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Journal

Biological research for nursing, 4(1)

ISSN

1099-8004

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Publication Date

2002-07-01

DOI

10.1177/1099800402004001004

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Presence and Predictors of Hepatitis C Virus RNA in the Semen of Homeless Men

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Although the possibility of sexual transmission of the hepatitis C virus (HCV) remains controversial, little is known of the associations of positive semen specimens with potential demographic and behavioral risk factors. Knowledge of these predictors may suggest factors that increase risk of HCV RNA in the semen. Semen and blood from 80 HCV-infected homeless men were evaluated for the presence of HCV RNA by means of branch DNA and transcription-mediated amplification analyses. Associations of selected demographic and behavioral characteristics of the participants with presence or absence of HCV in their semen were also assessed. HCV RNA was detected in the semen of 36% of the sample. Associations were found with HCV RNA in semen and older age, higher viral loads of HCV in blood, current alcohol and lifetime methamphetamine use, and having been vaccinated for the hepatitis B virus. Findings suggest that sexual transmission of HCV is plausible and shed light on the need to conduct more in-depth investigations.

Key words: hepatitis C, homeless men, semen

Although parenteral transmission of the hepatitis C virus (HCV) is well established (Des Jarlais and Schuchat 2001; Hagan and others 2001), the possibility of sexual transmission of HCV remains controver-

sial (Bresters and others 1993; Osmond and others 1993; Ward and others 2000). Data from several studies indicate no parenteral exposure in 30% to 40% of all HCV cases (McLindon and others 1995; Semprini and others 1998). Furthermore, several researchers have reported that HCV in noninjection drug users was related to sex with multiple partners; having exchanged

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sex for drugs, shelter, or money; duration of paid sex; and sexually transmitted disease (STD) infection (Nakashima and others 1992; Xi-Ping and others 1995; Hershow and others 1998).

A number of recent studies have reported the presence of HCV RNA in seminal fluid, thus providing a biological basis for sexual transmission. Prevalence of HCV RNA in the semen has ranged from low (less than 1%) (Fiore and others 1995) to high (38%) (Liou and others 1992; Leruez-Ville and others 2000). However, a fair number of studies have detected no HCV RNA in the semen of HCV-infected individuals (Hsu and others 1991; Fried and others 1992; Semprini and others 1998). Conflicting data may be explained in part by differences in the collection and/or storage of semen specimens prior to analysis (Levy and others 2000). For example, it has been conjectured that during the time between specimen collection, freezing, and subsequent RNA extraction, enzymes, such as proteases or lipases present in human secretions, may destroy the HCV virus and degrade the genome, thus destroying the template necessary for polymerase chain reaction amplification (Hsu and others 1991; Fiore and others 1995). In addition, researchers have found that previous qualitative and quantitative assays for HCV RNA may have been poorly standardized in terms of reaction, condition, primer sequences, and the use of controls in the study design (Ackerman and others 1998).

Only a few studies examined associations of positive semen specimens with potential demographic and behavioral risk factors (e.g., age, ethnicity, substance use). Caldwell and others (1996) reported that 12.5% of their sample had HCV RNA in their semen; however, no association was found between positive semen and HCV viral load in the blood. Fiore and others (1995) found that 1 of 11 patients had HCV RNA in their semen, but this was not related to HCV RNA in the blood, CD4 cell count, stage of HCV infection, or extent of liver damage. On the other hand, Leruez-Ville and others (2000) reported that patients with HCV RNA in their semen (38%) had higher median blood viral loads than those who were HCV RNA-negative. Knowledge of these predictors is important, as it may suggest factors that increase risk of HCV RNA being detected in the semen, and possibly increase the potential of sexual transmission of HCV to uninfected sexual partners.

In this study, we measured HCV RNA in semen and plasma samples from 80 HCV-infected homeless men by means of branch DNA (bDNA) and transcription mediated amplification (TMA) analysis. We also assessed the associations of selected demographic and behavioral characteristics of the participants with presence or absence of HCV in their semen.

Methods

Subjects and Setting

The sample for this study originally consisted of 106 homeless men who were referred from the John Wesley Community Health Medical Clinic in the Skid Row area of Los Angeles by medical care providers who were aware of the eligibility criteria for the study. Moreover, a flyer announcing the study was posted in the medical clinic. These men resided in shelters or hotels nearby. One hundred six of these men were eligible for the study: men were 18 to 65 years of age, possessed verifiable evidence of being HCV-positive by laboratory and medical record data (often based on high liver enzyme results), agreed to provide a semen and blood sample on site, and completed a 15-min questionnaire detailing potential demographic, biologic, and behavioral risk factors for HCV transmission. Men with a history of vasectomy and men who were judged incoherent or actively hallucinating by the study nurses were excluded. Two of the 106 (2%) eligible volunteers could not provide a semen specimen, and their data were excluded. Informed consent was obtained from all subjects participating in the study. Informed consents were reviewed and approved by the University of California, Los Angeles, Medical Institutional Review Board.

Procedures

After the study was explained and written informed consent was obtained, a study nurse screened potential participants to be sure they met the eligibility requirements. All eligible participants were then asked to collect a semen specimen privately, by masturbation, using standard collection methods (World Health Organization 1992). Semen specimens were frozen on dry ice immediately post collection. After semen specimen

collection, 10 mL of ethylenediamine-tetraacetic acid (EDTA)-preserved blood was collected. Subsequently, each man completed an interviewer-administered 15-min structured questionnaire. The participants were paid for providing the specimens and completing the survey and questionnaire. Semen specimens were preserved at -70°C on dry ice until HCV testing was performed; these and blood specimens were transported to the laboratory daily for batch analysis.

Paired semen and plasma specimens were analyzable for 80 of the 104 men sampled. The bDNA procedure requires serum or plasma, although TMA can be run on either cells or plasma. To accommodate running bDNA on every blood and semen sample, semen samples were allowed to liquefy on wet ice and then spun for 3 min at 2500 g at 4°C in an Allegra 64R centrifuge (Beckman Coulter, Fullerton, CA) to separate seminal plasma from cells so that testing could be performed on seminal plasma. However, only 6 semen specimens were found to be bDNA-positive (quantitative assay) compared to 29 semen specimens that were TMA-positive (nonquantitative assay). The small number of bDNA-positive semen specimens limited the ability to test for quantitative viral loads in blood versus semen.

Measures

Sociodemographic Characteristics

Information collected by the structured questionnaire included age, sex, race, education, veteran status, relationship status, country of birth, history of incarceration, health status, history of homelessness, drug treatment history, and length of time in the United States.

Biological Variables

Quantification of HCV RNA in blood plasma and semen was conducted according to standard operating procedures developed by Specialty Labs in Santa Monica, California. The Versant HCV RNA 3.0 bDNA assay was used for blood and semen specimens. This assay is a signal amplification nucleic acid probe technique for the direct quantification of HCV RNA and has been proven to be sensitive in blood plasma and serum for detecting a range of 521 to 8.3×10^6 HCV IU/mL (Germer and Zein 2001). In addition, to detect even lower amounts of HCV RNA, blood that

was negative using bDNA and all semen specimens were analyzed using VERSANT HCV-RNA qualitative assay based on TMA (Bayer Diagnostics, Emoryville, CA). This method is capable of detecting down to 5 IU/mL at 100% sensitivity (Ross and others 2001).

Nonsexual Behavioral Variables

Drug and alcohol use were measured by the revised Texas Christian University (TCU) Drug History Form (Simpson and Chatham 1995). This questionnaire has been tested with men and women with a history of drug addiction, prostitution, and homelessness. It records the frequency of use of 16 drugs by injection, or other means, during the past 6 months and also elicits information about lifetime use. Drugs assessed are heroin, street methadone, other opiates, cocaine, crack, methamphetamine and other amphetamines, inhalants, marijuana/hashish, hallucinogens, tranquilizers, barbiturates, other sedatives, designer drugs, and alcohol and nicotine. The CAGE questionnaire was administered to assess persons with a high likelihood of alcohol dependence or abuse. Injection drug use was defined as any use of drugs by injection, regardless of frequency, during the past 6 months (recent) and lifetime. Objective measures of drug use were not obtained, as they only provide relatively short-term evidence of drug use. Furthermore, our research team collected hair samples in a previous study of homeless women and found reasonable concurrence between self-report and objective evidence of cocaine use (Nyamathi and others 2001). Other behavioral risk factors that were assessed include sharing needles and syringes (works) and accessories, tattooing, piercing, and sharing personal hygiene implements, such as toothbrushes and razors.

Sexual Behavior

Participants' sexual behavior was assessed by a 13-item Sexual Activity Questionnaire, developed by Temoshok (1986), that measures types and frequency of sexual activity, particularly as it relates to protected and unprotected activity lifetime and during the past 6 months. Sexual risk behaviors included trading sex for money, nonuse of condoms, partner shooting drugs, vaginal sex, sexual activity in the past 6 months, and

Table 1. Associations between Key Characteristics and Finding Hepatitis C Virus (HCV) RNA in the Semen

	HCV-Positive Semen (<i>n</i> = 29)		HCV-Negative Semen (<i>n</i> = 51)		<i>P</i> Value
	Mean	<i>SD</i>	Mean	<i>SD</i>	
Sociodemographic factors					
Age	48.3	7.1	45.5	5.9	0.058
Education	12.2	1.4	12.2	2.3	0.998
Biological factor					
HCV viral load in blood	2,886,276	2,576,055	1,662,257	2,879,015	0.001 ^a

a. Based on a *t* test for group differences in means of log-transformed viral load.

lifetime STDs. Nature of partners, length of current relationships, and lifetime number of male and female sexual partners were also assessed.

Data Analysis

Differences in categorical sociodemographic and behavioral characteristics between those who did and did not have evidence of HCV RNA in their semen samples were examined with chi-square and Fisher exact tests. Differences between the 2 groups in continuous variables were assessed with independent sample *t* tests. Serum levels of HCV were log transformed for analysis, although untransformed values are presented in Table 1. To identify independent predictors of detecting HCV RNA in semen, stepwise backward logistic regression analysis was conducted using variables in Tables 1 to 3 that were associated with the outcome at the 0.10 level. The *P* value for retention was also set at 0.10.

Results

Presence of HCV RNA in Homeless Men

Paired semen and plasma specimens were analyzable for 80 of 104 men sampled. Of these men, HCV RNA was detected in the semen of 29 of 80 (36%) of the sample. Twenty four specimens were excluded as a result of clotted blood (4), HCV RNA–negative blood plasma (10), and unmanageability or indetermination due to the immediate freezing and subsequent failure

Table 2. Prevalence of Hepatitis C Virus (HCV) RNA in Semen as a Function of Major Sociodemographic and Biological Characteristics

	HCV-Positive Semen		<i>P</i> Value
	<i>n</i>	% ^a	
Sociodemographic factors			
Race			0.343
Black	14	30.4	
White	8	50.0	
Hispanic	7	41.2	
Veteran			0.183
Yes	17	43.6	
No	12	29.3	
Lifetime jail			0.264
Yes	27	35.1	
No	2	66.7	
Biological factor			
HIV-positive (self-report)			0.703
Yes	3	42.9	
No	26	35.6	

NOTE: The *P* value represents the significance of comparisons between HCV-positive and HCV-negative men on each of the factors.

a. Percentage of those with the factor who were positive (e.g., 30.4% of black participants had HCV-positive semen).

to liquefy, excessive foam, viscosity, or other characteristics of the semen (10).

Overall, for this sample of 80 men, the mean age was 46 ± 6.5 years and the mean educational attainment was 12 years (Table 1). Homeless men who had evidence of HCV RNA in their semen were found to be older than those who did not. Furthermore, virus load in blood plasma was associated positively with HCV RNA in the semen. As displayed in Table 2, no significant relationships were found between HCV RNA in the semen

Table 3. Prevalence of Hepatitis C Virus (HCV) RNA in Semen as a Function of Selected Substance Use and Behavioral Characteristics

	HCV-Positive Semen		<i>P</i> Value
	<i>n</i>	% ^a	
Drug-related risk factors			
Share needles			0.429
Yes	18	40.0	
No	11	31.4	
Share straws			0.555
Yes	10	32.3	
No	19	38.8	
Other risk behaviors			
Share toothbrushes			0.230
Yes	6	26.1	
No	23	40.4	
Tattoo			0.104
Yes	11	27.5	
No	18	45.0	
Sexual risk behaviors			
Trade sex for money			0.162
Yes	9	27.3	
No	20	42.6	
Use condom with nonregular partners			0.241
None of the time	8	32.0	
A little of the time	1	14.3	
Some of the time	2	25.0	
Most of the time	1	16.7	
All of the time	11	52.4	
Partner shot drugs			0.278
Yes	15	42.9	
No	14	31.1	
Vaginal sex			0.224
Yes	12	26.7	
No	3	50.0	
Sexually active past 6 months			0.060
Yes	15	28.9	
No	14	50.0	
More than 20 lifetime partners			0.028
Yes	12	26.1	
No	17	50.0	
Lifetime sexually transmitted disease			0.408
Yes	12	31.6	
No	17	40.5	
Lifetime alcohol			0.682
Yes	28	35.9	
No	1	50.0	
Alcohol past 6 months			0.013
≥ 2 times per week	18	51.4	
< 2 times per week	11	24.4	
Lifetime barbiturates			0.044
Yes	11	55.0	
No	18	30.0	

Table 3. Continued

	HCV-Positive Semen		<i>P</i> Value
	<i>n</i>	% ^a	
Lifetime cocaine			0.038
Yes	25	43.1	
No	4	18.2	
Lifetime crack			0.543
Yes	21	34.4	
No	8	42.1	
Lifetime methadone			0.018
Yes	7	70.0	
No	22	31.4	
Lifetime methamphetamine			0.006
Yes	15	57.7	
No	14	25.9	
Lifetime other amphetamines			0.046
Yes	12	52.2	
No	16	28.6	
Crack past 6 months			0.981
Yes	13	36.1	
No	16	34.4	

NOTE: The *P* value represents the significance of comparisons between HCV-positive and HCV-negative men on each of the factors. a. Percentage of those with the factor who were positive (e.g., 40% of those who share needles had HCV-positive semen).

and race/ethnicity, veteran status, or HIV-positive status. Furthermore, education and history of homelessness were not related to testing positive for HCV in the semen.

Drug Use and Sexual Risk Variables

Homeless men who were lifetime users of methamphetamine were more likely to have HCV RNA detected in their semen than their counterparts who did not use this particular drug (Table 3). Men who used barbiturates, cocaine, and methadone were also more likely to have HCV RNA detected in their semen compared to men who did not use these drugs. These findings were not demonstrated for lifetime noninjection drug users or heroin users. Homeless men who reported alcohol use at least twice a week in the past 6 months were more likely to have HCV RNA detected in their semen than those who drank less often. Lifetime alcohol revealed a similar, but somewhat attenuated, pattern. Other potentially unsafe parenteral risk factors, such as sharing needles, razors, straws, or toothbrushes, and having tattoos, were not found to be

Table 4. Logistic Regression Analysis of Factors Predicting Detection of Hepatitis C Virus (HCV) RNA in the Semen of Homeless Men (*n* = 80)

Variable	Semen HCV-Positive Unadjusted Odds Ratios (<i>n</i> = 80)			Semen HCV-Positive Adjusted Odds Ratios (<i>n</i> = 80)		
	Odds Ratio	95% Confidence Interval	<i>P</i>	Odds Ratio	95% Confidence Interval	<i>P</i>
Age	1.07	0.99–1.16	0.039	1.16	1.04–1.30	0.010
Lifetime methamphetamine use	3.90	1.45–10.46	0.007	10.24	2.30–45.64	0.002
Viral load	2.04	1.29–3.22	0.002	1.96	1.14–3.39	0.016
Alcohol past 6 months ^a	3.27	1.27–8.46	0.014	6.41	1.68–24.41	0.007
Sexually active past 6 months	0.41	0.16–1.05	0.064	0.24	0.06–0.92	0.037

a. Used alcohol at least twice a week during the previous 6 months.

associated with having evidence of HCV RNA in the semen.

In terms of sexual behaviors, only 1 important association was found. Men with 20 or more lifetime partners were less likely to have HCV RNA in their semen than those with fewer partners (Table 3).

Biological and Health History Factors

Homeless men who had HCV RNA in their semen had substantially higher blood levels of HCV compared to those not found to have HCV RNA in their semen. In particular, homeless men who tested positive for HCV RNA in their semen had mean and median HCV blood levels of 2,886,276 and 1,800,000 IU/mL, respectively. In contrast, those who tested negative had mean and median HCV blood levels of 1,662,257 and 791,500 IU/mL, respectively. Men who had completed a hepatitis B virus (HBV) vaccine series were also more likely to have HCV RNA in their semen than homeless men who had not completed this series.

Multivariable Analyses

From these analyses, our model showed that age, lifetime methamphetamine use, HCV plasma virus load, and frequent recent alcohol use were associated positively with evidence of HCV in the semen (Table 4). For example, men who used methamphetamine in their lifetime were more than 10 times as likely as nonusers to test positive for HCV RNA in semen, and men who used alcohol at least twice a week in the past 6 months were 6 times as likely to test positive for HCV RNA as men who drank less often. However,

lifetime number of sex partners was found to be associated negatively with HCV RNA in semen.

Discussion

Scientific and clinical literature reports a range of HCV-positivity in semen from low to no positives (Hsu and others 1991; Semprini and others 1998) to those reporting a high prevalence of HCV RNA in the semen of HCV-infected persons (Liu and others 1994; Leruez-Ville and others 2000). Our study, conducted with homeless men who were validated by laboratory or medical chart data to be HCV positive, revealed one of the highest rates of HCV in the semen, 36%. Rapid freezing and storage of semen plus choice of TMA and bDNA laboratory assays contributed to the successful detection of HCV RNA in the semen in the current study. The finding of 36% positive semen in the present study, as well as the findings of other investigators, provides a first step toward identifying a biological basis for sexual transmission of HCV.

Although investigators have been quick to conclude that sexual transmission of HCV occurs infrequently, if at all (Fried and others 1992), they also suggest a need to determine whether coinfections, such as STDs, or high levels of HCV viral load facilitate sexual transmission. Although our study did not support the relationship of HCV RNA detection in the semen with lifetime history of STDs or number of sexual partners, other studies have found associations of HCV infection with multiple sexual partners (Xi-Ping and others 1995) and duration of paid sex (Nakashima and others 1992). Moreover, we unexpectedly found an inverse association between recent sexual activity and HCV

RNA in semen. Although these findings are unexplainable, they do suggest the need for more research in this area.

We found no association with reported HIV-seropositivity and HCV RNA in the semen. This finding, based on self-report data, has been supported by Fiore and others (1995). In addition, Fiore and others reported no association of HCV RNA with CD4 cells, stage of HIV disease, or extent of liver damage. These findings, along with studies showing that spouses of HCV-infected individuals more often demonstrate HCV infection than spouses of non-HCV-infected partners (Liou and others 1992; Xi-ping and others 1995), provide support for further investigations directed at assessing the incidence of HCV among previously uninfected sexual partners of HCV-infected persons. These studies can shed more light on possible sexual transmission of HCV and aid in the development of guidelines to protect against the spread of HCV infection.

Although there is a paucity of research that assesses predictors of HCV RNA in the semen of HCV-infected persons, older age and longer duration of marriage appear to be factors associated with HCV antibody concordance among couples (Kao and others 1992). Although our sample did not lend itself to an examination of marital duration, we did find that homeless men who were older were more likely to have HCV RNA detected in their semen.

Our study did support an association between HCV RNA in semen and higher levels of HCV RNA in the blood. An association of HCV RNA in semen and higher loads of HCV in the blood is supported by contentions that a threshold effect may occur in which a minimal concentration of virus in the blood must be present before virus will begin to spill over into other body secretions (Dore and Kaldor 2000; Leruez-Ville and others 2000). This seems, in fact, quite likely, when one compares viral loads of HBV and HCV. For example, whereas HBV is well documented in saliva, urine, and semen, with copies ranging from 10^5 to 10^6 of HBV RNA in saliva and semen (Jenison and others 1987), viral titers of HCV in the blood have been much lower (Fried and others 1992). These findings have led scientists to conclude that the low levels of HCV in the blood do not produce sufficient viral shedding for HCV RNA to be detected in secretions, even when amplified by the polymerase chain reaction technique (Fiore and others 1995).

To our knowledge, relationships between current alcohol and lifetime methamphetamine use and having HCV RNA in semen have not previously been reported. These findings suggest that for persons who are destitute and quite vulnerable to escaping the pressures of the outside world, methamphetamine use may propel them into environments that encourage them to engage in continued risky behaviors. However, because other drugs were also associated with the presence of HCV RNA in the semen, the multiple drug use profile could be driving the methamphetamine results. This is likely to be the case, as methamphetamine use was associated with multiple drug use. Nevertheless, methamphetamine use has been associated positively with greater frequency of high-risk sexual behaviors (e.g., anal intercourse among heterosexuals and homosexuals alike) (Molitor and others 1999). The effect of recreational drug use on the immune system and reproductive tract are unclear currently; whether these exposures modify movement of HCV virions from the serum into the seminal compartment is an important question for future studies. It is also possible that methamphetamine use is a marker for aspects of sex risk not captured in our sex variables. For example, sex partners of methamphetamine users were more likely to be methamphetamine injectors than were sex partners of heroin users (Zule and Desmond 1999). Thus, methamphetamine might be a marker for the intersection of sexual and injection risk, making HCV transmission more likely (Ackerman and others 1998). Nevertheless, there is still no clear explanation for the association between methamphetamine use and the detection of HCV RNA in semen. This is likewise true for the finding that persons who had completed the HBV vaccination series were more likely to have HCV RNA detected in their semen than their counterparts who had not completed the HBV vaccination series.

There are several limitations in this study. These include self-report of HIV status and of drug and sexual activity. However, in a previous investigation, we found good concordance between self-reported cocaine use and objective evidence of cocaine use as measured by hair analysis (Nyamathi and others 2001). We attribute these findings to the strong and nonthreatening rapport that was developed between the homeless participants and the research staff. Another limitation is the West Coast perspective of these participants. Still another limitation is that homeless men are likely to

have compromised immune systems, although none of the homeless history variables made a difference. We did not collect data on length of time various drugs were used, frequency of use, or amounts of drugs used, all of which may affect the findings of the study. Finally, the small number of bDNA-positive semen specimens limited the ability to test for quantitative viral loads in blood versus semen.

In summary, 36% of semen tested from this HCV-infected homeless population was positive for HCV RNA. Findings of our study reveal that homeless persons with HCV infection who have used methamphetamine and alcohol during their lifetime may be at increased risk for the presence of HCV RNA in their semen and, thus, are perhaps more likely to transmit HCV to a sexual partner. Although much needs to be understood about nonparenteral transmission of HCV, there is growing concern that guidelines for the prevention of sexually transmitted HCV infection must be clearly developed. The possibility of sexual transmission of HCV is particularly worrisome among homeless adults, as more than 70% report having unprotected sex with multiple partners (Nyamathi and others 1999). Furthermore, Osmond and others (1993) contend that sexual behavior can inevitably transmit a significant proportion of new HCV infections in the United States annually. Even though sexual transmission occurs less often than parenteral transmission, the potential exists for a large pool of carriers who could generate a significant number of secondary cases by sexual spread. Finally, published advice is conflicting about whether the use of condoms among discordant HCV-infected couples is necessary (Bresters and others 1993). Findings of this study suggest sexual transmission is plausible and highlight the need to conduct more in-depth investigations among homeless and general populations to determine who may be at risk for sexual transmission. Results from such investigations may guide policy recommendations with regard to the use of condoms for HCV prevention.

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