Sexual Transmission of Hepatitis C Virus among Patients Attending Sexually Transmitted Diseases Clinics in Baltimore—An Analysis of 309 Sex Partnerships

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The prevalence of antibodies to hepatitis C virus (anti-HCV), the behavioral and laboratory-derived risk factors for anti-HCV, and the quantity and homology of HCV RNA were assessed among 1039 non–injection drug–using sexually transmitted disease (STD) patients representing 309 sex partnerships. Thirty-seven (7%) of 555 males and 19 (4%) of 484 females had anti-HCV. In logistic regression analyses, factors associated with anti-HCV included age ($P < .001$), greater numbers of lifetime sex partners ($P = .023$), human immunodeficiency virus infection ($P < .001$), *Trichomonas* infection ($P < .001$), cigarette smoking ($P < .001$), and male homosexual exposure ($P = .012$). Among couples, females whose sex partners were anti-HCV positive were 3.7 times more likely to have anti-HCV than females whose sex partners were anti-HCV negative ($P = .039$). The proportion of RNA homology between anti-HCV positive females and their male partners (94%) was higher than among randomly selected patients (82%). Sexual transmission of HCV may contribute to the high prevalence of anti-HCV reported in urban settings.

Hepatitis C virus (HCV) is the principal cause of non-A, non-B hepatitis worldwide [1–4]. In the United States, antibodies to HCV are found in <1% of blood donors, compared with 70%–90% of injection drug users, 50%–80% of hemophiliacs, and 6%–15% of patients attending sexually transmitted disease (STD) clinics [5–10]. Parenteral transmission (e.g., through blood transfusions or contaminated needles) of HCV is well established and accounts for the high rates of HCV among injection drug users and hemophiliacs. However, no parenteral exposure is recognized in ~50% of HCV cases [2].

In one study of community-acquired non-A, non-B hepatitis, cases lacking parenteral exposure to HCV were 11 times more likely than controls to have multiple sex partners [11]. This observation and the high prevalence of HCV found in patients attending STD clinics [8], prostitutes [12], male homosexuals [13], and sex partners of persons infected with both HCV and human immunodeficiency virus (HIV) [14] suggest that sexual transmission of HCV may occur. However, studies of monogamous sex partners of HCV-infected transfusion recipients have revealed little or no HCV transmission [15–19]. Thus, the extent to which sexual transmission accounts for cases of HCV with no parenteral exposures remains controversial.

Previously, we observed a high prevalence of anti-HCV among non–injection drug–using patients attending STD clinics in Baltimore [9]. However, in that investigation the anti-HCV status of sex partners was not assessed and information on possible risk factors was limited. In addition, since the completion of that study, more sensitive and specific assays for anti-HCV have been developed, and methods of tracking HCV transmission through comparison of viral nucleotide sequences and methods for quantifying HCV RNA in sera have been described [20–23]. In this investigation, we used these techniques to examine the evidence for sexual transmission of HCV in 309 sex partnerships representing 1039 patients attending Baltimore STD clinics, a population previously found to be at high risk for HCV and for whom information on potential cofactors was carefully collected.

Methods

From 1990 to 1992, 1330 patients attending STD clinics in Baltimore were recruited into a prospective study of the transmission and acquisition of genital ulcers and other sexually transmitted diseases [24, 25]. After enrollment, an extensive behavioral questionnaire was administered to subjects by study clinicians who also performed detailed physical examinations, including cultures of genital swabs, and serum collection. The

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questionnaire measured sexual risk factors, including the number of lifetime sex partners, age of first intercourse, condom use, and history of exchange of sex for money or drugs. Substance abuse was assessed with questions about substances used (e.g., alcohol, heroin, cocaine, cigarettes, or marijuana), the route of use (e.g., intravenous, other percutaneous, intranasal, or oral), the proximity of use ("ever" compared with "in the past 30 days"), and the frequency of use (for cigarettes and alcohol only). Patients were also asked to identify sex partners in the preceding 30 and 90 days. Sex partners were then confidentially contacted by study personnel, offered enrollment in the study, and assessed in the same manner. For this study, a partnership was defined as 2 patients of different gender (>95% of patients were heterosexual) who both acknowledged sexual intercourse at least once in the 90 days before their visits to the STD clinic.

Laboratory methods. Laboratory evaluation included cultures of genital swabs for *Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis*, and herpes simplex (for lesions) and serology for HCV, HIV, syphilis, and herpes simplex virus (by Western blot, as described by Ashley et al. [26]). Blood specimens were collected in the clinics and transported to a central laboratory, processed, and stored at −70°C within 7 h of collection. Testing for anti-HCV was done by a commercially available second-generation EIA (Ortho Diagnostics, Raritan, NJ); serum specimens repeatedly reactive by EIA were retested using a second-generation recombinant immunoblot assay (RIBA) (Ortho Diagnostics). Participants were considered anti-HCV positive if their sera were repeatedly reactive for anti-HCV on EIA and positive for anti-HCV by RIBA.

HCV RNA was detected by reverse transcriptase polymerase chain reaction (RT-PCR). For this procedure, HCV RNA was isolated from 200 μL of serum using 800 μL of a guanidine thiocyanate-, phenol-, and sarkosyl-containing lysis solution followed by extraction with chloroform. The aqueous phase was precipitated with 2-propanol and dissolved in 100 μL of water containing RNasin. Fifty microliters of the solution, 100 μL serum equivalent, was used in the nested RT-PCR. The nucleotide sequence corresponding to the 5′ noncoding region of HCV-1 was used to select primers for amplification. The outer primer set was 25 mer, NF5, 5′-GTGAGGAACTACTGTCTTCACGCAG-3′ (sense), and 25 mer, NR5, 5′-TGCTCATGGTGCACGGTCTACGAGA-3′ (antisense), while the inner primer set was 20 mer, KF2, 5′-TTCACGCAGAAAGCGTCTAG-3′ (sense), and 21 mer, NR4, 5′-CTATCAGGCAGTACCACAAGG-3′ (antisense).

The antisense primer of the outer set also served as primer for the RT-PCR reaction. One-tenth of the first PCR product was subjected to the second amplification, and 20 μL of the second reaction mixture was examined by ethidium bromide staining after agarose gel electrophoresis. Duplicate samples of the same extract were tested for all specimens. Any specimens with discrepant reactivities between two tests were retested. A specimen was considered positive when two of the four tests reacted positively. As positive and negative controls, three 10-fold serial dilutions of known positive and negative serum specimens were extracted in each assay along with experimental samples. The solutions from these extractions were then amplified by RT-PCR along with 1 additional specimen previously extracted from a known negative source. The test was considered invalid if any 1 of the negative controls was reactive or if either of the 2 samples with the highest concentration of HCV RNA was unreactive.

To quantify HCV in sera, a prototype branched (b) DNA signal amplification assay (provided by Chiron, Emeryville, CA) was used as described by Lau et al. [22]. The sandwich assay with signal amplification using bDNA is based on specific hybridization of viral RNA in the sample by synthetic oligonucleotides to the highly conserved 5′-untranslated region and core gene of HCV, which are immobilized on the surface of a microwell plate. Synthetic bDNA amplifier molecules and multiple copies of an alkaline-phosphatase–linked probe are hybridized to the immobilized complex, which is incubated with a chemiluminescent substrate. Light emission is measured and, since the signal is proportionate to the amount of HCV RNA, the quantity is determined from a standard curve. Each serum specimen was run in duplicate, and if the coefficient of variation was >20%, the assay was repeated. Specimens RT-PCR positive but negative on bDNA were considered to have $<50 \times 10^3$ Eq/mL of HCV RNA. Specimens RT-PCR negative and bDNA negative were considered to have no HCV RNA.

To compare the dominant HCV strains in sex partners, the nucleotide sequence encompassing the hypervariable domain in the junction between E1 and NS1/E2 region was directly sequenced after PCR. The outer primer set, CS2, 5′-CACGAATTCGGGGCTGGGAGTGAAGCAAT-3′ (antisense) and CS3, 5′-GGTAAGCTTATGGCATGGGATATGATGAT-3′ (sense), was used in the first round of PCR. The inner primer set, CS7, 5′-GTCCTGGCGGGCATAGCGTAT-3′ (sense) and CS8, 5′-CTCGGGACAGCCCGAAGAGTT-3′ (antisense), was used in the second round of PCR. The nested PCR product had 300 bases and was sequenced bidirectionally two times to assure consistency. The nucleic acid sequences of the PCR product were determined using an automated DNA sequencer (ABI 373A, Foster City, CA) and compared with sequencer software (Genecodes, Ann Arbor, MI). The percentage of RNA homology was calculated as the ratio of the number of nucleic acid discrepancies per number of base sequences aligned (between 200 and 230).

A commercially available EIA (Organon Teknika, Charleston, SC) was used to analyze serum samples for antibodies to HIV. Serum samples that were reactive on two EIAs were confirmed by Western blot analysis (Du Pont NEN, Wilmington, DE). Serum specimens were considered positive if two of the following three viral protein bands were present: p24, gp41, or gp120/160.

Statistical analysis. Information about demographic characteristics, STDs, and sexual behavior was analyzed and compared with the results of serologic tests. Data were analyzed separately for each sex because of prior information suggesting that sexual transmission of HCV to females may be more efficient than to males [9]. The variables (age, frequency of alcohol use, and number of lifetime sex partners) were categorized into three levels representing the upper, lower, and middle two quartiles. χ^2 tests (or Fisher's exact test when indicated) were used to evaluate associations of demographic and risk-behavior information with anti-HCV status in univariate analysis. Descriptive meth-

ods were used to compare the distributions of quantities of HCV RNA and the percent homology of RNA nucleic acid sequences, since small numbers of observations precluded application of parametric tests.

Risk factors associated with anti-HCV status on univariate analysis were further examined using logistic regression. Since the anti-HCV risk factors identified for males and females in the univariate analysis were similar and to minimize data sparseness, males and females were considered together in multivariate analysis. Multivariate analyses were also done separately for males and females and did not reveal significant differences (data not shown). With anti-HCV as the outcome and each of the factors associated with anti-HCV on univariate analysis included as covariates, logistic regression was done using a backward stepwise procedure with a probability inclusion criteria of 0.1. For this model, the continuous variables (age and number of lifetime sex partners) were divided into 2 groups representing the upper quartile at highest risk for anti-HCV and the remaining participants. The potential contribution of covariates to the model was also assessed by comparing the reduction in the −2 log likelihood to a χ^2 with the appropriate degrees of freedom. Interactions among covariates were evaluated by χ^2 analysis.

Because of a strong correlation between HIV in males and homosexual exposure, the adjusted estimate of the strength of the association of homosexual exposure with anti-HCV were calculated from a model inclusive of all other variates except HIV infection. Pregnancy and the frequency of alcohol use were not considered biologically meaningful, and serologic results positive for syphilis were intimately associated with HIV status. Thus, these factors were not included in the logistic model. PC SAS 6.04 software (SAS, Carey, NC) was used for all calculations. To assess for a possible lack of independence between observations created because of the inclusion of partnerships, the correlations of variates with anti-HCV were also calculated after adjustment for intrapartnership correlation: No significant differences ($P > .01$) were found.

Results

Study population. Between 1 November 1990 and 30 June 1992, 1330 patients were enrolled; 156 (86% of whom were anti-HCV positive) acknowledged a history of injection drug use and were excluded from further analyses of anti-HCV correlates. An additional 135 persons were not assessed because of missing information on injection drug use ($n = 9$), HCV status ($n = 72$), or sex practices ($n = 54$).

Anti-HCV was detected in 7% and 4% of non–injection drug–using males and females, respectively. To explore the basis for the high prevalence of anti-HCV observed among patients with no parenteral exposures, we ascertained the association of anti-HCV with the number of lifetime sex partners, history of sexually transmitted diseases, sexual behavior, nonparenteral substance abuse, and laboratory-derived markers of other infectious diseases (including HIV, syphilis, gonorrhea, herpes, chlamydia, and trichomonas).

Male participants. Males represented 53% of the study population and, compared with females, had a higher prevalence of anti-HCV ($P = .05$), anti-HIV ($P = .002$), and syphilis ($P = .006$). Males were also more likely than females ($P < .001$) to have >45 lifetime sex partners (highest quartile) and to have a history of crack cocaine use. Among males, anti-HCV was associated with older age ($P < .001$), a history of *Trichomonas* ($P = .002$) or syphilis ($P = .046$) infections, homosexual exposures ($P = .003$), and anal-receptive sex ($P = .03$; table 1). Anti-HCV was also more prevalent in males reporting >45 lifetime sex partners (the highest quartile; $P < .001$), in those who smoked cigarettes ($P < .001$) but not marijuana ($P = .978$), and in males acknowledging alcohol use more than once a week ($P = .006$). In addition, serologic evidence of syphilis ($P = .016$) and HIV infections ($P < .001$) was associated with anti-HCV.

Female participants. Females comprised 47% of the study population. As with males, anti-HCV in females was associated with increasing age ($P < .001$), greater numbers of lifetime heterosexual partners ($P < .001$), cigarette smoking ($P = .003$), and more than weekly use of alcohol ($P < .001$; table 2). Associations of anti-HCV with a history of pregnancy ($P = .03$) and with laboratory evidence of vaginal trichomonas infection ($P < .001$) were also noted.

Factors not significantly associated with anti-HCV in males or females included laboratory and clinical evidence of genital herpes, *Chlamydia*, and gonococcal infections, age of first intercourse, and history of crack cocaine use. Histories of exchanging sex for money or drugs were also associated with anti-HCV among females before we removed patients with a history of injection drug use from the analysis.

Multivariate analysis. To further explore the associations of various risk factors with anti-HCV, a logistic regression model was used. In this analysis, anti-HCV was associated with older age ($P < .001$), greater numbers of lifetime sex partners ($P = .023$), *Trichomonas* ($P < .001$) and HIV ($P < .001$) infections, and cigarette smoking ($P < .001$; table 3). In this model, the associations of sex and male homosexuality became nonsignificant. The association of male homosexual exposure with anti-HCV became nonsignificant when HIV infection was included in the model, since 24 (63.2%) of 38 males with homosexual exposure were HIV seropositive ($P < .001$). The logistic regression was repeated without HIV in the model, and a history of male homosexual exposure remained significantly associated with anti-HCV ($P = .012$).

Sex partners. To better explore the potential for sexual transmission of HCV, 309 discrete heterosexual partnerships were identified among the 1039 STD patients. These partnerships consisted of 295 males and 301 females and included 19 (51.3%) of the 37 anti-HCV–positive males and 12 (63.1%) of the anti-HCV–positive females from the total study population. Fourteen males and 8 females had 2 sex partners. Because of the strong association of anti-HCV with injection drug use, a partnership was excluded when the patient being considered for possible sexual transmission of

Table 1. Univariate analysis of risk factors associated with anti-HCV among 555 non–injection drug–using males attending STD clinics in Baltimore, 1990–1992.

Risk factor	No. (%)	OR (95% CI)	*P*
Age, years			
13–21	167 —	—	—
22–30	264 (6.4)	—	—
>30	124 (16.1)	—	<.001
STD history			
Trichomonas			
No	510 (5.7)	1.0	—
Yes	45 (17.8)	3.6 (1.6–8.0)	.002
Syphilis			
No	500 (6.0)	1.0	—
Yes	53 (13.2)	2.4 (1.0–5.6)	.046
Gonorrhea			
No	247 (4.9)	1.0	—
Yes	306 (7.8)	1.7 (0.8–3.4)	.158
Nongonococcal urethritis			
No	387 (6.7)	1.0	—
Yes	159 (5.7)	0.8 (0.4–1.8)	.647
Lifetime sex partners			
1–10	171 (3.5)	—	—
11–45	248 (5.6)	—	—
>45	136 (12.5)	—	.001
Cigarette smoking history			
No	216 (1.8)	1.0	—
Yes	339 (9.7)	5.7 (2.2–14.7)	<.001
Marijuana smoking history			
No	436 (6.6)	1.0	—
Yes	119 (6.7)	1.0 (0.5–2.2)	.978
Alcohol use			
Less than monthly	212 (4.2)	—	—
Intermediate	139 (4.3)	—	—
More than once a week	204 (10.8)	—	.006
Sex with males			
No	517 (5.8)	1.0	—
Yes	38 (18.4)	3.6 (1.6–8.5)	.003
Anal-receptive sex			
No	498 (6.0)	1.0	—
Yes	15 (13.3)	2.3 (1.1–4.7)	.03
Laboratory evaluation			
Syphilis			
Negative	465 (5.6)	1.0	—
Positive	67 (13.4)	2.6 (1.2–5.7)	.016
HIV			
Negative	482 (5.4)	1.0	—
Positive	37 (21.6)	4.8 (2.2–10.0)	<.001

NOTE. Except as indicated, data are no. positive (% positive) for anti-HCV. OR = odds ratio, CI = confidence interval, HIV = human immunodeficiency virus. Continuous variables (age, no. of sex partners, alcohol use) were categorized into upper and lower quartiles and statistical comparisons made using Cochran-Mantel-Haenszel χ^2 test. Difference between total for each characteristic and 555 represents missing data.

Table 2. Univariate analysis of risk factors associated with anti-HCV among 484 non–injection drug–using females attending STD clinics in Baltimore, 1990–1992.

Risk factor	No. (%)	OR (95% CI)	*P*
Age, years			
13–19	152 —	—	—
20–28	223 (4.5)	—	—
>28	109 (8.3)	—	<.001
STD history			
Trichomonas			
No	299 (3.0)	1.0	—
Yes	178 (5.6)	1.9 (0.8–4.7)	.159
Syphilis			
No	463 (3.9)	1.0	—
Yes	18 (5.7)	1.4 (0.2–11.4)	.722
Gonorrhea			
No	296 (3.7)	1.0	—
Yes	186 (4.3)	1.2 (0.5–2.9)	.748
Lifetime sex partners			
1–5	237 (1.7)	—	—
6–20	204 (4.4)	—	—
>20	43 (14.0)	—	<.001
Cigarette smoking history			
No	211 (0.9)	1.0	—
Yes	273 (6.2)	6.9 (1.9–25.0)	.003
Marijuana smoking history			
No	416 (3.8)	1.0	—
Yes	68 (4.4)	1.1 (0.3–4.1)	.824
Alcohol use			
Less than monthly	239 (2.1)	—	—
Intermediate	141 (2.1)	—	—
More than once per week	104 (10.6)	—	<.001
Ever pregnant			
No	132 (0.8)	1.0	—
Yes	352 (5.1)	7.2 (1.3–41.0)	.03
Laboratory evaluation			
Syphilis			
Negative	432 (3.7)	1.0	—
Positive	34 (8.8)	2.5 (0.7–8.8)	.15
Seropositive HIV			
Negative	453 (3.5)	1.0	—
Positive	13 (7.7)	2.4 (0.3–18.2)	.38
Trichomonas culture			
Negative	373 (2.1)	1.0	—
Positive	96 (11.4)	5.8 (2.5–13.4)	<.001

NOTE. Except as indicated, data are no. positive (% positive) for anti-HCV. OR = odds ratio, CI = confidence interval, HIV = human immunodeficiency virus. Difference between total for each characteristic and 484 represents missing data. Continuous variables (age, number of sex partners, alcohol use) were categorized into upper and lower quartiles and statistical comparisons made using Cochran-Mantel-Haenszel χ^2 test.

HCV had a history of injection drug use. First, partnerships in which the male had a history of injection drug use were excluded. The remaining male patients whose female sex partners were anti-HCV negative had a prevalence of anti-HCV (7.7%) similar to that in males whose sex partners were anti-HCV positive (7.1%; table 4). Then, the partnerships in which the female patient had a history of injection drug use were excluded from the original 309. The remaining females whose male sex partners were anti-HCV positive were 3.7 times more likely to have anti-HCV (10.2%) than those whose sexual partners were anti-HCV negative (3.0%; P = .039).

Table 3. Multivariate analysis of risk factors associated with anti-HCV among patients attending STD clinics in Baltimore, 1990–1992.

Risk factor	Adjusted OR	Adjusted CI	Adjusted *P*
Age >28 years	3.8	2.1–6.8	<.001
More than 24 lifetime sex partners	1.9	1.1–3.5	.023
HIV infection	4.4	1.9–10.3	<.001
Trichomonas infection	3.3	1.7–6.3	<.001
Cigarette smoking	4.5	1.9–10.8	<.001

NOTE. OR = odds ratio, CI = confidence interval. Age groups and no. of lifetime sex partners are divided into quartile at highest risk for anti-HCV and remaining patients. 69 patients (5 anti-HCV positives) were not included because of missing information on one covariate. In recognition of similarities in male and female anti-HCV risk factors identified in univariate analysis and to minimize data sparseness, males and females were considered together in multivariate analysis. Multivariate analyses were also done for males and females separately and did not reveal meaningfully different results (data not shown). Because of strong interaction between HIV and homosexual exposures, values for homosexual exposures are calculated from model inclusive of all other variates shown except HIV infection. Alcohol use was closely associated with smoking ($P < .001$) and was not included in this model. In another model, inclusion of alcohol use did not change reported associations, except to decrease level of significance (an overadjustment) for smoking ($P = .005$).

HCV RNA testing. To further explore evidence that HCV was sexually transmitted from males to females, the presence, quantity, and sequence homology of HCV RNA was assessed in the 5 partnerships in which sexual transmission was presumed and compared with those without apparent sexual transmission. These partnerships represent the five instances when a non–injection drug–using female patient and her sex partner were anti-HCV positive (table 4). HCV RNA was detected by RT-PCR in 4 of 5 non–injection drug–using anti-HCV–positive females and all of their 5 male sex partners compared with 7 of 10 males who did not transmit HCV to their female partners. The mean titers of HCV RNA were slightly higher by bDNA analysis in the 5 males who appeared to transmit HCV to their female partners (2.5×10^6 Eq/mL) compared with the 10 males who failed to transmit HCV to their female partners (1.1×10^6 Eq/mL). However, there was overlap among these values.

The degree of homology of HCV RNA was assessed in 3 HCV-positive sex partnerships and compared with 10 randomly created pairs of HCV-positive males who were attending the clinic during the same time but were not sex partners. The 10 control partnerships were constructed by randomly selecting 10 anti-HCV–positive males from the list of 43 who did not appear to transmit HCV to their sex partners, sequencing HCV RNA from the sera, and comparing 10 randomly created pairs from these males. The mean percentage of RNA homology was higher in the sex partners (94.4%) than in the randomly selected male pairs (82.4%), with very little overlap in the distribution.

Discussion

The basis for the higher prevalence of anti-HCV found in inner-city populations in the United States is unclear. In Baltimore, anti-HCV has been reported among 18% of patients attending the Johns Hopkins Emergency Department [27], 15% of patients attending the STD clinics [9], 9% of females attending the Johns Hopkins prenatal clinics [28], and 88% of participants in a study of injection drug use [6]. Injection drug use is a proven route of HCV transmission and contributes to the increased prevalences of anti-HCV. However, even after excluding injection drug users, the rate of anti-HCV was 13.3% among persons attending the Johns Hopkins Emergency Department and 9.6% among patients visiting STD clinics.

More than 50% of persons infected with HCV will develop chronic liver disease, which may progress to cirrhosis or hepatocellular carcinoma [10, 29, 30]. Understanding the basis for transmission of HCV in urban populations in the United States is essential to reduce the morbidity and mortality attributed to this disease. This investigation provides some evidence that sexual transmission of HCV occurs among patients attending the Baltimore STD clinics. Females with anti-HCV–positive sex partners were 3.7 times more likely to have anti-HCV than females with anti-HCV–negative sex partners. In addition, the similarities of the nucleic acid sequences of these females and their male partners suggest a common source of HCV, such as would be expected with sexual transmission [23, 31]. Several of the risk factors associated with anti-HCV in this population were also consistent with sexual transmission of the virus. Anti-HCV was more frequent in male and female patients who had greater numbers of sex partners (even after adjusting for age and other associated factors), and anti-HCV was associated with other sexually transmitted diseases, such as HIV and *Trichomonas* infections.

Studies that have found little or no transmission of HCV

Table 4. Anti-HCV status of non–injection drug–using sexually transmitted disease patients by the anti-HCV status of their heterosexual sex partners, Baltimore, 1990–1992.

	Contact				
Patients	Positive	Negative	Total	OR*	*P*
Males					
Positive	1 (7.1)	13 (92.9)	14	1.0	—
Negative	18 (7.7)	214 (92.3)	232	0.9	>0.1
Females					
Positive	5 (10.2)	44 (89.8)	49	3.7	.039
Negative	7 (3.0)	227 (97)	234	1.0	—

NOTE. Except as indicated, data are no. (%).

* Odds ratio (OR) represents relative risk of non–injection drug–using patients being anti-HCV positive depending on anti-HCV status of their heterosexual partners.

to sex partners differ from ours in that they evaluated long-term, monogamous sex relationships [15–19]. Though it is difficult to make direct comparisons, it is reasonable to assume that the number of sex partners, the prevalence of other sexually transmitted diseases, and perhaps even the frequency and types of sexual activity would be different in STD patients than in hemophiliacs and transfusion recipients and their spouses. If the sexual transmission of HCV, like HIV and hepatitis B, is enhanced in the presence of genital ulceration or other STDs, then the more frequent occurrence of these conditions in STD patients may explain the greater estimate of sexual transmission derived from studies in this setting [32–35]. In addition, since sexual transmission of HCV in any setting appears to be infrequent, a large number of partnerships must be studied to demonstrate features indicative of transmission. For example, in one study of sexual transmission of HCV among sex partners in California, no association of sexual behavior with anti-HCV was found [18]. However, since only 3 cases of anti-HCV appeared to have been sexually acquired, the statistical power to detect such associations would be limited. Likewise, a study of another STD patient population in San Francisco failed to demonstrate evidence of sexual transmission of HCV in its analysis of risk factors [8]. However, in the San Francisco STD study, sex partnerships were not assessed, and there were only 43 cases of HCV in non–injection drug–using patients, suggesting that the investigation might have limited power to find such associations.

Ninety-five percent of patients in our investigation were African-American. Although racial differences in the prevalence of hepatitis B and HCV have been reported in the United States, the basis for the increased prevalence of viral hepatitis in minority populations is unknown [8, 27, 36, 37]. Because of the ethnic homogeneity of our study group, associations of anti-HCV with race or ethnicity could not be made. In addition, the results of our study may not be completely generalizable to populations of different racial compositions.

Males whose sex partners were anti-HCV positive were not more likely to have anti-HCV than those whose sex partners were anti-HCV negative. This observation may mean that female-to-male transmission of HCV is less efficient than from male to female, as previously shown for gonococcal, *Chlamydia*, and HIV infections [38–41]. However, because of the cross-sectional study design, the direction of transmission can only be inferred. In addition, another unmeasured risk factor may account for some of the HCV cases in males. In this study, males not only had a higher rate of anti-HCV, but also had a higher prevalence of HIV, syphilis, and risk behaviors, such as smoking and crack cocaine use. Collectively, these factors may represent an unmeasured HCV risk factor, such as unacknowledged injection drug use, and explain why for males an association with the HCV status of the sex partner was not found.

For many STDs, such as HIV and hepatitis B, increased prevalence rates are found in males with past homosexual exposure [42–45]. However, only modestly increased rates of HCV have been found in other studies of male homosexuals [6, 13, 46–48]. In this investigation, anti-HCV was found in 18% of males with past homosexual exposure; these males were 3.6 times more likely to have anti-HCV than male STD patients with only heterosexual partners. Although this association became nonsignificant when HIV was included in the multivariate model, because of the interrelatedness of male homosexual exposure and HIV, this most likely represents an overadjustment [49]. In a separate logistic regression model inclusive of all factors except HIV, homosexual exposures remained associated with anti-HCV.

Other studies of HCV transmission have also compared RNA sequence homologies [23, 50]. Although homologies $\leq 100\%$ have been reported, methods of making these comparisons are not standardized. The proportion of homology would be affected by the locus of the genome amplified, and thus, may not be readily compared between different studies.

The quantities of HCV RNA in this study were somewhat higher in the male partners of the females who appeared to have acquired HCV sexually (that is, were not injection drug users and had sex partners who were anti-HCV positive) compared with the male partners of females who did not appear to acquire the infection sexually (females who were anti-HCV negative but had anti-HCV–positive male partners). However, there were only 15 partnerships assessed and there was considerable overlap in the range of values for each group. Recently, in another study the quantity of HCV RNA did appear to correlate with transmission of HCV from mothers to their infants [51]. Although there was also some overlap in the range of values in the perinatal transmission study, the differences in that study were statistically significant. Additional studies are needed to delineate the importance of quartiles of HCV RNA in virus transmission.

In this investigation, cigarette smoking was associated with anti-HCV. Cigarette smoking has been linked with other infectious diseases, including HIV, and biologically plausible mechanisms have been proposed to explain these observations [52–54]. In addition, cigarette smoking may reflect other high-risk behaviors. For HCV, the most important risk behavior to consider is unacknowledged parenteral drug use. In this study, information on drug use was obtained in a nonjudgmental way by an interviewer with a carefully designed questionnaire, which explicitly measured for injection drug use. However, in this study unreported drug use could have contributed to the high prevalence of anti-HCV and its association with factors such as cigarette smoking. In addition, the data on sex partners were limited by the possible sexual contacts between patients and persons other than their stated sex partner, a limitation not shared by studies of long-term monogamous sex relationships, and by the fact that patients were considered sex partners even if sexual contact was rare.

Nonetheless, because of the high prevalence of anti-HCV among non-injection drug-using STD patients, the association of anti-HCV with having a greater number of sex partners and, for females, with having an anti-HCV-positive male partner, and the similarities in HCV RNA sequences between HCV-positive females and their sex partners, we do not believe that unacknowledged injection drug use fully explains the high prevalence of anti-HCV observed in the STD population. Rather, in this setting, sexual transmission of HCV appears to contribute to the high rates of HCV. Prospective studies of sex partners attending STD clinics and of other high-risk urban populations are needed to further clarify the high rates of HCV found among inner city residents in the United States.

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