MAJOR ARTICLE

Iatrogenic Transmission of Hepatitis C Virus (HCV) by an Anesthesiologist: Comparative Molecular Analysis of the HCV-E1 and HCV-E2 Hypervariable Regions

Yonat Shemer-Avni,[1,3,a] Michal Cohen,[4,a] Ayelet Keren-Naus,[1,3] Emanuel Sikuler,[2,3] Negba Hanuka,[1,3] Arie Yaari,[1,3] Eithan Hayam,[3] Larisa Bachmatov,[4] Romy Zemel,[4] and Ran Tur-Kaspa[4,5]

[1]Clinical Virology Unit and [2]Department Medicine B, Faculty of Health Sciences, and [3]Soroka Academic Medical Center, Ben Gurion University, Beer Sheva, and [4]Molecular Hepatology Lab, Felsenstein Medical Research Center, Sackler Faculty of Medicine, Tel-Aviv University, and [5]Department of Medicine D and Liver Institute, Rabin Medical Center, Petach Tikva, Israel

Background. Transmission of hepatitis C virus (HCV) from infected health care workers to patients rarely occurs. In 2003, a cluster of patients with HCV infection was identified at a medical center in Israel. All patients had a common history of various surgical procedures performed during the period 2001–2003. All patients had been anesthetized by an anesthesiologist who was an injection drug user and was infected with genotype 2a HCV. Screening was initiated by the hospital to identify newly infected patients with HCV infection and to determine the source of the iatrogenic HCV infection outbreak using comparative molecular analysis of the HCV E1 and HCV E2 hypervariable regions (HVR1 and HVR2).

Methods. A total of 1200 patients who were anesthetized by the anesthesiologist (the related group) and 873 hospital personnel and patients anesthetized by other anesthetists (the unrelated group) were examined. Serum samples were screened for anti-HCV antibodies, HCV RNA, and genotype. Sequence analysis of HVR1 and HVR2 was performed after reverse-transcriptase polymerase chain reaction.

Results. HCV type 2a was found in 33 patients in the related group but in only 1 patient in the unrelated group. The differences between the sequences isolated from the related group serum samples and the sequences isolated from genotype 2a control group serum samples (obtained from 15 patients) were highly statistically significant. The genetic distances from the anesthesiologist sequence were 1.4%–4.4% in the HVR1 and 0%–3% in the HVR2 in the related group serum samples, whereas in the HCV genotype 2a control group serum samples, the genetic distances were 22%–45% and 10%–35%, respectively.

Conclusions. Molecular analysis revealed sequence similarity of HVR1 and HVR2 in the related group, suggesting that the anesthesiologist with chronic HCV infection may have transmitted HCV to 33 patients.

Chronic hepatitis C virus (HCV) infection is a blood-born disease. Therefore, it is most efficiently transmitted through large or repeated percutaneous exposure to infected blood. In countries where blood donations are screened for HCV, injection drug use is a major risk factor for acquiring HCV infection, leading to a high prevalence of chronic HCV infection in this population [1]. Occupational, perinatal, and sexual exposures can also result in transmission of HCV, although they are less efficient modes of transmission than injection drug use. An additional source of HCV transmission is nosocomial outbreaks of HCV infection, resulting from unsafe injection practices or contaminated equipment [2, 3]. Although iatrogenic HCV infection involving transmission of HCV from an infected health care provider to patients is a rare event, it causes great distress to patients and staff and can give rise to expensive legal claims.

Molecular analysis has been used to investigate the epidemiology of HCV transmission using sequence analysis of different regions of the HCV genome.

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[a] Y.S.A. and M.C. contributed equally to this study.

Reprints or correspondence: Dr. Shemer Avni Yonat, Dept. of Virology, Faculty of Health Sciences, Ben Gurion University, POB 653, Beer Sheva, 84105, Israel (Yonat@bgu.ac.il).

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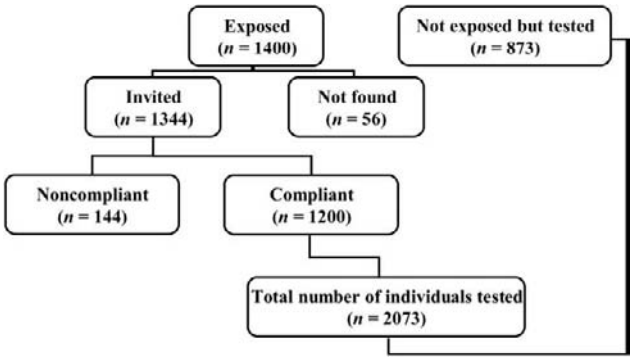

Figure 1. Description of the screen for hepatitis C virus performed during August 2003.

Genotyping and time of divergence of various HCV genotypes isolated from different geographical regions are determined by sequences in the highly conserved 5′-noncoding region and the NS5 region of the virus genome [4]. Sequence analysis of the hypervariable regions at the junction between the coding regions for envelope glycoproteins E1 and E2 (HVR1) and of the E2 gene (HVR2), as well as sequence analysis of the NS5 region, have been used to investigate vertical or horizontal transmission of HCV between pairs of individuals, providing evidence for a common source of infection in several large-scale outbreaks of HCV infection [5–7].

Molecular analyses of infections transmitted from patient to patient in a hospital have been reported for patients receiving dialysis, administration of contaminated anti-D immunoglobulin, and surgical procedures [7–13]. HCV transmission by an HCV-infected surgeon and by an anesthesiologist assistant [14, 15] has been described, as well.

In 2003, we identified a cluster of HCV-infected patients at a medical center in Israel. All patients had a common history of surgical procedures performed during the period 2001–2003. Epidemiological investigation of >2000 individuals indicated an HCV-infected health provider as the source of infection. We describe here the HCV infection outbreak and the phylogenetic investigation that linked the health care provider to the newly acquired HCV infections.

MATERIALS AND METHODS

Study population. The screened population consisted of 2 groups. The related group comprised 1200 patients (among 1344 patients that were actively contacted by the hospital to be screened) who underwent surgical procedures at the medical center (Soroka Academic Medical Center, Beer Sheva, Israel) during the period 2001–2003 and were anesthetized by a health care provider who was chronically infected with genotype 2a HCV. The unrelated group (i.e., the control group) comprised 873 individuals and included health care providers from the hospital and patients who were anesthetized by other health care providers in the same hospital; all were anxious to be examined. All serum samples collected from patients associated with the outbreak and collected from control subjects were obtained during the screening period (2 weeks), including serum samples obtained from the source of the outbreak. The time period between HCV acute infection and the collection of the serum samples was variable, ranging from 3.5 to 20 months.

All serum samples collected were tested immediately for anti-HCV antibodies. In parallel, aliquots were frozen at −70°C for HCV RNA monitoring, genotyping, and E2 gene sequencing (figures 1 and 2).

The HCV screening was performed in the clinical virology laboratory of the Soroka Academic Medical Center. In addition, 15 patients that were known to carry genotype 2a HCV before 2001 were included in the study as unrelated control subjects for the analysis of the HCV E2 HVR sequence. These 15 isolates were taken from the frozen serum archive of the Clinical Virology Unit, Soroka Hospital (Beer Sheva, Israel). The study was approved by the Helsinki committee of the Soroka Academic Medical Center.

Screening for antibodies to HCV. Anti-HCV antibodies were determined using a third-generation test and Axsym apparatus according to the manufacturer's instructions (Abbot Diagnostics Laboratories). Samples that tested in the gray zone range were further tested for HCV antibodies by RIBA (Ortho) according to the manufacturer's instructions and for HCV RNA. In addition, because a long period between HCV infection and seroconversion may occur, all serum samples that tested negative for anti-HCV antibodies and were obtained

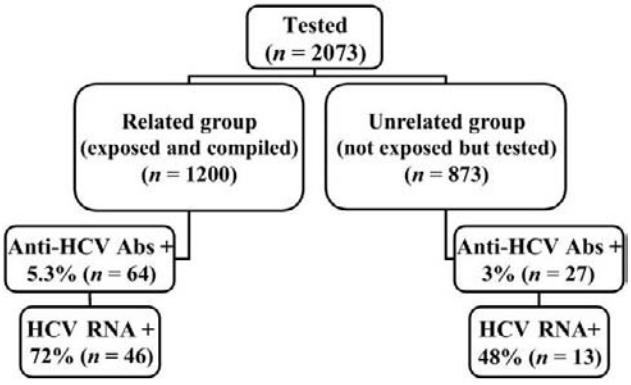

Figure 2. Flow chart of tests for hepatitis C virus (HCV) infection in the related and unrelated groups. The related group consisted of patients who underwent surgical procedures at the medical center during the period 2001–2003 and were anesthetized by a health care provider infected with HCV. The unrelated group consisted of health care providers from the hospital, as well as patients who were anesthetized by other health care providers in the same hospital. Abs, antibody; +, positive.

Table 1. List of primers used for amplification of hepatitis C virus (HCV) E2 hypervariable region.

Primer	Sequence	Position	Isolate
E2G2R1	ACGTCCACGATGTTTTGGTGGAG	2414-2392	HCV 2a AF238483
E2G2F1	ACACCAAYGGMAGCTGGCACAT	1550-1571	HCV 2a AF238483
E2G2R2	GCCCATTCCGTGGTGGAGTGCA	2336-2315	HCV 2a AF238483
E2G2F3	TTCAACTCGTCAGGATGTCCCG	1645-1666	HCV 2a AF238483
E2HVR1FO	GGCCATCGTATGGCTTGGGATATGATG	1250-1275	HCV 2a AF238483
E2HVR1RO	GCTGTCCGGTTGATGTGCCAGCTGCCATTGGT	1552-1777	HCV 2a AF238483
E2HVR1FI	GCTTGGGATATGATGATGAACTGGTC	1260-1285	HCV 2a AF238483
E2HVR1RI	TTGATGTGCCAGCTGCCATTGGTGT	1573-1549	HCV 2a AF238483

from patients who underwent anesthesia <6 months before the screening period were tested for HCV RNA.

HCV RNA detection and genotyping. Total RNA was extracted from 200 μL of serum and was prepared using an HCV specimen preparation kit (Amplicor; Roche Diagnostic). HCV RNA was detected using HCV amplification and detection kits (Cobas HCV Amplification and Detection; Roche Diagnostic). In HCV RNA–positive samples, HCV genotypes were determined by sequencing the 5′ untranslated region of the PCR product (MBC; HyLab). Sequencing of the HVR1 to HVR2 region was performed for serum samples with genotype 2a.

HCV E1-E2 sequence analysis. Sequence analysis was performed on the hypervariable regions of the E2 gene, spanning 2 regions: 190 nucleotides of HVR1 (1336–1454; AF238483) (performed at Rabin Medical Center, Petach Tikva, Israel) and the 660-nucleotide fragment (1645–2336; AF238483) encompassing HVR2, including the double-stranded RNA–activated protein kinase region (PKR) domain (performed at Soroka Academic Medical Center). These regions are known to exhibit a high degree of variability and are used to distinguish between HCV isolates of the same subtype and for determination of quasispecies (the coexistence of different sequences in the same patient). It is assumed that a longer period of infection results in a higher mutation rate [16]. Each of the 2 regions was analyzed in a different laboratory using different serum samples that were obtained from the same patient. Total RNA was extracted from 200–500 μL of serum using a QIAamp UltraSense kit (Qiagen GmbH). The primers used for the amplification are listed in table 1. The HVR2-PKR region RT-PCR was performed at 42°C for 1 h, using Superscript II (Invitrogen), 10 pmol of E2G2R1 primer, and 10 μL of RNA in total volume of 30 μL. One μL of cDNA and primers E2G2R1 and E2G2F1 were used for the first PCR reaction. The nested PCR was performed with 2 μL from the first PCR and primers E2G2R2 and E2G2F3 in a total volume of 50 μL. All PCR amplifications were performed with Takara Ex Taq (Takara) to eliminate Taq-sequencing mistakes. The cDNAs were analyzed on a gel, cleaned on a column, and sent for sequencing (MBC; HyLab) from both ends, using primer E2G2F3 for the 5′ end and primer E2G2R2 for the 3′ end. The 2 sequences obtained for each patient were aligned to ensure the correct nucleotide sequence. The HVR1 was analyzed on a limited number of serum samples; RNA was isolated as described earlier and amplified as described by Sandres et al. [17]. In brief, RT-PCR was performed using the AccessQm TM RT-PCR system (Promega), and primers E2HVR1FO and E2HVR1RO. The nested PCR was performed with primers E2HVR1FI and E2HVR1RI (table 1), and the PCR product was purified and sequenced as described earlier.

Statistical analysis. Pairwise analysis was performed using multiple-sequence alignment of HVR2-PKR (660 nucleotides). The ClustalW program [18] was used to determine the genetic distance, divergence of nucleotide substitutions per 100 nucleotide sites, and neighbor-joining slanted phylogram tree analysis. Comparison between means of nucleotide variability was performed using Student's *t* test for independent samples. Results were considered to be statistically significant at $P < .05$.

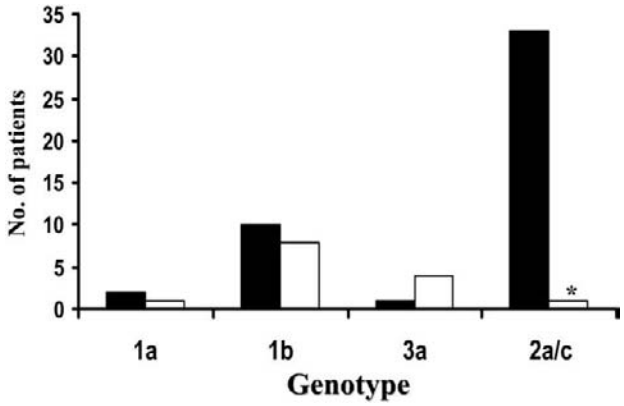

Figure 3. Genotype distribution among the related group *(black columns)* and unrelated group *(white columns).* *This patient had genotype 2a/c HCV infection according to laboratory records, but at the time of screening, the patient had completed therapy and had test results that were negative for hepatitis C virus RNA.

Table 2. Hepatitis C virus (HCV) genotype distribution among 64 anti-HCV antibody–positive patients in the related group.

Variable	No. of patients
HCV RNA positive	46
Nondocumented HCV infection[a]	
All	39
With genotype 1b	6
With genotype 2a	33
Documented HCV infection before 2001[b]	
All	7
With genotype 1b	4
With genotype 1a	2
With genotype 3a	1

NOTE. Laboratory records of HCV genotypes of patients from the related group were extracted.

[a] The nondocumented HCV infection group includes patients for whom no record of HCV infection before the screening in 2003 was found.

[b] The documented HCV infection group includes patients from the related group who had records of HCV infection before 2001.

RESULTS

Analysis of the HCV outbreak. In August 2003, a cluster of newly infected patients with HCV infection with a common history of various surgical procedures performed during the period 2001–2003 in the Soroka Medical Center was identified. All patients were anaesthetized by the same anesthesiologist. The physician was known to be an injection drug user and was chronically infected with genotype 2a HCV. He worked in the hospital for 2 years (2001–2003) and was released from work 4 months before the patient screening period because of excessive use of anesthetics. Three strategic guidelines were adopted by the hospital board: (1) to identify all patients cared for by this physician, (2) to treat those patients who were infected by the virus without any charge, and (3) to take full responsibility for this event. A computerized database consisting of all patients who underwent surgical procedures and were anesthetized by this physician was created (1400 patients). Invitations for serological testing for HCV were sent to 1344 patients by courier (figure 1). Patients were contacted by telephone, as well, to ensure their compliance. A special clinic for HCV screening was operated 24 h per day. Numerical findings were published in daily press conferences. During a 2-week period, a total of 2073 individuals were screened for HCV (figure 2), including 1200 compliant patients (compliance rate, 89%; the related group) and 873 patients and staff who wished to be tested (the unrelated group).

In the related group, 64 (5.3%) of 1200 patients had test results that were positive for anti-HCV antibody, and 46 (72%) of these 64 patients had results that were positive for HCV RNA (figure 2). One anti-HCV antibody–negative sample was RNA positive at the time of the screening. However, this patient became anti-HCV antibody positive 1 year later; the window period (i.e., the time from infection to the appearance of anti-HCV antibodies) was 2 years. In the unrelated group, 27 (3%) of 873 patients were anti-HCV positive; of these patients, 13 (48%) were HCV RNA positive. Thus, the prevalence of HCV RNA–positive serum samples in the related and unrelated groups was 3.8% and 1.5%, respectively. The prevalence of both markers of HCV infection was significantly higher in the related group ($P < .05$).

HCV genotype distribution between the 2 groups varied to a great extent (figure 3). In the related group, the most abundant genotype was genotype 2a (found in 33 [72%] of 46 patients), which was identical to the genotype found in the suspected source of infection. The prevalence of genotype 2a in the unrelated group was significantly lower (found in 1 [7%] of 14 subjects; $P < .01$). In contrast, the difference in the number

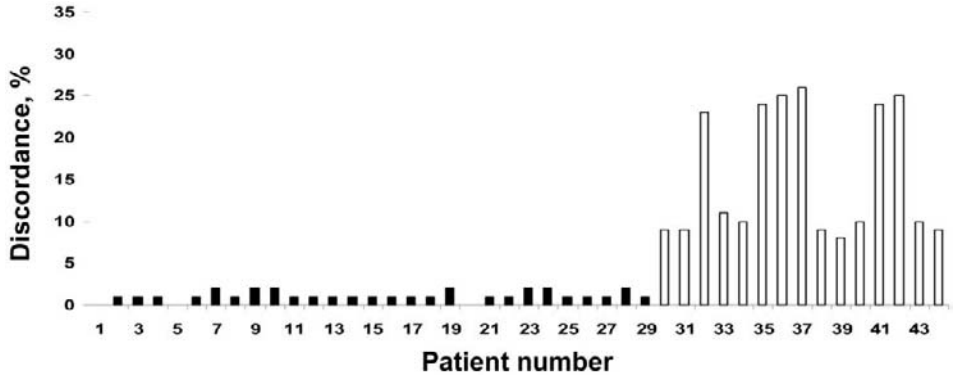

Figure 4. Sequence analysis of the hepatitis C virus (HCV) E2 hypervariable and double-stranded RNA–activated protein kinase region (E2 HVR2-PKR). The E2 HVR2-PKR region was sequenced and the genetic distances between the sequence from patient 1 and the sequences from related patients (patients 2–29; *black columns*) and unrelated patients (patients 30–44; *white columns*), all of whom were infected with genotype 2a HCV, were calculated as percentage of discordance of nucleotides from patient 1.

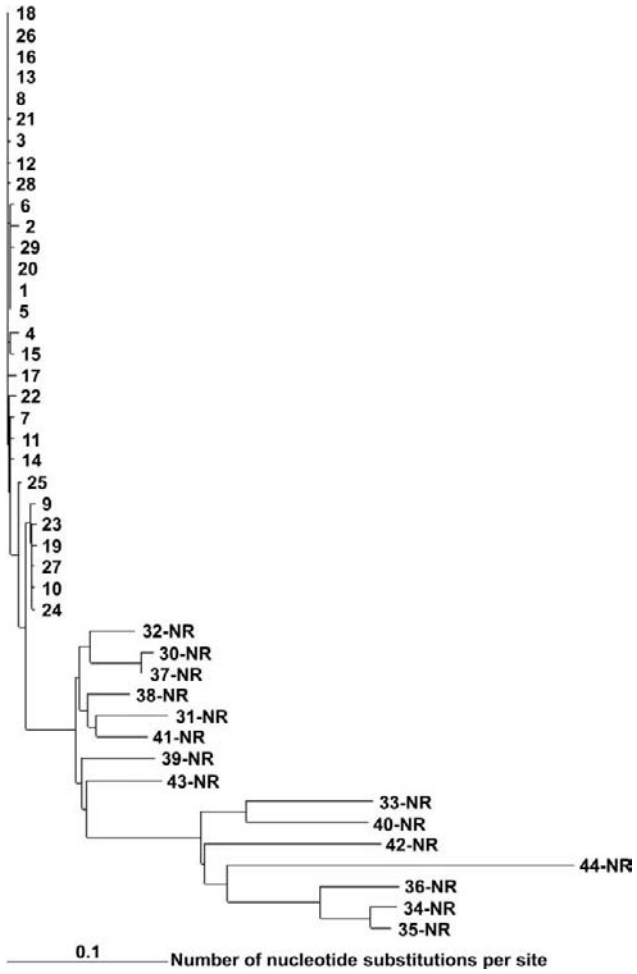

Figure 5. Phylogram tree showing the rate of variation of nucleotides (hepatitis C virus [HCV] E2 hypervariable and double-stranded RNA–activated protein kinase region) within the outbreak patients (patients 1–29) and the unrelated control subjects (NR-30 to NR-44); patients with HCV infection were infected with genotype 2a/c HCV before 2001.

of patients with results that were positive for genotype 1 HCV in the 2 populations was insignificant. In the related group with genotype 2a HCV infection, the surgical procedure undergone at the time of HCV acquisition varied and included gynecological (14 patients), orthopedic and trauma (8), general surgical (8), and other procedures (neurosurgery, plastic surgery, and a urological procedure; 1 patient each). The interval between the surgical procedure and screening was a mean ($\pm$SD) of 14 $\pm$ 4 months (range, 4–20 months).

Table 2 depicts the analysis of HCV genotype distribution in patients from the related group only, according to the laboratory records that were available (from 1996 and after). Genotype 2a HCV was found exclusively in the newly identified patients with HCV infection, whereas patients with HCV infection that had been recorded before the 2001 outbreak had genotypes 1a, 1b, and 3a.

Analysis of HVR2 in the genotype 2a HCV–infected population. To establish the transmission of HCV between the source of infection and the infected patients, sequence analysis of the HVR1 and HVR2-PKR regions of E2 was performed. Sequence analysis of the HVR2-PKR region was performed as part of the epidemiological study by the Clinical Virology Laboratory at Soroka Medical Center. Two groups of patients were analyzed for the 660-nucleotide HVR2-PKR region. The first group comprised HCV carriers with genotype 2a who were enrolled as a result of screening. Sequences from 28 of 33 patients (patients 2–29) were obtained (figure 4). The negative control group comprised 15 genotype 2a HCV carriers who had been infected before 2001 or patients who were discovered in 2003 but were not treated by the infected health care provider (patients 30–44) (figure 4). All sequences were aligned with the sequence obtained from the source of infection (patient 1). Although patients who were associated with the HCV outbreak showed a $\leqslant$2% difference, the control group exhibited differences ranging from 9% to 35% ($P < .003$). Pairwise analysis for the E2 fragment nucleotide sequences for the genotype 2a HCV cohort that had most nonsynonymous substitutions were concentrated in the control group (figure 5).

Sequence analysis of the HVR1 region was performed as a service to the Israeli state prosecution by the molecular hepatology laboratory at the Rabin Medical Center. The analysis was performed on 6 new serum samples that were collected from 6 patients in the related group and on a serum sample from the infected health care provider. Both laboratories performed the analysis independently, and HCV RNA was extracted from different blood samples. Thus, 6 patients were tested in parallel for the HVR1 and HVR2-PKR regions of HCV. HCV RNA was isolated, amplified, and analyzed for the sequence diversity of the HVR1 and HVR2 regions. We used a panel of sequences of HCV isolates retrieved from the gene bank as controls. The HCV sequences were aligned with the isolated HCV sequence from the serum sample obtained from the anesthesiologist. The genetic distance differences between the sequences isolated from the related group serum samples and those retrieved from the gene bank were highly statistically significant (figure 6). The genetic distances were 1.4%–4.4% in the HVR1 region and 0%–2% in the HVR2-PKR region in the related group serum samples, whereas in the control group serum samples, they were 22%–32% and 10%–35%, respectively. It is of interest that the sequence homology, determined in the various isolates from the related group infected serum samples, was conserved both in the HVR1 (95.6%–98.6%) and HVR2 (98%–100%) regions.

DISCUSSION

Of 1200 patients who were at risk of iatrogenic HCV infection during a period of 2 years (the related group), 46 (3.8%) were

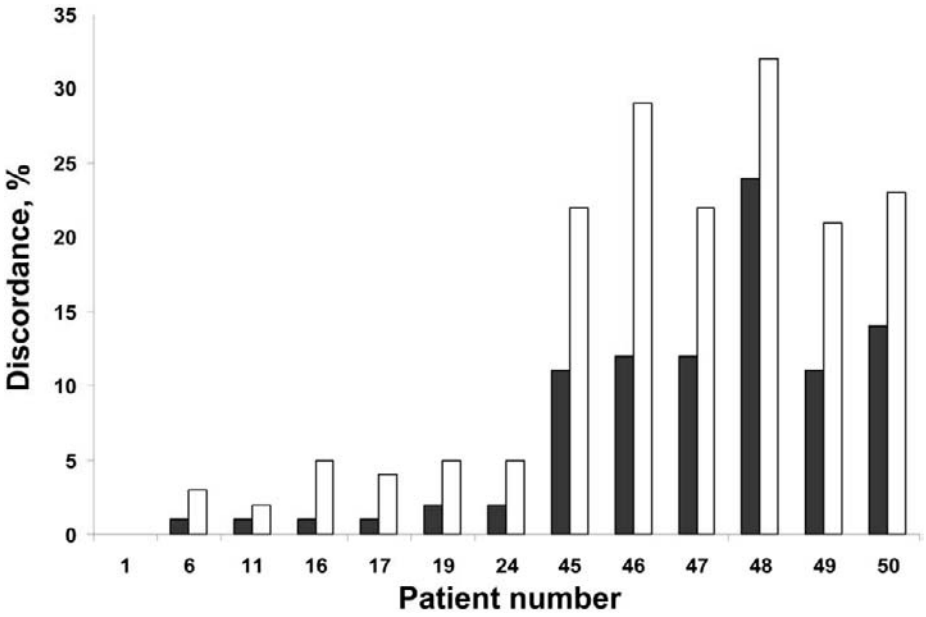

Figure 6. Nucleotide distance from the source of infection for hepatitis C virus (HCV) E1 hypervariable region (HVR1) and HCV E2 hypervariable and double-stranded RNA–activated protein kinase region (HVR2-PKR) . Nucleotide sequence differences of HVR1 and HVR2-PKR regions, obtained from patients in the related group (patients 1, 6, 11, 17, 19, and 24), and sequences retrieved from the gene bank (patients 45–50; accessions numbers: HC-J6CH_E2_AF177036, Q2A_E2_AF348702, Td-6_E2_D00944, BEBE1_E2_D50409, MD2A-2_E2_AF238482, and AY746460_E2_ AY746460, respectively) were compared with the sequences of HVR1 *(white columns)* and HVR2-PKR *(black columns)* regions that were obtained from the source of infection.

found to be infected with HCV. Of 873 individuals in the unrelated group, 27 (1.5%) were infected with HCV. This prevalence is higher than the previously described HCV antibody prevalence of 0.44% in Israeli blood donors [19]. In the unrelated group, genotype 1 was most frequent (found in 64% of subjects), similar to the previously reported prevalence of HCV genotypes detected in the same region in Israel [20, 21]. It is noteworthy that only 1 case of genotype 2a/c HCV infection was found in this unrelated group. In the related group, however, 71% of HCV-positive patients were infected with genotype 2a HCV. The fact that genotype 2a, which is an infrequent genotype in Israel [20], was detected in the serum samples from the infected health care provider and was detected almost exclusively in serum samples from the related group supported our theory that the source of the outbreak was the anesthesiologist. This was further established by the comparative molecular analysis of HVR1 and HVR2-PKR regions of the HCV genome. Evaluation of relatedness among samples of genotype 2a HCV was performed by comparing HVR1 and HVR2 sequences isolated from the related group serum samples with a panel of controls, including genotype 2a HCV–infected serum samples from unrelated patients, as well as sequences retrieved from the database. Pairwise analysis was performed using multiple sequence–alignment programs to determine the genetic distance, expressed as divergence of nucleotide substitutions per 100 nucleotide sites [18]. The genetic distances were 1.4%–4.4% in the HVR1 and 0%–2% in the HVR2 in the related group serum samples, whereas in the control group, they were 22%–32% and 10%–35%, respectively. It is of interest that, in the related group, the sequence homology was conserved both in the HVR1 (95.6%–98.6%) and HVR2 (98%–100%) regions. This study compares the sequence variability of both HVR1 and HVR2 in an outbreak of HCV infection.

Previously reported comparisons of nucleotide distance variation among related and nonrelated serum samples (for HVR1 of HCV genotypes 1a, 2b, and 3) demonstrated 0%–5% of nucleotide distance in the related groups, compared with 6.1%–93% in the nonrelated group [11, 12, 14]. These figures are in agreement with our results, confirming the detection of the source of the HCV infection outbreak as the anesthesiologist.

We could not perform an accurate analysis of the rates of evolution of the nucleotide sequence of genotype 2a during HCV infection, as was shown for genotype 1b [16]. However, it should be emphasized that most serum samples collected from patients associated with the outbreak were obtained at the same period, including the samples obtained from the source of the outbreak. The time period between acute HCV infection and serum sample collection was variable, ranging from 3.5 to 20 months. Most patients had been infected for >1 year, with a mean ($\pm$SD) interval of 14 $\pm$ 1.9 months. However, no apparent correlation was found between the time of infection and the rate of nucleotide variability. For example, 2 samples obtained from patients 5 and 20 and the sample obtained from the infectious source (patient 1) had the same

nucleotide sequence. However, patient 20 had been infected for only 3.5 months, whereas patient 5 had been infected for 12 months.

The means by which HCV outbreaks occur and spread vary. According to the Centers for Disease Control and Prevention, the major source of HCV infection (accounting for 60% of cases) is injection drug users. Occupational hazards account for 4% of cases, the source of infection is unknown in 10%, and nosocomial, iatrogenic, and perinatal infections account for 1% (reviewed by Shepard et al. [22]). Various paths of HCV transmission in medical settings have been described. Patient-to-patient transmission of HCV during medical procedures [7, 9, 23] and through HCV-contaminated intravenous anesthetic ampoules used to treat multiple patients have been described [24]. Another source of iatrogenic transmission is from health care provider to patient [12, 13, 25], as well as from patient to health care provider [15]. An HCV infection outbreak in 2 hospitals in Valencia, Spain, was reported [26]. That report and unpublished data mentioned in a recent publication [27] describe HCV infection in >200 patients, attributed to an anesthetist who was an HCV carrier and an intravenous morphine addict for many years. Our molecular studies provide evidence that an anesthesiologist who was an injection drug user infected with genotype 2a HCV transmitted HCV infection to 33 patients. During a period of 2 years, 1400 patients were at risk, and 33 were genuinely infected by the source. The low viral load (10^5 copies/mL) detected in the anesthesiologist's serum samples might explain the relatively small number of infected patients. The outbreak of HCV infection described here demonstrates the severe consequences of an anesthesiologist who is an active injection drug user having access to anesthetic ampoules for intravenous use.

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Potential conflicts of interest. All authors: no conflicts.

References

1. Wasley A, Alter MJ. Epidemiology of hepatitis C: geographic differences and temporal trends. Semin Liver Dis **2000**; 20:1–16.
2. Okuda K, Hayashi H, Kobayashi S, Irie Y. Mode of hepatitis C infection not associated with blood transfusion among chronic hemodialysis patients. J Hepatol **1995**; 23:28–31.
3. Sanchez-Tapias JM. Nosocomial transmission of hepatitis C virus. J Hepatol **1999**; 31(Suppl 1):107–12.
4. Simmonds P, Smith DB, McOmish F, et al. Identification of genotypes of hepatitis C virus by sequence comparisons in the core, E1 and NS-5 regions. J Gen Virol **1994**; 75(Pt 5):1053–61.
5. Bruguera M, Saiz J-C, Franco S, et al. Outbreak of nosocomial hepatitis C virus infection resolved by genetic analysis of HCV RNA. J Clin Microbiol **2002**; 40:4363–6.
6. de Ledinghen V, Trimoulet P, Mannant PR, et al. Outbreak of hepatitis C virus infection during sclerotherapy of varicose veins: long-term follow-up of 196 patients (4535 patient-years). J Hepatol **2007**; 46: 19–25.
7. Massari M, Petrosillo N, Ippolito G, et al. Transmission of hepatitis C virus in a gynecological surgery setting. J Clin Microbiol **2001**; 39: 2860–3.
8. Heinsen A, Bendtsen F, Fomsgaard A. A phylogenetic analysis elucidating a case of patient-to-patient transmission of hepatitis C virus during surgery. J Hosp Infect **2000**; 46:309–13.
9. Lesourd F, Izopet J, Mervan C, et al. Transmissions of hepatitis C virus during the ancillary procedures for assisted conception. Hum Reprod **2000**; 15:1083–5.
10. Rispeter K, Lu M, Behrens SE, Fumiko C, Yoshida T, Roggendorf M. Hepatitis C virus variability: sequence analysis of an isolate after 10 years of chronic infection. Virus Genes **2000**; 21:179–88.
11. Cody SH, Nainan OV, Garfein RS, et al. Hepatitis C virus transmission from an anesthesiologist to a patient. Arch Intern Med **2002**; 162: 345–50.
12. Ross RS, Viazov S, Roggendorf M. Phylogenetic analysis indicates transmission of hepatitis C virus from an infected orthopedic surgeon to a patient. J Med Virol **2002**; 66:461–7.
13. Ross RS, Viazov S, Thormahlen M, et al. Risk of hepatitis C virus transmission from an infected gynecologist to patients: results of a 7-year retrospective investigation. Arch Intern Med **2002**; 162:805–10.
14. Esteban JI, Gomez J, Martell M, et al. Transmission of hepatitis C virus by a cardiac surgeon. N Engl J Med **1996**; 334:555–60.
15. Ross RS, Viazov S, Gross T, Hofmann F, Seipp HM, Roggendorf M. Transmission of hepatitis C virus from a patient to an anesthesiology assistant to five patients. N Engl J Med **2000**; 343:1851–4.
16. Duffy M, Salemi M, Sheehy N, et al. Comparative rates of nucleotide sequence variation in the hypervariable region of E1/E2 and the NS5b region of hepatitis C virus in patients with a spectrum of liver disease resulting from a common source of infection. Virology **2002**; 301: 354–64.
17. Sandres K, Dubois M, Pasquier C, et al. Genetic heterogeneity of hypervariable region 1 of the hepatitis C virus (HCV) genome and sensitivity of HCV to alpha interferon therapy. J Virol **2000**; 74:661–8.
18. Thompson JD, Higgins DG, Gibson TJ. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res **1994**; 22:4673–80.
19. Stern M, Manny N, Klein A, Naparstek Y, Shraga S, Tur-Kaspa R. A study of Israeli blood donors, asymptomatic carriers of hepatitis C virus, in relation to liver. Isr J Med Sci **1995**; 31:277–9.
20. Shemer-Avni Y, el Astal Z, Kemper O, et al. Hepatitis C virus infection and genotypes in Southern Israel and the Gaza Strip. J Med Virol **1998**; 56:230–3.
21. Hanuka N, Sikuler E, Tovbin D, et al. Hepatitis C virus infection in renal failure patients in the absence of anti-hepatitis C virus antibodies. J Viral Hepat **2002**; 9:141–5.
22. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis **2005**; 5:558–67.
23. Kozarek RA. Transmission of hepatitis C virus during colonoscopy. N Engl J Med **1997**; 337:1848–9.
24. Tallis GF, Ryan GM, Lambert SB, et al. Evidence of patient-to-patient transmission of hepatitis C virus through contaminated intravenous anaesthetic ampoules. J Viral Hepat **2003**; 10:234–9.
25. Ross RS, Viazov S, Roggendorf M. Risk of hepatitis C transmission from infected medical staff to patients: model-based calculations for surgical settings. Arch Intern Med **2000**; 160:2313–6.
26. Bosch X. Spanish anaesthetist infects patients with hepatitis C. Brit Med J **1998**; 316:1625.
27. Wróbel B, Torres-Puente M, Jiménez N, et al. Analysis of the over-dispersed clock in the short-term evolution of hepatitis C virus: using the E1/E2 gene sequences to infer infection dates in a single source outbreak. Mol Biol Evol **2006**; 23:1242–53.