NOSOCOMIAL TRANSMISSION OF HEPATITIS C VIRUS ASSOCIATED WITH THE USE OF MULTIDOSE SALINE VIALS

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ABSTRACT

OBJECTIVE: To identify the source of an outbreak of acute hepatitis C virus (HCV) infection among 3 patients occurring within 8 weeks of hospitalization in the same ward of a Florida hospital during November 1998.

DESIGN: A retrospective cohort study was conducted among 41 patients hospitalized between November 11 and 19, 1998. Patients' blood was tested for antibodies to HCV, and HCV RNA–positive samples were genotyped and sequenced.

RESULTS: Of the 41 patients, 24 (59%) participated in the study. HCV genotype 1b infections were found in 5 patients. Three of 4 patients who received saline flushes from a multidose saline vial on November 16 had acute HCV infection, whereas none of the 9 patients who did not receive saline flushes had HCV infection ($P = .01$). No other significant exposures were identified. The HCV sequence was available for 1 case of acute HCV and differed by a single nucleotide (0.3%) from that of the indeterminate case.

CONCLUSION: This outbreak of HCV probably occurred when a multidose saline vial was contaminated with blood from an HCV-infected patient. Hospitals should emphasize adherence to standard procedures to prevent blood-borne infections. In addition, the use of single-dose vials or prefilled saline syringes might further reduce the risk for nosocomial transmission of blood-borne pathogens (*Infect Control Hosp Epidemiol* 2003;24:122-127).

Several outbreaks of nosocomial transmission of hepatitis C virus (HCV) have been linked to breaches in standard precautions for blood-borne infections during nursing procedures[1] or interventions such as colonoscopy,[2] surgery,[3] and dialysis.[3] In other reports of HCV outbreaks, although the route of transmission could not be identified, failure to follow standard procedures was suspected in most instances.[4-11] In reports on other types of outbreaks, saline flushes of indwelling peripheral intravenous cannulas have been linked to nosocomial transmission of pathogens other than HCV.[12-16]

In August 1999, three patients diagnosed as having HCV infection within 8 weeks of being discharged from the same hospital in southern Florida were reported to the Florida Department of Health. All three patients had been hospitalized in the same ward of a 150-bed hospital between November 11 and 19, 1998. The results of previous tests for hepatitis A and B were negative. Hospital officials conducted an internal investigation, including testing of most hospital staff members for antibody to HCV (anti-HCV), but were unable to identify the source of the outbreak. All serum samples from staff members were negative for anti-HCV. Subsequently, the Florida Department of Health was asked to investigate the outbreak to identify its source. We conducted an epidemiologic investigation of patient-to-patient transmission of HCV that identified exposure to saline flushes as a risk factor for infection.

METHODS

Case Definitions and Case Ascertainment

An acute case was defined as a patient hospitalized in the ward between November 11 and 19, 1998, with (1) a positive antibody test against HCV; (2) onset of jaundice after November 26, 1998; (3) serum aminotransferase levels greater than 1,000 IU/L; and (4) no evidence of prior HCV infection. A chronic case was defined as a patient hospitalized in the ward between November 11 and 19, 1998, with a diagnosis of HCV infection before admission. An indeterminate case was defined as a patient hospitalized in the ward between November 11 and 19, 1998, with (1) a positive antibody test against HCV and (2) no evidence of jaundice, elevated serum aminotransferase levels, or prior HCV infection.

Patients hospitalized in the ward between November 11 and 19, 1998, were contacted either directly or through their primary physicians. Patients were informed of the possible nosocomial transmission of hepatitis C and were offered a test for HCV infection. Serum samples of patients were tested first by enzyme immunoassay (EIA) (HCV-EIA version 3.0, Ortho-Clinical Diagnostics, Rochester, NY).

Samples that were repeatedly reactive by EIA were subsequently confirmed by RIBA HCV 3.0 Strip Immunoblot Assay (Chiron Corp., Emeryville, CA).

Review of Medical Records and Patient Interviews

The medical records of all patients hospitalized in the ward between November 11 and 19, 1998, were reviewed. Data on medical history, diagnosis, diagnostic procedures, surgical interventions, medications, and other treatments were collected. Any medication or diagnostic procedure that involved access to the patient's vascular system was recorded with the day and time of exposure. These exposures included intravenous medication, infusions, transfusions, phlebotomy, and capillary blood sugar tests.

Patients who tested positive for anti-HCV were interviewed regarding symptoms, medical history, and risk factors for hepatitis C. Risk factors were defined as any of the following: injection drug use, history of sexually transmitted diseases, hemodialysis, blood transfusions or blood components before 1992, organ transplants before 1992, or receipt of clotting factor concentrates produced before 1987.[17]

Retrospective Cohort Study

A retrospective cohort study was conducted among patients who were tested for anti-HCV, using the case classification as the outcome variable. Indeterminate and chronic cases were excluded from the analysis because their status as a source or recipient of infection could not be determined with certainty. Exposures shared by acute case patients were analyzed by day and time of exposure. Six time periods per day of 1 to 6 hours each were defined to compare exposures. Longer time periods were chosen for hours during which few medical or nursing interventions occurred, and shorter time periods were chosen for times of frequent exposures. P values (Fisher's exact test) were calculated using Epi-Info software (version 6.0; Centers for Disease Control and Prevention, Atlanta, GA).

Genotyping and Sequencing of HCV

Anti-HCV–positive specimens were tested for HCV RNA by nested reverse transcription polymerase chain reaction (RT PCR) amplification of the 5' noncoding region.[18,19] PCR products were separated by electrophoresis on a 2% agarose (GIBCO-BRL, Carlsbad, CA) and by positive specimens identified with ethidium bromide staining.

HCV genotypes were determined for HCV RNA–positive specimens from the 300-nucleotide–long sequence of the nonstructural coding region 5b (NS5b).[19-21] PCR products were purified and sequenced using an automated DNA sequencer (ABI 377, Applied Biosystems Inc., Foster City, CA). Genotypes were defined using a multiple sequence alignment program (Pileup, Wisconsin Genetic Computer Group, Madison, WI).[22]

To determine the relatedness of HCV isolates, quasi-species of the hypervariable region 1 were determined by limiting dilution PCR for each HCV RNA–positive specimen as described below. Nested RT PCR was performed to determine the endpoint titer, using the following primers: first round 1350F1 (forward primer 5'-GCTACTCCGGATCCC ACAAGC-3') and 1633R (reverse primer 5'-TCATTGCAGTTCAGGGCCGT-3'), and second round 1426F1 (forward primer 5'-TATTCCATGGTGGGGAACTGG -3') and 1619R (reverse primer 5'-GCAGTCCTGTTGATG TGCCA-3'). Multiple PCR reactions (30 to 40) were then performed at twice the serum dilution of the endpoint PCR titer. Each PCR amplicon was purified, and the nucleotide sequence determined as described previously.

Pairwise analysis of nucleotide sequences was performed using multiple sequence alignment programs (Pileup and Pretty, Wisconsin Genetic Computer Group). Distribution of nucleotide distances was calculated using Jukes–Cantor distance correction (Evolutionary Distance, Wisconsin Genetic Computer Group).

Site Inspection and Review of Procedures

The ward was visited on August 25 and 26, 1999, and the materials used to administer medications were inspected. Staff assignment sheets were reviewed to determine which patients shared the same nurses during each shift. Staff members were asked to demonstrate procedures that involved phlebotomy, intravenous medication, or any other access to the vascular system of patients, such as blood sugar testing.

RESULTS

Case Ascertainment

Between November 11 and 19, 1998, a total of 41 patients were hospitalized on the ward. By the time the investigation was started in August 1999, 15 (37%) of the patients had died; none of these patients had hepatitis or liver disease listed as a cause of death on the death certificate. Two (5%) of the patients could not be located. Thus, 24 (59%) of the patients were available to provide blood samples for testing. Five (21%) of the 24 samples were positive for anti-HCV.

Review of Medical Records and Patient Interviews

Twenty-one women and 20 men comprised the 41 patients hospitalized in the ward between November 11 and 19, 1998. Patients' ages ranged from 18 to 93 years, with a median of 77 years. The mean age of the 24 patients (12 women and 12 men) who were tested for anti-HCV was 72 years versus a mean age of 83 years for the 17 patients (9 women and 8 men) who were not tested. The mean length of hospitalization was 10 days. Admission diagnoses included a variety of cardiac, gastrointestinal, traumatic, and gynecologic problems. Most admissions resulted from geriatric health problems. Thirty-nine (95%) of the patients had an indwelling peripheral intravenous cannula, 10 (24%) of the patients underwent surgery, 6 (14%) of the patients underwent gastroenterologic interventions, 5 (12%) of the patients underwent radiologic imaging with contrast medium, 3 (7%) of the patients had a central venous catheter,

TABLE 1

CHARACTERISTICS OF PATIENTS WITH HEPATITIS C VIRUS INFECTION AS OF AUGUST 1999 WHO WERE HOSPITALIZED IN THE SAME WARD BETWEEN NOVEMBER 11 AND 19, 1998

Patient	Age (y)	Gender	Anti-HCV Test Before Admission	Onset of Jaundice	Admission Diagnosis	Dates of Stay in Ward	Risk Factors?	Case Classification
A	34	M	Negative 11/95	1/17/99	Asthma	11/11/98–11/17/98	No	Acute
B	18	F	Not done	12/5/98	Guillain–Barré syndrome	11/12/98–11/19/98	No	Acute
C	51	F	Not done	1/7/99	Asthma	11/12/98–11/19/98	No	Acute
D	82	M	Not done	None	Chronic obstructive pulmonary disease	11/13/98–11/18/98	Unknown	Indeterminate
E	66	F	Positive 1990	1990	Pancreatitis	11/13/98–12/13/98	Yes	Chronic

Anti-HCV = antibody to hepatitis C virus.

and 6 (15%) of the patients underwent other invasive interventions.

According to the medical history and laboratory results, 3 patients had acute cases (patients A, B, and C), 1 patient had an indeterminate case (patient D), and 1 patient had a chronic case (patient E) of hepatitis C (Table 1). All 5 case-patients received intravenous medications through indwelling peripheral intravenous cannulas, but they did not have the same medications in common. None of the case-patients underwent surgery, gastroscopy, dialysis, or a blood transfusion during their hospitalization in the ward. The exposures shared by all 3 acute case-patients (patients A, B, and C) were having an indwelling peripheral intravenous cannula, receiving any kind of intravenous medication, receiving saline flushes of the indwelling peripheral intravenous cannula, and undergoing venous phlebotomy. Patients A and B had intramuscular medication, and patients A and C received nebulizer treatments. Patient B underwent a spinal tap, and patient C underwent subcutaneous insulin and capillary blood tests.

The onset of signs and symptoms of acute hepatitis C occurred in patients A, B, and C during December 1998 and January 1999; none of these patients had identified risk factors for HCV infection. Patient D died in November 1999 without developing signs or symptoms of hepatitis. This patient had received multiple blood transfusions from 1997 to 1999 but not during his hospitalization in the ward in November 1998. Although this patient could not be interviewed, his relatives and primary care physicians reported no knowledge of any risk factors for HCV infection. Patient E had been diagnosed as having HCV infection in 1990; she had received multiple blood transfusions in 1975 in Cuba and reported no other risk factors for HCV infection.

Retrospective Cohort Study

Twenty-four patients were included in the cohort study; 3 of these patients had acute hepatitis C (patients A, B, and C) and were considered acute cases. The remaining 19 patients tested negative for anti-HCV. Patients D and E were excluded from the analysis. Exposure to indwelling peripheral intravenous cannulas was tested for the entire period of hospitalization because the dates on the installation or exchange of the cannulas were not available. The only exposure that yielded a trend for an association with acute HCV infection was saline flush of indwelling peripheral intravenous cannulas. During six time periods of 1 to 6 hours each, all 3 acute case-patients received a saline flush. During those times, the only exposure significantly associated with acute HCV infection was the saline flush on November 16 at 10:00 pm (Table 2). This was one of four times during which patient D, the indeterminate case-patient, received a saline flush before the 3 acute case-patients.

Genotyping and Sequencing of HCV

The genotype of all five samples was 1b. Samples from patients B, D, and E were PCR positive and were sequenced. Two samples could not be sequenced (patients A and C) because viral material was no longer available. In a pairwise analysis, the sequences of the NS5b region of the HCV from patients B and D differed by a single nucleotide (0.3%), and the HCV from patient E differed from the virus from patients B and D by 9 nucleotides (3%).

Relatedness of HCV isolates was evaluated by hypervariable region 1 quasi-species analysis. A single hypervariable region 1 quasi-species with identical sequence was obtained from the HCV from patient B by limiting dilution PCR (n = 20 amplicons). Multiple quasi-species variants were isolated from patient D (n = 16 amplicons) and patient E (n = 19 amplicons) with sequence variation between each other ranging from 0% to 2%. The hypervariable region 1 quasi-species sequences of the HCV from patients B and D varied by a single nucleotide. The nucleotide identity was 99% between the sequences of the HCV from patients B and D. The hypervariable region 1 sequences obtained by limiting dilution PCR from the HCV from patient E had only 72% identity with the HCV from patients B and D.

Site Inspection and Review of Procedures

The ward had three rooms with two beds and 12 single-bed rooms, and was divided into two sections with nine

TABLE 2
EXPOSURES* AMONG PATIENTS WITH HEPATITIS C VIRUS INFECTION HOSPITALIZED IN THE SAME WARD BETWEEN NOVEMBER 11 AND 19, 1998

Type and Time of Exposure	Acute HCV Infections Among Exposed	Acute HCV Infections Among Unexposed	P Value‖
Intravenous injection, Nov. 11–19	3/12	0/10	.14
Indwelling peripheral intravenous cannula, Nov. 11–19†	3/19	0/2	.73
Venous blood test, Nov. 11–19	3/20	0/2	.74
Saline flush, Nov. 11–19	3/14	0/8	.24
Nov. 14: 6:00–10:00 pm‡	3/7	0/2	.42
Nov. 14–15: 10:00 pm–1:00 am§	3/7	0/2	.42
Nov. 15: 12:00–6:00 pm‡	3/6	0/3	.24
Nov. 15: 10:00 pm§	3/5	0/4	.12
Nov. 16: 9:00 am–3:00 pm‡	3/7	0/6	.12
Nov. 16: 10:00 pm‡	3/4	0/9	.01

HCV = hepatitis C virus.
*Exposures shared by at least 2 cases.
†Total is 21 patients instead of 22 patients as for other exposures because information was not available for 1 patient.
‡Time periods ≤ 6 hours during which all 3 acute case-patients received a saline flush within 1 hour after the indeterminate case-patient received a saline flush.
§Time periods ≤ 6 hours during which all 3 acute case-patients received a saline flush.
‖Fisher's exact test.

patients in each section. There were two nursing teams; each team was primarily responsible for one of the two sections. Patients C and E shared one room, which was located across the hall from the room of patient A in the first section. Patients B and D stayed in two adjacent single-bed rooms in the second section.

The hospital used a computerized system for drugs and nursing supplies that kept individual accounts of all items used for each patient. This system prevented the sharing of medications and nursing supplies. The only medication that was not individually stored and assigned for patient use was saline solution used to prepare flushes of indwelling peripheral intravenous cannulas. The saline solution was in 30-mL multidose vials that were stored on a medication cart and used by all nurses in both sections of the ward. Only one vial was opened at a time. Saline flushes were performed before and after each intravenous medication or by schedule, usually once per nursing shift.

Nurses were able to explain the standard precautions for the prevention of blood-borne infections and stated that they adhered to the precautions. According to interviews with the nurses, the saline flushes were prepared by inserting a single-use needle into the multidose vial and drawing approximately 3 mL into a single-use syringe. The needle was then discarded, and the syringe was connected directly to the indwelling peripheral intravenous cannulas, injecting the saline solution. On the basis of the nurse assignment sheets, the 3 acute case-patients did not share the same nurse during any shift.

DISCUSSION

The results of this epidemiologic investigation suggest that three patients were infected with HCV by injection of saline solution from a multidose vial that might have been contaminated with the blood of another patient. Five

lines of evidence support this conclusion. First, the HCV infection of all five case-patients had genotype 1b, which has an estimated prevalence of 17% among HCV-infected individuals in the general U.S. population.[19] Second, the sequencing results demonstrate a close genetic resemblance between the viruses of one acute case-patient (patient B) and the indeterminate case-patient (patient D), suggesting patient-to-patient transmission. Third, saline solution was the only substance for intravenous use in the ward that was kept in multidose vials to be used for more than one patient. Fourth, the epidemiologic analysis identified exposure to saline flushes as the only significant risk factor for acute HCV infection. Fifth, the indeterminate case-patient received a saline flush before the three acute case-patients on November 16 at 10:00 pm, which is consistent with the possibility of the saline solution being contaminated with the blood of the indeterminate case-patient.

Although all five patients with HCV infection had genotype 1b, the sequencing results indicated that patient E (the chronic case) was not connected to patients B (an acute case) and D (the indeterminate case). The larger variation of quasi-species for the virus from patient D would be consistent with the hypothesis that his infection was of longer duration than patient B's infection.[23] If this outbreak resulted from a single incident, patient D was the most likely source for infection of patients A, B, and C.

We could not determine exactly how the saline solution was contaminated with HCV, especially because the nurses said they adhered to the standard precautions against transmission of blood-borne pathogens and denied filling the same syringe twice from a multidose vial. The medication records in the hospital did not always indicate the order in which patients received saline flushes during a given time. However, there were four times when the indeterminate case-patient received a saline flush before the

three acute case-patients. A possible scenario is that the 3-mL saline flush in the source patient was not sufficient to clear the indwelling peripheral intravenous cannula, and the nurse used the same syringe to draw an additional 3 mL from the multidose vial for a second flush. When the syringe was connected to the indwelling peripheral intravenous cannula the first time, the syringe might have become contaminated. Thus, using the same syringe to draw an additional 3 mL would subsequently contaminate the contents of the vial. Similar scenarios have been postulated for other nosocomial transmissions of blood-borne pathogens.[6,13,24,25] In some outbreaks, investigators found that nurses would reuse syringes or cannulas for saline flushes for the same patient.[24,26] This can occur with both needleless and traditional needle systems.[16]

This study has several limitations. First, 17 (41%) of the 41 patients could not be tested for anti-HCV because 15 (37%) of the patients had died before the investigation started. However, no history of HCV infection was documented in the medical records of these patients, and the death certificates did not mention liver disease. Second, we lacked sequencing data to determine how closely the virus genomes of patients A and C were related to the other samples. Third, the investigation started too late after the event for nurses to be able to remember any breaches in standard procedures. In addition, nurses might have been hesitant to admit such behaviors because they were aware that lawsuits were pending against the hospital. Fourth, we cannot exclude the possibility of intentional violation of standard procedures, such as described in a hepatitis C outbreak in Spain that involved drug addiction.[27] However, the only occasion that any of the five patients with HCV infection received drugs with the potential for abuse or addiction was on November 14 when patient A received lorazepam.

Previous reports of nosocomial HCV transmission have suggested multidose saline solution vials as a source for transmission, but epidemiologic associations were not established.[4,6,10,11] Saline flushes and other intravenous multidose medications have also been reported to transmit blood-borne pathogens such as malaria, human immunodeficiency virus, or bacteria.[1,13-16,24-26,28-35] For at least four reports of nosocomial transmission of blood-borne pathogens, investigators were able to epidemiologically link the transmission to saline flushes of indwelling peripheral intravenous cannulas. However, none of these reports included HCV.[12,13,15,25]

According to an Institute of Medicine report, medical errors occur at a high rate, and systems need to be designed to make it difficult for staff to make errors.[36] Ideally, adherence to standard procedures would prevent contamination of a multidose vial or its contents. However, handling errors occur even among trained staff with adequate procedures in place, particularly during busy periods.[3] Two changes could reduce, although not completely eliminate, the probability of contaminated saline flushes[24,37,38]: (1) multidose vials could be replaced by single-dose vials or (2) single-use syringes prefilled with saline solution would reduce the need for vials entirely. Such products are available, and some hospitals have their pharmacy departments fill the syringes and deliver them in sterile packages to the units.[37] As a result of this investigation, the affected hospital has replaced multidose saline vials with single-dose vials. A cost–benefit analysis for such interventions is needed, but the additional cost of single-use saline vials or prefilled syringes is likely to be small.

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