.572 of the PHYLIP package (J. Felsenstein, Phylogeny Inference package, version 3.5; Department of Genetics, University of Washington, Seattle). Phylogenetic trees were constructed from the above distance matrices with the NEIGHBOR program (neighbor-joining algorithm). Bootstrap values were calculated using SEQBOOT (100 resamplings), followed by DNADIST, NEIGHBOR, and CONSENSE in order to generate the majority rule consensus tree.

**Statistical methods.** Attack rates were calculated for patients exposed and not exposed to potential risk factors and compared using, when possible, relative risk. Ninety-five-percent confidence intervals of relative risk were computed by the exact method. Fisher’s exact test was used to compare groups mean of nucleotide sequence divergence. Two-tailed P values of <0.05 were considered statistically significant. Calculations were performed using STATA (STATA statistical software, release 5.0; Stata Corporation, College Station, Tex.).

**Nucleotide sequence accession numbers.** The sequences described in this study have been submitted to the EMBL data bank and assigned accession no. AJ310571 to AJ310608 and AJ409828 to AJ409866.

**RESULTS**

**Cases of HCV infection.** Of the 16 patients who underwent surgery on 8, 9, or 10 January 1998, 5 tested anti-HCV and HCV RNA positive in March 1998. The other 11 patients tested anti-HCV and HCV RNA negative then and 6 months later.

All anti-HCV-positive women underwent surgery in the morning of 9 January 1998. One of them, the first patient of the day, was a 51-year-old woman known to have chronic hepatitis C at the time of the intervention. The four cases with acute, type C hepatitis were identified following admission to the hospital in March 1998 (two patients aged 67 and 33 years) or following epidemiological investigation (two patients aged 67 and 47 years).

All surgical personnel tested anti-HCV negative. The sexual partners of the anti-HCV-positive women tested anti-HCV and HCV RNA negative. No other risk factor for a blood-borne infection was identified by interviewing the four outbreak patients.

The review of infection control procedures adopted in the gynecological surgery unit did not show failures in the infection control routine. Decontamination, disinfection, and sterilization procedures were appropriate. Fresh sucker heads and/or suction catheters were reportedly used on each patient. Breathing circuits were replaced after the operation on the patient with chronic HCV infection. Finally, new high-efficiency filters (Hygroboy; DAR, Mirandola, Italy) were fitted on the patient circuits of anesthesia equipment at each operation.

In the operating room, a two-way peripheral venous catheter was inserted before surgery following skin cleaning and preparation. One channel was used for administering fluids, and the other was used for intravenous anesthetics; an antireflux valve in the latter prevents blood reflux during infusion. Occasionally, anesthetics were administered through the other channel (which does not have an antireflux valve). Intraoperative blood pressure was always monitored with a cuff sphygmomanometer placed on the arm where the peripheral venous catheter was inserted. Finally, multidose anesthetic vials (propofol and fentanyl) were used.

**Virological data.** All samples from the five anti-HCV-positive patients were HCV RNA positive. The patients were all infected with HCV type 1b. For each patient, the nucleotide sequences of 10 to 20 clones, corresponding to partial fragments of the E1-E2 and the NS5 regions, were analyzed and aligned. A total of 185 clones were sequenced: 111 for the E1-E2 region (20 from the putative source patient, 58 from the outbreak patients, and 33 from the control patients) and 74 for the NS5 region (12 from the putative source patient, 32 from the outbreak patients, and 30 from the control patients). Mean percent divergence values of each pairwise comparison between the putative source patient and the outbreak patients ranged from 3.63 to 5.32 for E1-E2 and from 2.70 to 4.48 for NS5, respectively. The percent divergence among sequences of the same nucleotide fragments between the putative source patient and the control patients was significantly higher (t test for E1-E2 region [including HVR-1] = 5.2893; P = 0.001; t test for NS5 region = 3.2737; P = 0.01). The phylogenetic tree reconstruction of cloned sequences is shown in Fig. 1.

**Epidemiological data.** According to regional records, no case of acute HCV infection was reported in 1997 through 12 January 1998 among women undergoing operations in a gynecological unit.

Outbreak patients were defined as those individuals with previously unknown HCV serostatus who had antibodies to HCV and homology of HCV RNA sequences in serum or
plasma samples by RT-PCR and genotyping in March 1998. The source patient was traced among those patients who already were anti-HCV positive at the time of the operation and showed molecular homology in the HCV genome with those of the outbreak patients.

Based on genotyping, sequencing, and phylogenetic analysis of HCV RNA-positive samples, the woman with chronic hepatitis C is the putative source patient and the four anti-HCV-positive women are cases of recent HCV infection. On 9 January 1998, the day-specific attack rate of HCV infection was 80% (four of five). Among the risk factors investigated, those common to all the patients who underwent surgery on the morning of 9 January were administration of atropine, fentanyl, and propofol. However, propofol was not administered to the patient who did not become infected.

The retrospective cohort study conducted among the patients who underwent surgical intervention on 8, 9, and 10 January showed that having undergone surgery in the morning session of 9 January was the sole significant risk factor ($P = 0.003$).

**DISCUSSION**

This study describes the molecular epidemiology of a cluster of cases of primary HCV infection that involved four gynecological patients who underwent surgery on the same day in the same gynecological surgery unit.
The investigation identified a woman with chronic hepatitis C as the putative source patient. She was the first of the six patients of the operative session of 9 January: all the other women but one became outbreak patients. The phylogenetic analysis of viral sequences from the source and outbreak patients revealed very close homology between viral sequences, consistent with nosocomial transmission; this highlights the potential of molecular strategies to unambiguously trace cases of HCV transmission from a common source of infection.

It is extremely likely that HCV infection resulted from a point source and that it occurred in the operating room. Among the risk factors identified, the source patient and outbreak patients shared only the administration of propofol. This anesthetic was not administered to the woman who did not seroconvert.

Review of infection control procedures showed the possibility of a blood reflux in the peripheral venous line during blood pressure monitoring.

Accordingly, if the syringe used to administer propofol to the source patient was also used to draw propofol from the multidose vials and administer it to the subsequent patients, blood contamination of the syringe is likely to have resulted and to have determined the spread of HCV infection. However, the surgical team members were adamant in stating that the syringe used to draw fluid from the multidose vials was changed after each patient.

Despite the well-known risks of blood-borne infection transmission to both patients and operators, reuse of disposable syringes and multidose drug vials in anesthesia remains a major concern, as microscopic amounts of blood in the intravenous tubing can cause contamination of syringes, needles, and drug vials (18).

Contaminated multidose vials have been implicated in the transmission of hepatitis B (13, 14, 20), hepatitis C (24), and bacterial infections (5). Indeed, as in-use contamination of propofol multidose vials is not uncommon (6, 7), specific precautions have been suggested to prevent propofol-related infection because its lipid base can support bacterial growth in case of contamination (21).

In conclusion, two factors seem to have been decisive in causing this cluster of HCV infection: the operating schedule and a common vehicle of infection. Had the source patient been the last to be operated on, HCV infection might never have occurred. This case emphasizes the risk of nosocomial transmission and the importance of universal infection control procedures in the operating room. Some health care procedures, i.e., surgical and dental treatments, have recently been indicated as risk factors for acute HCV (17), likely due to breaches in infection control measures. The present study highlights the usefulness of molecular epidemiological strategies and particularly of cloning and sequencing techniques for the investigation of outbreaks of HCV infection.

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