The Low Risk of Hepatitis C Virus Transmission Among Sexual Partners of Hepatitis C-Infected Hemophilic Males: An International, Multicenter Study

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To study the transmission rate of hepatitis C virus (HCV) in the female sexual partners of antibody-positive hemophilic males, 106 partners from three hemophilia centers located in Europe, America, and Australia were tested for HCV seropositivity using a first-generation enzyme-linked immunosorbent assay (ELISA-1) and, subsequently, a second-generation ELISA (ELISA-2) and a supplemental recombinant immunoblot assay. Additionally, the cohort was tested for the presence of antibody to the human immunodeficiency virus type-1 and hepatitis B virus markers. No female partner was HCV antibody-positive using the ELISA-1 test, whereas five were seropositive by the ELISA-2 test. Three of these five female partners were seropositive on the supplemental test, the remaining two having indeterminate results, for an overall prevalence of 2.7%. Thus, even with the use of sensitive testing, the prevalence of HCV infection remains low in this cohort, showing that the efficiency of heterosexual transmission of HCV is poor.

THE HEPATITIS C VIRUS (HCV) shares many of the epidemiologic characteristics with the hepatitis B virus (HBV) and the human immunodeficiency virus (HIV-1), both of which can be sexually transmitted. Before HCV serology was available, the results of a case-controlled study performed in the United States showed that individuals with multiple heterosexual partners had a greater risk for non-A non-B hepatitis (NANBH) than those who were less sexually active.[1] However, more recent serologic surveys performed with anti-HCV assays in heterosexual partners of infected individuals, patients with sexually transmitted diseases, and sexual partners of male homosexuals indicated that HCV was not efficiently transmitted by the sexual route,[2-6] although one study showed an increased transmission to the female if the male was coinfected with HIV-1.[6] One possible explanation for these discrepancies is that first-generation assays for anti-HCV used in these studies were not sensitive enough to detect all cases of HCV infection.[7] In general, the diagnostic sensitivity of second-generation assays based on viral polypeptides of both structural and nonstructural regions of HCV appears to be superior to that of first generation assays.[8-10] Recent studies, for example, have shown a 14% increased detection rate of HCV infection in blood donors using the second-generation serologic tests.[11,12]

It has been known since the early 1970s that hemophilia patients who frequently infused coagulation factor concentrates manufactured from pooled plasma had intermittently elevated liver aminotransferases. These abnormalities were attributed mainly to NANBH.[13,14] With the cloning of the HCV genome and the subsequent development of serologic tests for HCV, hemophilic cohorts were tested for the prevalence of HCV antibody.[15,16] Cohort studies have shown that using the first-generation anti-HCV c-100 enzyme-linked immunosorbent assay (ELISA) at least 80% of hemophilic patients are positive.[17-19] By posttransfusion follow-up studies, seropositivity seems to indicate ongoing infection with the virus and not immunity.[20]

Because a high percentage of males with hemophilia are infected with HCV, their heterosexual partners represent a cohort in which to study the sexual transmission of HCV. Thus, 106 female sexual partners of multitransfused anti-HCV–positive hemophiliacs from three hemophilia centers in Australia, Italy, and the United States were tested for the presence of anti-HCV using first- and second-generation immunoassays.

PATIENTS AND METHODS

Patients. One hundred and six long-term female sexual partners (38 from Australia, 35 from Italy, and 33 from the United States) of anti-HCV–positive hemophilic males were tested for the presence of HCV antibodies, antibody to HIV-1, and antibodies to HBV after informed consent was obtained. These pairs were chosen because they had all been in a relatively long relationship with the anti-HCV–positive hemophilic males (Table 1). All the males had infused factor concentrate before virally inactivated materials became available in 1985/1986. Two hemophilic males but no female partner received the hepatitis B vaccine. Only one female partner (described under Results) related previous high-risk behavior as the sexual partner of an intravenous drug user, although she denied drug use herself. The demographics of the group are summarized in Table 1.

Serologic testing. Three immunoassays were used to detect anti-HCV in thawed sera frozen at −20°C. All sera were tested by both first- and second-generation ELISA for anti-HCV (Ortho HCV ELISA test system, Raritan, NJ). Sera reactive for anti-HCV in either assay were further tested by a second-generation immunoblot assay (recombinant immunoblot assay [RIBA] HCV, second-generation assay; Chiron Corporation, Emeryville, CA).

The first-generation ELISA detects antibodies directed against the dominant nonstructural viral epitope of HCV, produced by yeast clones as a fusion protein (c100-3) between the c100 polypeptide of HCV and human superoxide dismutase (SOD). The

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Submitted January 2, 1992; accepted March 16, 1992.

Supported in part by US Public Service Grant No. MCI-252002-01 (D.B.B.).

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0006-4971/92/8002-0009$3.00/0

Table 1. Demographics of the Female Partners (n = 106)

Median age	36.6 yr (range, 18-73.5 yr)
Median length of exposure to HCV-positive hemophiliac	147 mo (range, 5- > 240 mo)
Hepatitis B serology	
HBsAg positive	0/66*
Anti-HBc positive	6/106 (5.7%)
Anti-HBs positive	6/66* (9.1%)
Anti-HBs and anti-HBc positive	5/66
HIV serology	
Anti-HIV-1 positive	4/66† (6.2%)

*Female partners (n = 40) not tested.

†Denominator represents only the partnerships where the hemophilic male was anti-HIV-1 positive. All 106 hemophiliacs were tested.

second-generation ELISA detects antibodies directed against the nonstructural SOD fusion polypeptide c200, which incorporates the nonstructural peptides c33c and c100-3, and the structural SOD fusion peptide c22-3 of HCV.[9,10] The second-generation RIBA detects antibodies directed against five recombinant antigens, namely, 5-1-1 polypeptide of HCV, which is produced in *Escherichia coli* clones of HCV as a fusion protein with SOD; the SOD fusion protein c100-3; the c33c peptide produced in *E coli;* the c22-3 peptide produced in yeast; and the SOD protein itself, which is in the RIBA as a control. Sera reacting against any two antigens except the SOD protein were called reactive. Sera reacting against any one antigen were called indeterminate, while those not reacting against any antigen or to SOD were called nonreactive. All ELISA-positive sera were additionally tested with the RIBA assay.

HIV-1 testing was performed using an ELISA method (Abbott, North Chicago, IL) and confirmed with Western Blot. HBV serology was tested with commercially available radioimmunoassays (Abbott).

RESULTS

As shown in Table 2, among the 106 female sexual partners of anti-HCV–positive male hemophilic patients, none were reactive for anti-HCV by the first-generation ELISA, and five were positive by the second-generation test. Analysis by RIBA showed confirmed positivity in three samples for a prevalence rate of 2.7%.

The three females who were anti-HCV–positive in both the ELISA-2 and the RIBA had other possible risk factors. One from the United States was a past partner of an intravenous drug abuser, although she denied intravenous drug use herself. She currently is asymptomatic on antiretroviral therapy with persistently normal aminotransferases.

Table 2. HCV Reactivity in the Five ELISA HCV-Positive Female Partners

Partner	ELISA 1st-Gen	ELISA 2nd-Gen	RIBA 2nd-Gen 5-1-1	RIBA 2nd-Gen c100-3	RIBA 2nd-Gen c33c	RIBA 2nd-Gen c22	Overall Results
1 (US)	Neg	Pos	Neg	Neg	2+	2+	Pos
2 (Italy)	Neg	Pos	Neg	Neg	1+	3+	Pos
3 (Italy)	Neg	Pos	Neg	Neg	1+	4+	Pos
4 (Italy)	Neg	Pos	Neg	Neg	Neg	1+	Ind
5 (Australia)	Neg	Pos	Neg	Neg	Neg	3+	Ind

Abbreviations: Neg, negative; Pos, positive; Ind, indeterminate.

The second was a nurse in a geriatric unit and the third had an episode of jaundice in 1956 and blood transfusions in 1976. The latter two, both from Italy, also had normal aminotransferases. None of the three anti-HCV–positive females had positive HBV serology, but one was HIV-1 seropositive (partner no. 1 in Table 2). Of the hemophilic male partners of these three anti-HCV–positive females, all were anti-hepatitis B surface (HBs) positive from previous exposure to factor concentrate containing HBV, and one was anti–HIV-1 positive. These three couples used condoms either not at all or seldomly. The length of the partnerships were 36 months, 47 months, and 504 months, respectively.

In this cohort, four female partners of 66 HIV-1–seropositive hemophilic males were found to be HIV-1 positive (6.1%), including one anti-HCV–positive female. As seen in Table 3, the presence of HCV seropositivity in the female partner did not correlate with the presence of HIV-1/HCV coinfection in the hemophilic male. The difference between the number of females that were infected with HIV-1 (6.1%) and HCV (2.7%) was not significant by the Fisher exact test.

As shown in Table 1, no female partners were found to be carriers of HBsAg, although 5.7% were positive for antibody to hepatitis B core (HBc) antigen, and 9.1% were positive for antibody to HBsAg. It is noteworthy that of the six females that were anti-HBc and/or anti-HBs positive, their six male partners also had positive anti-HBc and/or anti-HBs serology. One of these males (from Italy) had received the hepatitis B vaccine.

DISCUSSION

Our study shows that the prevalence of HCV infection in a high-risk cohort of female partners of hemophilic males infected with HCV is 2.7%, similar to that reported by Eyster et al.[6] This is four to six times the reported prevalence in blood donors from Australia, Italy, and the United States.[21-23] Reports from the Transfusion Safety Study Group and the National Cancer Institute Multicenter Study of AIDS in the United States found that only a small proportion (less than 3.5%) of the heterosexual partners of hemophiliacs had been infected by HCV despite long contact with the index cases.[6,24] One of these studies used the second-generation ELISA or more sensitive RIBA tests,[6] the other did not.[24] Similar studies were also performed among sexual partners of HCV-seropositive drug addicts and patients with NANB chronic hepatitis and similar conclusions were reached.[2,3,19] Because the HCV transmission rate was small in our study group, any compar-

Table 3. HIV and HCV Serology in the Hemophilic Males and HCV Serology in Their Female Partners

Anti-HCV–Positive Hemophilic Males	Female Partners* HCV+	Female Partners* HCV−
Anti-HIV–negative (n = 40)	2	38
Anti-HIV–positive (n = 66)	1	65

*Differences not significant by Fisher exact test.

ison of sexual habits between the anti-HCV–negative and –positive couples is difficult. While the three couples in whom heterosexual transmission of HCV may have occurred practiced protected intercourse rarely, many other couples in whom the female was anti-HCV negative also did not use condoms frequently.

Our study shows that with the increased diagnostic capability of the newer serologic assays for anti-HCV, an increased prevalence may be found in high-risk cohorts. However, the prevalence of HCV-infected partners of hemophilic index patients still remained low, indicating that HCV was not efficiently transmitted by the heterosexual route and perhaps less effectively than other sexually transmissible viruses such as HIV-1 and HBV. A similar conclusion was reached by Tor et al.[3] Considering that all three female partners with anti-HCV investigated by us may have had other risk factors for acquiring HCV in addition to sexual activity with HCV-infected persons, the role of heterosexual transmission of HCV in this setting could be even smaller than it appears.

The low prevalence occurred even though the rate of anti–HIV-1 coexisting with anti-HCV in the hemophiliac patients was high (62.2%), a condition thought to facilitate the sexual transmission of HCV.[3,6,24] Contrary to the observations of Eyster et al,[6] two of three cases of confirmed HCV seropositivity in the female partners occurred in the absence of HIV seropositivity in the male partner. Hence, we could not show that coinfection of HIV and HCV in the hemophiliac patient was likely to be associated with transmission of HCV to the female partners.

Why in this setting HCV was not efficiently transmitted by sexual route is difficult to explain. Perhaps the intensive education that this cohort has undergone concerning the importance of protected intercourse to avoid HIV transmission may have aided in decreasing transmission rates of HCV. Certainly in counselling HCV-seropositive patients and their sexual partners the importance of condom use should be advised until more information concerning HCV sexual transmission is obtained.

ACKNOWLEDGMENT

The authors thank Margaret Arpano for her technical help and Mary Robichaud for her valued assistance in the preparation of the manuscript.

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