American Journal of Gastroenterology
© 2004 by Am. Coll. of Gastroenterology
Published by Blackwell Publishing

ISSN 0002-9270
doi: 10.1111/j.1572-0241.2004.04150.x

Lack of Evidence of Sexual Transmission of Hepatitis C among Monogamous Couples: Results of a 10-Year Prospective Follow-Up Study

Carmen Vandelli, M.D., Francesco Renzo, M.D., Luisa Romanò, Ph.D., Sergio Tisminetzky, M.D., Marisa De Palma, M.D., Tommaso Stroffolini, M.D., Ezio Ventura, M.D., and Alessandro Zanetti, Ph.D.
Dipartimento di Medicina Interna, Università di Modena e Reggio Emilia, Modena, Italy; Istituto di Virologia, Università degli Studi di Milano, Italy; ICGEB, Trieste, Italy; Centro Trasfusionale, Azienda Policlinico di Modena, Modena, Italy; Divisione di Gastroenterologia, Ospedale San Giacomo, Roma, Italy; and Istituto di Virologia, Università degli Studi di Milano, Italy

The risk of sexual transmission of hepatitis C virus (HCV) infection was evaluated among 895 monogamous heterosexual partners of HCV chronically infected individuals in a long-term prospective study, which provided a follow-up period of 8,060 person-years. Seven hundred and seventy-six (86.7%) spouses were followed for 10 yr, corresponding to 7,760 person-years of observation. One hundred and nineteen (13.3%) spouses (69 whose infected partners cleared the virus following treatment and 50 who ended their relationship or were lost at follow-up) contributed an additional 300 person-years. All couples denied practicing anal intercourse or sex during menstruation, as well as condom use. The average weekly rate of sexual intercourse was 1.8. Three HCV infections were observed during follow-up corresponding to an incidence rate of 0.37 per 1,000 person-years. However, the infecting HCV genotype in one spouse (2a) was different from that of the partner (1b), clearly excluding sexual transmission. The remaining two couples had concordant genotypes, but sequence analysis of the NS5b region of the HCV genome, coupled with phylogenetic analysis showed that the corresponding partners carried different viral isolates, again excluding the possibility of intraspousal transmission of HCV. Our data indicate that the risk of sexual transmission of HCV within heterosexual monogamous couples is extremely low or even null. No general recommendations for condom use seem required for individuals in monogamous partnerships with HCV-infected partners.

INTRODUCTION

Since the discovery of the hepatitis C virus (HCV) genome more than a decade ago, the possibility for its sexual transmission has not been unequivocally established. Several studies, mostly providing prevalence data, addressed this topic, yielding conflicting results. The rate of HCV infection in sexual partners of anti-HCV-positive individuals has been shown to range between very low and as high as 30% (1–27). These discordant findings may partly be explained by the confounding effect of nonsexual transmission routes, such as unrecognized (or undisclosed) parenteral transmission due to sharing of toothbrushes, razors, needles, and syringes. Moreover, since prevalence data reflect a cumulative incidence of infection over time, it does not offer an accurate relationship in time between viral exposure and the acquisition of infection. Prospective studies, therefore, represent the most adequate tool to directly assess the actual risk of infection in a given setting. Two retrospective-prospective studies, carried out in long-term male monogamous sexual partners of German (28) and Irish (29) women, inadvertently infected by HCV-contaminated anti-D globulin at a young age revealed, after nearly two decades of follow-up, null, or very low-HCV infection rates, respectively. Pooling the findings for these two cohorts, revealed a crude rate of HCV seroconversion among the 487 male partners involved, of 0.001 per 1,000 person-years (30). On the contrary, a rather higher rate of infection (12 per 1,000 person-years) was found in a cohort of 449 monogamous heterosexual Italian couples followed for a mean of 13.4 months (31). Few prospective data are available. The aim of this study was to prospectively evaluate the risk of acquiring HCV infection in a large cohort of heterosexual monogamous partners of HCV chronically infected individuals.

MATERIALS AND METHODS

Study Population

We investigated 895 heterosexual couples comprising one partner positive for anti-HCV antibody and HCV-RNA (index patient), and one partner negative for HCV markers. Couples were recruited from the Department of Internal Medicine of the University of Modena and Reggio Emilia (Italy) in

September 1991, by screening spouses of 967 HCV infected patients followed in the clinic.

HCV negative partners were informed of the possible routes of HCV transmission, including that of sexual transmission, and were invited to enter in a follow-up protocol consisting of yearly clinical visits for a prospective evaluation of the risk of acquiring HCV infection. Liver function tests and anti-HCV antibody were evaluated at each such visit and participants showing seroconversion to anti-HCV, or with elevated transaminase levels, were further assayed for HCV-RNA. At the same time participants were administered precoded questionnaires investigating sexual behavior (*i.e.*, frequency of condom use, mean number of sexual contacts per week, sex during menstruation, anal intercourse, extramarital relationships) and potential sources for parenteral exposure.

Follow-up duration was 10 yr. Partners declaring extramarital relationships were excluded from the study.

Participants were not advised to initiate condom use, but were strongly invited to avoid sharing personal hygiene items such as toothbrushes, razor blades, and nail scissors.

The study was approved by the Institutional Review Committee of the Hospital of Modena.

Case Definition

Transmission of HCV from an index patient to the susceptible partner was considered to have occurred when anti-HCV and/or HCV-RNA were detectable in the serum of such a previously negative partner, and when the NS5b region sequences derived from index patients and their respective spouses were homologous and consistent with a transmission event.

Laboratory Tests

Blood samples were drawn from all participants at the time of enrollment and at each yearly visit. Sera were divided in two aliquots: one was initially assayed for anti-HCV by a first-generation enzyme-linked immunoadsorbent assay (ELISA, Ortho Diagnostic Systems, Raritan, NJ); the second aliquot was stored at −80°C and used retrospectively for HCV-RNA detection by nested reverse-transcriptase polymerase chain reaction (RT-PCR), once this assay became available. Serum HCV-RNA was detected by an in-house RT-PCR assay with nested primers of the 5′ noncoding region of the HCV genome (32). HCV-RNA load was measured by branched DNA version 2.0 assay (Quantiplex HCV RNA, Bayer Diagnostics, Emeryville, CA) according to the manufacturer's instructions. In later follow-up visits, HCV antibodies were assayed by second- and third-generation ELISA (Ortho Diagnostic System, Raritan, NJ). HCV genotypes of partners who acquired infection and their respective spouses were determined with a line probe assay (INNO-LiPA-HCV, lnnogenetics, Ghent, Belgium).

Sequence Analysis

HCV sequencing and phylogenetic analysis were performed as follows: extracted RNA was amplified by nested RT-PCR using primers derived from the NS5b region of the HCV genome. Briefly, the cDNA synthesis of the NS5b region was performed with primer Hep102 (5′-AGCATGATGTTATCAGCTCC-3′) and then amplified by PCR with primer sense Hep101 (5′-ATACCCGCTGCTTTGACTC-3′) and primers antisense Hep102 for the first step and primer sense Hep101 and antisense Hep105 (5′-ATACCTAGTCATAGCCTCCGTGA-3′) for the second step. The PCR product of 381 bp (nt 8,258–8,638) from the NS5b region was purified and sequenced on Applied Biosystem ABI373 automated sequencer. For phylogenetic analysis, four sequences obtained from the two couples infected with concordant HCV genotypes were compared with eight sequences (four of genotype 2a and four of genotype 1b) derived from unrelated patients attending the same hospital and with two prototype sequences sampled from GenBank (accession number AF169005 for genotype 2a and AF356827 for genotype 1b). The whole set of 14 sequences was resampled by boostrapping 1,000 times and then analyzed with PHYLIP package, version 3.5c (33). The degree of divergence between the sequences was estimated by the Kimura 2-parameter method, with a transition-transversion ratio of 2:0 (DNADIST of the PHYLIP package) (34). The set of distance matrices was then analyzed by unweighted pair group method with arithmetic mean (UPGMA) as implemented by the NEIGHBOR program of the PHYLIP package. The final unrooted consensus tree was drawn by the CONSENSE program of the same package.

Anti-HIV antibody and IgM antibodies to CMV and EBV were detected by commercially available kits (Enzygnost Anti-HIV 1/2 Plus SP2, DADE Boehring, Marburg, Germany; Enzygnost Anti-CMV IgM, DADE Boehring, Marburg, Germany; EBV VCA IgM Immunowell GenBio, SpA Italian Laboratory BOUTY, Milan, Italy).

RESULTS

A total of 967 sexual partners of HCV infected patients were consecutively identified and tested for anti-HCV antibody. Of these, 33 (3.4%) were anti-HCV positive and were excluded from the study, 39 (4%) despite negativity for anti-HCV were excluded because they did not complete the questionnaire while 895 (92.6%) fit the requirement for enrollment. Twenty-eight of the 33 (84.5%) spouses found anti-HCV positive at the baseline, reported major risk factors for HCV exposure such as multiple blood transfusions prior to the introduction of anti-HCV donor screening, surgery and histories of intravenous drug abuse, or parenteral therapy with glass syringes. HCV genotypes of the 5 spouses with no apparent risk factors except living together with an infected partner, were discordant in 3 couples and concordant in the remaining 2 couples. No samples are available for phylogenetic analysis of the HCV sequences derived from the 2 couples with concordant genotypes. As shown in Table 1, gender distribution was 364 (40.7%) males and

Table 1. Demographic Characteristics and Clinical and Virologic Features of the Index Patients and Their Spouses at Enrollment

	Index	Spouse
Gender (M/F ratio)	531/364	364/531
Age (yr)		
Mean ± SD	46.02 ± 11.9	44.2 ± 11.3
Range	(18–65)	(19–60)
Years of marriage	23.7 ± 12.02	
Anti-HIV/anti-CMV IgM/anti-EBV IGM negative	895	895
HCV-RNA positive	895	0
Viral load		
≤1 MEq/L	119	0
>1 ≤ 2,5 MEq/L	173	0
>2,5 MEq/L	603	0
Liver histology		
Minimal changes	44	–
Mild	84	–
Moderate	479	–
Severe	211	–
Cirrhosis	77	–

531 (59.3%) females, mean age at enrollment was 44.2 ± 11.3 yr, and the mean duration of marriage was 23.7 ± 12.02 yr. All subjects (index patients and spouses) tested negative for HIV 1 and 2 antibodies, IgM anti-CMV, and IgM anti-EBV. The mean level of HCV-RNA of the index patients was 6.7 ± 1.7 MEq/L. Liver histology showed minimal changes in 44 (5%) index patients, mild hepatitis in 84 (9.4%), moderate hepatitis in 479 (53.4%), severe hepatitis in 211 (23.6%), and cirrhosis in 77 (8.6%) patients. Two hundred and thirty index patients have undergone α-interferon treatment; 69 (30%) achieved long-term responses. Spouses of these index patients were excluded from the analysis since they were no longer at risk of HCV exposure. Taken together, 776 (93.9%) partners completed the 10-yr follow-up period, corresponding to 7,760 person-years of observation. The remaining 119 (6.1%) spouses: 69 whose partners cleared the virus following treatment and 50 spouses who interrupted their relationship (n = 18) or were lost (n = 32) at some point during follow-up, contributed an additional period of observation of 300 person-years. Thus, the overall follow-up was 8,060 person-years. All partners denied anal intercourse, sex during menstruation, condom use, and no genital lesions were observed at each visit. The reported average weekly rate of intercourse was 1.8.

Three spouses acquired HCV infection in the course of the follow-up period, corresponding to an incidence rate of 0.37 per 1,000 person-years.

The characteristics of the three spouses who became anti-HCV and HCV-RNA positive after 7, 8, and 9 yr, respectively, of follow-up and of their HCV-infected partners are shown in Table 2. One putative incident case (couple 1) was a female who reported having had dental implant 3 months before anti-HCV seroconversion. This participant's HCV genotype (2a) was discordant from that of her husband (1b). The second case (couple 2) was a nurse who became HIV and HCV positive after injuring herself with a needle infected from a HIV/HCV-coinfected patient. Her genotype (1b) was concordant with that of her husband, who, however, was anti-HIV negative. The third infected partner (couple 3) was a male who denied any parenteral risk factor; his genotype (2a) was concordant with that of his wife. Thus, the HCV infection rate during the follow-up for concordant genotypes was 0.25 per 1,000 person-years.

The aligned sequences of 381 bp encompassing the NS5b region obtained from both couples with concordant HCV genotypes were used to build a phylogenetic tree based on the Kiramura distance method and the UPGMA clustering. As shown in Figure 1, the sequences form two distinct clusters, one for the sequences of genotype 1b and another for the sequences of genotype 2a. However, further grouping within each cluster clearly separated the index patients (PTa and PTb) from their respective spouses (SPTa and SPTb), both supported by a boostrap value higher than 750/1,000. Thus, despite the fact that both partners carried the same genotype, HCV strains were not linked.

DISCUSSION

This large prospective study provides strong evidence that sexual transmission of HCV infection among long-term monogamous couples is a very rare event. Actually, the results of our epidemiological and molecular investigations showed that no partner was infected by the sexual route during 10 yr of monogamous partnership with an infected individual. We have to underline that in 33 of the 967 (3.4%) couples initially investigated both partners were anti-HCV positive. Thus we cannot exclude with certainty that HCV could have been transmitted earlier during their relationship. However a number of epidemiological considerations favor the hypothesis that the sexual route is highly inefficient also in these couples. These include the evidence that the anti-HCV prevalence found in these spouses of infected patients is comparable to that of the general population matched by age, sex, and geographical area (35), and the fact that the most anti-HCV positive spouses had major risk factors for

Table 2. Characteristics of the Couples in Whom HCV Seroconversion Occurred During Follow-Up

	Gender	Genotype	Risk Factor
Couple 1			
Index	M	1b	
Partner	F	2a	Dental implant
Couple 2			
Index	M	1b	
Partner	F	1b	Needle-stick injury
Couple 3			
Index	F	2a	
Partner	M	2a	Not identified

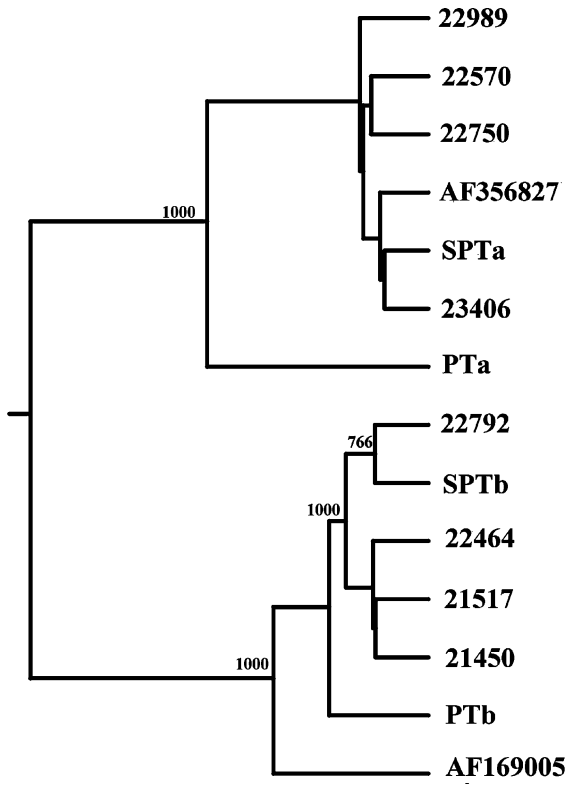

Figure 1. Phylogenetic tree of 14 isolates, based upon the NS5b region of the HCV genome. The analysis compares four 381-nt long sequences obtained from index patients (PTa and PTb) and their respective partners (SPTa and SPTb) with eight sequences (four of genotype 2a and four of genotype 1b, respectively) obtained from unrelated controls, and with two sequences sampled from GenBank (AF169005 for genotype 2a and AF356827 for genotype 1b). Boostrap values greater than 750/1,000 are reported.

HCV exposure in addition of living together with an infected partner.

Of the three spouses who acquired HCV infection during the follow-up period, one female partner did not present the same genotype of her chronically infected male partner, thus clearly excluding sexual transmission. It is possible that this woman acquired the infection iatrogenically, as she reported a history of invasive dental procedure carried out 3 months prior to her anti-HCV seroconversion. In the two couples with concordant genotypes, sequence analysis of the NS5b region coupled with phylogenetic analysis showed that newly infected partners presented viral isolates different from those of the infected index patients. This finding is inconsistent with intraspousal transmission of HCV. Needle-stick injury represented the most likely mode of exposure to HCV for the nurse (SPTa) who became HIV and HCV coinfected (blood sample from the suspected patient this nurse drew was not available for molecular analysis). No evidence of risk factors other than sexual were reported by the male (SPTb) who became infected with the same HCV genotype (2a) as his wife. However, molecular analysis of the HCV isolates derived from these two partners suggests that they were not linked to each other, providing evidence for a different source of infection. Therefore, it seems reasonable to affirm that the chance of HCV sexual transmission within heterosexual monogamous couples is very uncommon or even nonexistent. This finding is in agreement with the very low risk of sexual transmission of HCV observed in retrospective cohorts of female partners of hemophiliacs (9, 10), as well as in the German and Irish long-term cohorts of monogamous male partners of women infected with HCV-contaminated anti-D immunoglobulin (28, 29). These rates are markedly lower than those observed in an Italian prospective cohort study of 499 monogamous heterosexual couples followed up for a mean of 13.4 months, where the incidence of infection in sexual partners was 12 per 1,000 person-years (31). In another study carried out in Taiwan on 112 couples with an average partnership duration of 46 months, the incidence of infection was 2.3 per 1,000 person-years (35). Differences in the magnitude of risk of acquiring HCV among sexual partners, reported in different studies, may reflect differences in sexual risk behavior or differences in the rates of exposure to nonsexual sources of HCV infection. Recently, it has been shown that HCV infection among spouses is more likely associated to a common exposure to HCV infection risk factors rather than to sexual transmission (27). In this study, exposure to a common percutaneous risk factor (*i.e.*, sharing personal hygiene items and glass syringes) was estimated to be 12.4 times higher among couples in whom both partners were infected with HCV compared to those with only one infected partner, suggesting that modes of viral transmission other than the sexual route may play a key role in the intraspousal spread of HCV infection. All partners enrolled in our study were strongly advised to avoid sharing of personal hygiene items, such as toothbrushes, razor blades, and nail clippers. The lack of evidence of person-to-person transmission of HCV revealed in this study may reflect the adherence of couples to these recommendations and may indirectly support the soundness of these measures for the prevention of HCV transmission between sexual partners.

In conclusion, the results of our epidemiological and molecular study support the evidence that the transmission of HCV through the sexual route is highly inefficient, providing a basis for recommendations on the management of HCV-discordant couples. Individuals who are in long-term monogamous relationships with HCV-infected partners should be informed regarding the very low risk of acquiring HCV sexually, but no general recommendations for condom use seems to be required.

ACKNOWLEDGMENTS

This study was supported by a grant of the Association for the study of Infectious and Metabolic Diseases of the Liver (ASPImF). Molecular analyses were performed with a grant supplied by the Centre for Bio-Molecular Interdisciplinary

Studies and Industrial Application (CISI), Centre of Excellence, University of Milan.

Reprint requests and correspondence: Carmen Vandelli, M.D., Dipartimento di Medicina Interna, Policlinico, Via del Pozzo, 71-41100 Modena, Italy

Received August 5, 2002; accepted October 3, 2003.

REFERENCES

1. Tedder RS, Gilson RJ, Briggs M, et al. Hepatitis C virus: Evidence for sexual transmission. Br Med J 1991;302:1299–302.
2. Shev S, Wejstal R, Wahl M, et al. The lack of transmission of NANB/C hepatitis between acute and chronically infected patients and their heterosexual partners. Scand J Infect Dis 1991;23:407–11.
3. Brettler DB, Mannucci PM, Gringeri A, et al. The low risk of hepatitis C virus transmission among sexual partners of hepatitis C-infected hemophilic males: An international, multicenter study. Blood 1992;80:540–3.
4. Peano GM, Fenoglio LM, Menardi G, et al. Heterosexual transmission of hepatitis C virus in family groups without risk factors. Br Med J 1992;305:1473–4.
5. Gordon SC, Patel AH, Kulesza GW, et al. Lack of evidence for the heterosexual transmission of hepatitis C. Am J Gastroenterol 1992;87:1849–51.
6. Kao JH, Chen PJ, Yang PM, et al. Intrafamilial transmission of hepatitis C virus: The important role of infections between spouses. J Infect Dis 1992;166:900–3.
7. Bach N, Bodenheimer HC Jr. Transmission of hepatitis C: Sexual, vertical or exclusively blood-borne? Hepatology 1992;16:1497–9.
8. Osmond DH, Padian NS, Sheppard HW, et al. Risk factors for hepatitis C virus seropositivity in heterosexual couples. JAMA 1993;269:361–5.
9. Bresters D, Mauser-Bunschoten EP, Reesink HW, et al. Sexual transmission of hepatitis C virus. Lancet 1993;342:210–1.
10. Hallam NF, Fletcher ML, Read SJ, et al. Low risk of sexual transmission of hepatitis C virus. J Med Virol 1993;40:251–3.
11. Gabrielli C, Zannini A, Corradini R, et al. Spread of hepatitis C virus among sexual partners of HCVAb positive intravenous drug users. J Infect 1994;29:17–22.
12. Akahane Y, Kojima M, Sugai Y, et al. Hepatitis C virus infection in spouses of patients with type C chronic liver disease. Ann Intern Med 1994;120:748–52.
13. Seeff LB, Alter HJ. Spousal transmission of the hepatitis C virus? Ann Intern Med 1994;120:807–9.
14. Skidmore SJ, Collingham KE, Drake SM. Brief report: Sexual transmission of hepatitis C. J Med Virol 1994;42:247–8.
15. Chang TT, Liou TC, Young KC, et al. Intrafamilial transmission of hepatitis C virus: The important role of inapparent transmission. J Med Virol 1994;42:91–6.
16. Garcia-Bengoechea M, Cortes A, Lopez P, et al. Intrafamilial spread of hepatitis C virus infection. Scand J Infect Dis 1994;26:15–8.
17. 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;171:768–75.
18. Nakashima K, Ikematsu H, Hayashi J, et al. Intrafamilial transmission of hepatitis-C virus among the population of an endemic area of Japan. JAMA 1995;274:1459–61.
19. Healey CJ, Smith DB, Walker JL, et al. Acute hepatitis C infection after sexual exposure. Gut 1995;36:148–50.
20. Kao JH, Hwang YT, Chen PJ, et al. Transmission of hepatitis C virus between spouses: The important role of exposure duration. Am J Gastroenterol 1996;91:2087–90.
21. Salleras L, Bruguera M, Vidal J, et al. Importance of sexual transmission of hepatitis C virus in seropositive pregnant women: A case-control study. J Med Virol 1997;52:164–7.
22. Caporaso N, Ascione A, Stroffolini T. Spread of hepatitis C virus infection within families. Investigators of an Italian Multicenter Group. J Viral Hepat 1998;5:67–72.
23. Neumayr G, Propst A, Schwaighofer H, et al. Lack of evidence for the heterosexual transmission of hepatitis C. Q J Med 1999;92:505–8.
24. Wejstal R. Sexual transmission of hepatitis C virus. J Hepatol 1999;31(Suppl 1):92–5.
25. Maida MJ, Daly CC, Hoffman I, et al. Prevalence of hepatitis C infection in Malawi and lack of association with sexually transmitted diseases. Eur J Epidemiol 2000;16:1183–4.
26. Ackerman Z, Ackerman E, Paltiel O. Intrafamilial transmission of hepatitis C virus: A systematic review. J Viral Hepat 2000;7:93–103.
27. Stroffolini T, Lorenzoni U, Menniti-Ippolito F, et al. Hepatitis C virus infection in spouses: Sexual transmission or common exposure to the same risk factors? Am J Gastroenterol 2001;96:3138–41.
28. Meisel H, Reip A, Faltus B, et al. Transmission of hepatitis C virus to children and husbands by women infected with contaminated anti-D immunoglobulin. Lancet 1995;345:1209–11.
29. Power JP, Davidson F, O'Riordan J, et al. Hepatitis C infection from anti-D immunoglobulin. Lancet 1995;346:372–3.
30. Gross JB. Hepatitis C: A sexually transmitted disease? Am J Gastroenterol 2001;96:3051–3.
31. Piazza M, Sagliocca L, Tosone G, et al. Sexual transmission of the hepatitis C virus and efficacy of prophylaxis with intramuscular immune serum globulin. A randomized controlled trial. Arch Intern Med 1997;157:1537–44.
32. Tisminetzky SG, Gerotto M, Pontisso P, et al. Genotypes of hepatitis C virus in Italian patients with chronic hepatitis C. Int Hepatol Commun 1994;2:105–12.
33. Felsenstein J. PHYLIP (Phylogeny Inference Package) version 3.5c. Department of Genetics, University of Washington, Seattle, 1993.
34. Brown AJL. Methods in evolutionary analysis of viral sequences. In: Morse SS, eds. The evolutionary biology of viruses. New York, Raven Press; 1994:75–84.
35. Bellentani S, Tiribelli C, Saccoccio G, et al. Prevalence of chronic liver disease in the general population of northern Italy. Hepatology 1994;20:1442–49.
36. Kao JH, Liu CJ, Chen W, et al. Low incidence of hepatitis C virus transmission between spouses: A prospective study. J Gastroenterol Hepatol 2000;15:391–5.