Original Report

Risk Factors for Hepatitis C Virus Infection among Nonintravenous-Drug-Using Heterosexuals Attending a Clinic for Sexually Transmitted Disease in Italy

Rosamaria Corona, MD;* Federico Caprilli, MD;† Maria Elena Tosti, BSc;‡ Giulio Gentili, MD;†
Elisabetta Franco, MD;§ Laura Zaratti, BSc;§ Guido Palamara, MD;† Amalia Giglio, MD;†
Grazia Prignano, BSc;† Tommaso Stroffolini, MD;‡ Paolo Pasquini, MD;‡ Maria Grazia Rastelli, MD;¶
and Alfonso Mele, MD‡

ABSTRACT

Objective: To evaluate prevalence of and risk factors for hepatitis C virus (HCV) infection and the role of genital ulcer disease in the sexual transmission of HCV among 1280 nonintravenous-drug-using heterosexuals attending a sexually transmitted disease clinic.

Methods: Serum samples were tested by a third-generation enzyme-linked immunosorbent assay (ELISA) test. Reactive sera were confirmed using a RIBA assay. The independent effect of different variables on the likelihood to be anti-HCV positive was assessed by multiple logistic regression analysis.

Results: The overall anti-HCV prevalence was 4.9%, a rate nearly ten times as high as the 0.5% recently found in a national sample of young male adults 18 to 26 years old. The prevalence was 9.6% in subjects with positive syphilis serology and 3.8% in those without a history of sexually transmitted disease. Multiple logistic regression analysis showed that age greater than 34 years (OR 2.9; 95% CI 1.1–7.6), low educational level (OR 2.0; 95% CI 1.1–3.5), positive syphilis serology (OR 2.1; 95% CI 1.04–4.1), and anti-HIV positivity (OR 4.6; 95% CI 1.2–18.7) were all independent predictors of HCV infection.

Conclusions: These findings are consistent with sexual transmission of HCV. Syphilis infection may be a cofactor in HCV transmission or, alternatively, may be a strong indicator of high-risk sexual behavior.

*Servizio di Epidemiologia, Istituto Dermopatico dell'Immacolata (IDI-IRCCS); †Istituto Ospedaliero Dermosifilopatico di S.Maria e S.Gallicano; ‡Laboratorio di Epidemiologia e Biostatistica, Istituto Superiore di Sanità; §Dipartimento di Sanità Pubblica, Università degli Studi Tor Vergata, Rome, Italy; and ¶Azienda Sanitaria, Livorno, Italy.

Address correspondence to Dr. Rosamaria Corona, Servizio di Epidemiologia, Istituto Dermopatico dell'Immacolata (IDI-IRCCS), Via Monti di Creta 104, 00167 Rome, Italy.

Hepatitis C virus (HCV) is the cause of most parenterally transmitted non-A, non-B hepatitis. Transmission by blood transfusion, intravenous drug use, surgical intervention, and occupational exposure is well documented, whereas the risk of sexual transmission is not well defined.

High anti-HCV prevalence has been reported in patients with sexually transmitted diseases (STDs),[1-3] in homosexuals with multiple sexual partners,[4,5] and in spouses of patients with type C chronic liver disease.[6] In contrast, little or no transmission of HCV was found in sexual partners of HCV infected hemophiliacs.[7,8] A study on acute non-A, non-B anti-HCV positive hepatitis in the United States showed an association with multiple sexual partners,[9] whereas a weak and not significant association was found in a similar study done in Italy.[10]

Genital ulcers or erosions have been shown to be cofactors for hepatitis B virus (HBV) transmission,[3,11,12] but little information is available on the role of these factors for HCV transmission.

The aim of the present study, which used a sensitive third-generation test for HCV antibody, was to evaluate the prevalence of and risk factors for HCV infection among heterosexuals attending a clinic for sexually transmitted diseases in Rome, who denied blood transfusion and intravenous drug use. Particular attention was paid to the role of genital ulcerative diseases.

METHODS

Study Population

The study was conducted at the STD outpatient clinic of the S. Maria e S. Gallicano hospital in Rome, from February to December 1989 and from January 1993 to April 1994, as part of a study of human immunodeficiency virus (HIV) seroprevalence. Homosexual or bisexual men, intravenous

drug users, and blood transfused patients were excluded from the study.

During the first period, all the patients seen at the STD clinic were enrolled in the study; in the second period, all the patients reporting genital ulcers or erosions, and, on a random day a week, all the patients attending the clinic were recruited. After informed consent was obtained, the patients provided a blood sample and were administered an anonymous questionnaire, by a trained interviewer, to assess basic sociodemographic information (age, sex, education), sexual preference, number of sexual partners and condom usage in the year before the visit, history of intravenous drug use, blood transfusion, and history of STDs. Ulcerative diseases included syphilis and genital herpes infection. Non-ulcerative STDs included gonorrhea, genital warts, nongonococcal urethritis due to *Chlamydia trachomatis* or *Ureaplasma urealyticum*, vaginal or cervical infection due to *Trichomonas vaginalis*, *Candida albicans*, or *Chlamydia*, scabies, and *Phthirus pubis* infestation.

Laboratory Methods

The serum samples collected in both study periods were stored at $-20°C$ until tests were performed. All samples were tested with the third-generation Ortho HCV 3.0 enzyme-linked immunosorbent assay (ELISA) (Ortho Diagnostic Systems, Raritan, New Jersey). Reactive sera were also tested with an additional, more specific assay (Chiron RIBA HCV 3.0 SIA, Ortho Diagnostic Systems) consisting of a qualitative immunoblot assay that utilizes HCV antigens, immobilized as individual bands onto test strips. The two recombinant antigens (c33c and NS5) and two of the synthetic peptides (c100p and 5-1-1p) are derived from putative nonstructural regions of the virus, whereas the third peptide (c22p) corresponds to the putative nucleocapsid (core) viral protein. Sera were confirmed as positive when at least two HCV bands were reactive.

Syphilis serologic testing was performed with the rapid plasma reagin screening test and the hemagglutination test for *Treponema pallidum* and an enzyme immunoassay test for IgM and IgG for confirmation. Darkfield microscopy was performed on any lesion or rash compatible with primary or secondary syphilis.

The diagnosis of genital herpes was clinical. Serologic testing for chlamydial infection was made by ELISA. Vaginal and urethral specimens were either examined by Gram stain or cultured for *Neisseria gonorrhoeae*.

Statistical Analysis

Differences between proportions were tested by chi-square and Fisher's exact test, where appropriate. The strength of association of each considered variable with anti-HCV positivity was estimated by calculating crude odds ratios (OR) by univariate analysis. A logistic regression model was used to identify variables independently associated with anti-HCV positivity. Only variables significantly associated with anti-HCV positivity in the univariate analysis were entered into the model. For each variable the reference category was the most favorable level of exposure (youngest age, female sex, highest education level, lowest number of sexual partners, use of condoms, negative history of STDs). Because of a strong correlation between reactive syphilis serology and anti-HBc, for the purpose of assessing the strength of association between HCV infection and serologic results positive for syphilis, this last variable was not included in the model. BMDP Statistical Software 1990 (Berkeley, CA) was used for all calculations.[13]

RESULTS

A total of 1771 patients were asked to participate in the study (1090 in 1989 and 681 in 1993-1994). Of these, 975 (89.4%) in the first period and 522 (76.7%) in the second period were enrolled. The patients who accepted were similar to those who refused with regard to sex, age, number of sexual partners in the year previous to the clinical visit, and history of STDs.

A total of 782 (80%) of 975 patients who provided a blood sample in the first period and 498 (95%) of 522 in the second period were tested for anti-HCV. The median age of the 1280 study subjects was 32 years (range, 15-78 y). The general characteristics of the two groups of patients included in the study are shown in Table 1. The two groups were similar with respect to sex, age, number of sexual partners in the previous year, and history of STDs. The 1993-1994 group had a higher level of education and reported more frequent use of condoms as compared with the 1989 group. Of 141 patients with genital herpes, 119 (84%) had current disease at the time of enrollment into the study, and 22 (16%) reported a past history of genital herpes. The overall anti-HCV prevalence was 4.9%. The anti-HCV prevalence was 6.0% in 1989 and 3.2% in 1993-1994 (P = 0.03). After controlling for education level and diagnosis, this difference still remained (P = 0.05).

The prevalence was 9.6% in patients with positive syphilis serology and 3.8% in those without a history of STDs (OR 2.8, 95% CI 1.3-5.4). Three of 12 (25%) HIV-positive patients were anti-HCV positive versus 59 of 1246 (4.7%) anti-HIV negative (P = 0.001). Among 161 patients who tested positive for syphilis, four (2.5%) were also anti-HIV positive. Of these, two (50%) were also anti-HCV positive, whereas 14 of 157 (8.9%) HIV negative had anti-HCV antibodies (P = 0.05).

By univariate analysis, a statistically significant association was found between anti-HCV positivity and age greater than 24 years, less than 8 years of education, anti-HBc positivity, anti-HIV positivity, and positive syphilis serology. Three or more sexual partners in the previous year, history of non-ulcerative STD, and present or past

Table 1. General Characteristics of Nonintravenous-Drug-Using Heterosexuals Attending a Clinic for Sexually Transmitted Diseases in Rome, Italy

Characteristics	Study Year		P
	1989 n = 782 (%)	1994 n = 498 (%)	
Sex			
Female	209 (27)	148 (30)	0.245
Male	573 (73)	350 (70)	
Age			
15–24	166 (21)	99 (20)	
25–34	278 (36)	186 (37)	0.758
≥35	338 (43)	213 (43)	
Years of education			
>8	362 (47)	291 (59)	0.000
≤8	414 (53)	206 (41)	
Number of partners in previous year			
0–2	531 (68)	355 (71)	0.194
≥3	250 (32)	142 (29)	
Use of condom in previous year			
Yes	156 (21)	141 (28)	0.002
No	604 (79)	357 (72)	
Anti-HIV			
Negative	758 (99)	488 (99)	0.860
Positive	7 (1)	5 (1)	
Anti-HBc			
Negative	591 (76)	405 (82)	0.011
Positive	185 (24)	88 (18)	
Anti-HCV			
Negative	735 (94)	482 (97)	0.024
Positive	47 (6)	16 (3)	
Diagnosis			
No history of STD	362 (46)	238 (50)	0.000
History of non-ulcerative STD	248 (32)	103 (22)	
Current genital herpes	36 (5)	83 (17)	
History of genital herpes	14 (2)	8 (2)	
Primary syphilis	4 (0.5)	2 (0.4)	
Secondary syphilis	5 (0.6)	4 (0.8)	
Latent syphilis	113 (14)	38 (8)	

history of genital herpes were not associated with anti-HCV positivity (Table 2).

Adjustment of each variable for the confounding effect of the other variables was done by multivariate analysis. The logistic regression model included all the variables found significantly associated by univariate analysis. Increasing age, low education level, positive syphilis serology, and anti-HIV positivity were all independent predictors of HCV infection (Table 3). The adjusted odds ratios for positive syphilis serology and HIV positivity were 2.1 (95% CI 1.04–4.14) and 4.75 (95% CI 1.21–18.7), respectively. A second logistic model including a term for interaction between HIV and syphilis positivity was run. The estimated odds ratio for the interaction was 4.6 (95% CI 0.23–92.3).

DISCUSSION

In this investigation, an overall anti-HCV prevalence of 4.9% was found in heterosexual patients in a clinic for

STDs who denied intravenous drug use and blood transfusion. This rate is nearly ten times as high as the 0.5% found in a national sample of young adult males aged 18 to 26 years tested in 1990,[14] and even higher than the prevalence found in 1995 among Roman blood donors aged 18 to 60 years, which was 0.26% for donors at their first donation, and 0.015% for habitual donors tested with a third-generation test (Solinas S. Unpublished data).

The authors evaluated the evidence for sexual transmission of HCV by estimating the association between anti-HCV positivity and other diseases for which the sexual route of transmission is well known. In addition, the role of potential cofactors for sexual transmission of HCV was assessed by estimating the association between anti-HCV and STDs causing genital ulcers. The most striking finding of this study was the independent association linking positive syphilis serology with anti-HCV positivity.

This study confirms the results of Thomas et al,[3] who reported a more than twofold increased risk of HCV infections in nonintravenous-drug-using male patients with

Table 2. Prevalence of Anti-HCV Positivity among 1280 Nonintravenous-Drug-Using Heterosexuals Attending a Clinic for Sexually Transmitted Diseases in Rome, by Sociodemographic Characteristics of Subjects and Potential Risk Factors

Characteristics	No. Positive/No. Tested (%)	OR	95% CI
Sex			
Female	18/357 (5.0)	1	
Male	45/923 (4.9)	1.0	0.5–1.8
Age			
15–24	5/265 (1.9)	1	
25–34	22/464 (4.7)	2.6	0.9–8.9
≥35	36/551 (6.5)	3.6	1.4–12
Years of education			
>8	21/653 (3.2)	1	
≤8	42/620 (6.8)	2.2	1.2–3.9
Number of partners in previous year			
0–2	40/886 (4.5)	1	
≥3	23/392 (5.9)	1.3	0.8–2.3
Use of condom in previous year			
Yes	12/297 (4.0)	1	
No	48/961 (5.0)	1.3	0.6–2.5
Anti-HIV			
Negative	59/1246 (4.7)	1	
Positive	3/12 (25.0)	6.7	1.1–28
Anti-HBc			
Negative	27/996 (2.7)	1	
Positive	36/273 (13.2)	5.5	3.2–9.5
Diagnosis			
No history of STD	23/600 (3.8)	1	
History of non-ulcerative STD	17/351 (4.8)	1.3	0.6–2.5
Genital herpes	7/141 (5.0)	1.3	0.5–3.2
Positive syphilis serology	16/166 (9.6)	2.8	1.3–5.4

OR = odds ratio derived by univariate analysis; CI = confidence interval.

positive syphilis serology, and those of Nakashima and colleagues,[2] who found a significantly higher prevalence of HCV in patients with STDs who reported a history of at least one episode of syphilis.

Table 3. Variables Associated with Anti-HCV Positivity among 1280 Nonintravenous-Drug-Using Heterosexuals Attending a Clinic for Sexually Transmitted Diseases in Rome

Variables	OR	95% CI
Age		
15–24	1	
25–34	2.4	0.9–6.5
≥35	2.9	1.1–7.6
Years of education		
>8	1	
≤8	2.0	1.1–3.5
Anti-HIV		
Negative	1	
Positive	4.8	1.2–19
Diagnosis		
No history of STD	1	
History of non-ulcerative STD	1.2	0.6–2.3
Genital herpes	1.2	0.5–2.9
Positive syphilis serology	2.1	1.04–4.1

OR = odds ratio derived by multiple logistic regression model. Each variable is adjusted for the confounding effect of all others listed in the table.
CI = confidence interval.

Several studies on sexual transmission of HBV have already shown an association between syphilis and HBV infection.[3,12,15] Syphilis may be a risk factor even for HCV infection for several reasons. First, syphilis can be regarded as an indicator of high-risk sexual behavior. Second, it may be a cofactor in sexual transmission of HCV, as the disruption of the epithelial surface resulting in a genital ulcer may in fact facilitate virus transmission.

The hypothesis of a synergy between HIV and syphilis in the transmission of HCV infection was explored by running a multiple logistic model that included a term for interaction. The odds ratio for interaction of 4.6 was high, but, because of the low statistical power due to the small number of subjects positive for both HIV and syphilis, its estimate was imprecise, as indicated by the wide confidence interval, and not statistically significant.

Association of HCV infection with present or past history of genital herpes, which also causes genital ulceration, was not found. These results are consistent with those of other authors.[3] Moreover, even HBV has not been found to be associated with genital herpes.[3,12] It is speculated that some biologic factors linked to the genital herpes infection may not facilitate the transmission of other sexually transmitted viruses. For example, the painful herpetic lesion may be a deterrent from sexual

activity. Alternatively, genital herpes could merely be a less indicative surrogate marker of high-risk sexual behavior than syphilis. The authors did not use serology for the diagnosis of genital herpes because of the weak correlation between a positive test and clinical disease. In addition, the residual cross-reactivity between herpes simplex virus (HSV)-2 and HSV-1 excludes its use for diagnosis in absence of symptoms.

High prevalence of anti-HCV was found in anti-HBc-positive subjects, and, although only 12 of 1280 (0.9%) subjects were anti-HIV positive, as many as 25% of these had anti-HCV antibodies. These findings suggest common routes of transmission of the three viruses. In addition, the strong association between HCV and HIV infection, reported in several studies, supports the hypothesis that HCV transmission is more efficient when HIV is present.[3,4,7]

Behavioral factors, such as multiple sexual partners, have been shown to increase sexual transmission of HBV.[9,11,12,16] In the present study, no association was found between HCV infection and having three or more sexual partners in the year before the visit; this observation confirms the results reported by Weinstock and co-workers.[17] In contrast, Thomas found an increased risk of HCV infection for patients reporting more than 24 lifetime sexual partners.[18] However, because of the striking differences in the definition of the exposure levels used in the various studies, regarding the number of sexual partners, a comparison of these results is difficult.

Age greater than 24 years and having less than 8 years of education were also independent risk factors for HCV infection. Low education level is a good indicator of low socioeconomic status, which is not a risk factor by itself, but may identify lifestyles and behaviors that increase the likelihood of HCV infection.

The lack of association between HCV infection and the use of condoms and multiple sexual partners could be due to the fact that information on these variables was collected only for the year prior to the clinic visit; hence, the data may not reflect past unprotected sexual activity when HCV infection may have been acquired.

In conclusion, the higher than expected prevalence of HCV and its association with syphilis suggest sexual transmission of the virus. The association with a positive syphilis serology may indicate syphilis as a cofactor in the transmission of HCV, or, alternatively, as a powerful indicator of high-risk sexual behavior.

ACKNOWLEDGMENT

REFERENCES

1. Corona R, Prignano G, Mele A, et al. Heterosexual and homosexual transmission of hepatitis C virus: relation with hepatitis B virus and immunodeficiency virus type 1. Epidemiol Infect 1991; 107:667–672.
2. Nakashima K, Kashiwagi S, Hayashi J, et al. Sexual transmission of hepatitis C virus among female prostitutes and patients with sexually transmitted diseases in Fukuoka, Kyushu, Japan. Am J Epidemiol 1992; 136:1132–1137.
3. Thomas DL, Cannon RO, Shapiro CN, Hook EW, Alter MJ, Quin TC. Hepatitis C, hepatitis B, and human immunodeficiency virus infections among nonintravenous-drug-using patients attending clinics for sexually transmitted diseases. J Infect Dis 1994; 169:990–995.
4. Tedder RS, Gilson RJC, Briggs M, et al. Hepatitis C virus: evidence for sexual transmission. BMJ 1991; 302:1299–1302.
5. Osmond DH, Charlebois E, Sheppard HW, et al. Comparison of risk factors for hepatitis C and hepatitis B virus infections in homosexual men. J Infect Dis 1993; 167:66–71.
6. Akahane Y, Kojima M, Sugai Y, et al. Hepatitis C virus in spouses of patients with type C chronic liver disease. Ann Intern Med 1994; 120:748–752.
7. Eyster ME, Alter HJ, Aledort LM, Quan S, Hatzakis A, Goedert J. Heterosexual co-transmission of hepatitis C virus (HCV) and human immunodeficiency virus (HIV). Ann Intern Med 1991; 115:764–768.
8. Bresters D, Mauser-Bunschoten EP, Reesink HW, et al. Sexual transmission of hepatitis C virus. Lancet 1993; 342:210–211.
9. Alter MJ, Coleman PJ, Alexander WJ, et al. Importance of heterosexual activity in the transmission of hepatitis B and non-A, non-B hepatitis. JAMA 1989; 262:1201–1205.
10. Mele A, Sagliocca L, Manzillo G, et al. Risk factors for acute non-A, non-B hepatitis and their relationship to antibodies for hepatitis C virus: a case control study. Am J Public Health 1994; 84:1640–1643.
11. Rosenblum LS, Hadler SC, Castro KG, Lieb S, Jaffe HW, and the Belle Glade Study Group. Heterosexual transmission of hepatitis B virus in Belle Glade, Florida. J Infect Dis 1990; 161:407–411.
12. Corona R, Caprilli F, Giglio A, et al. Risk factors for hepatitis B virus infection among heterosexuals attending a sexually transmitted disease clinic in Italy: role of genital ulcerative diseases. J Med Virol 1996; 48:262–266.
13. BMDP: Statistical Software Manual. Berkeley: University of California Press, 1990; 1047–1076.
14. D'Amelio R, Stroffolini T, Matricardi PM, et al. Low prevalence of anti-HCV antibodies among Italian Air Force recruits. Scand J Infect Dis 1995; 27:12–14.
15. McQuillan GM, Townsend TR, Fields HA, Carrol M, Leahy M, Polk BF. Seroepidemiology of hepatitis B virus infection in the United States. 1976 to 1980. Am J Med 1989; 87:5S–10S.
16. Alter MJ, Ahtone J, Weisfuse I, Starko K, Vacalis TD, Maynard JE. Hepatitis B virus transmission between heterosexuals. JAMA 1986; 256:1307–1310.
17. Weinstock HS, Bolan G, Reingold AR, Polish LB. Hepatitis C infection among patients attending a clinic for sexually transmitted diseases. JAMA 1993; 269:392–394.
18. Thomas DL, Zenilman JM, Alter HJ, et al. Sexual transmission of hepatitis C virus among patients attending sexually transmitted diseases clinics in Baltimore: an analysis of 309 sex partnerships. J Infect Dis 1995; 171:768–775.