Hepatitis C virus: molecular and epidemiological evidence of male-to-female transmission

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ABSTRACT

Introduction: There is general consensus that hepatitis C virus is efficiently transmitted by the parenteral route, whereas data on viral transmission by sexual or non-sexual intrafamilial contact are conflicting. **Objective and Method:** The aim of this study was to investigate the transmission of hepatitis C virus in nine heterosexual couples. **Result:** The mean age of the couples was 43.7 years. When interviewed, all of the women denied the presence of risk factors for acquisition of the infection, whereas the cause of infection in the nine husbands could be attributed to blood transfusions in two of them (22.2%), use of intravenous and inhaled drugs in six (66.7%), acupuncture in one (11.1%), and tattooing in one (11.1%). All men and none of the women reported sexual relations with sex professionals. The mean homology score (Non Structural 5b-hepatitis C virus) was 98.4%. Among the nine couples with matching subtypes, one (11.1%) was infected with subtype 1a, three (33.3%) with subtype 1b, and five (55.5%) with subtype 3a. Shared personal hygiene items showed a much higher correlation with the possible route of transmission and were better supported by the sequence homology data than the other associated risk factors. Three (33.3%) couples shared toothbrushes, seven (77.8%) shared razor blades, eight (88.8%) shared nail clippers, and six (66.7%) shared manicure cutters. **Conclusion:** Sharing of personal hygiene items was a confounding factor in the discussion of sexual hepatitis C virus transmission and the hypothesis of male-to-female transmission was supported in this study.

Keywords: sexually transmitted diseases; phylogeny; hepatitis C.

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INTRODUCTION

Alter *et al*. published the first study discussing the possibility of sexual transmission of hepatitis C virus (HCV), with multiple sex partners being identified as a risk factor.[1] However, the contribution of sexual transmission of HCV remains controversial. Data regarding the sexual transmission of HCV vary widely, with frequencies ranging from 0 to 27%. However, most studies report a percentage of 0 to 3%.[1-5] The findings of studies involving specific populations, such as HIV-positive patients, patients with sexually transmitted diseases (STDs), drug users, homosexuals and sex professionals, differ from those obtained for the general population, with the observation of a marked increase in the risk of HCV transmission.[1,3,6]

In the case of HCV-positive patients, it is still not possible to clearly distinguish between those who present a potential of sexually transmitting HCV and those who do not. In addition, for long-lasting relationships in which the index case presents low viremia and a discordant partner, the possibility of transmission of the infection in the future cannot be ruled out.[7] Halfon *et al*. demonstrated the risk of male-to-female transmission of HCV and recommended the use of protection for discordant couples with high-risk sexual practices such as anal sex.[8] Rooney and Gilson reported an estimated risk of HCV infection of 1.5 (ci: 1.05-2.2) per decade of marriage. In addition, women with HCV-positive partners presented a 3.7-fold higher chance of contracting the disease.[4]

In the present study, we investigated nine heterosexual couples, husband and wife, with a clinical and laboratory diagnosis of HCV infection. The men reported one or more risk factors for the acquisition of HCV, whereas their female sexual partners reported

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no exposure or risk factors for contracting the disease. The mean homology score (NS5b) was 98.4%. Sharing personal hygiene items was frequent in this population and contributed as a confounding factor to the discussion of sexual transmission. The risks of intrafamilial transmission were also discussed.

PATIENTS AND METHODS

Patients

Between January 1999 and December 2002, forty-five heterosexual couples with a clinical and laboratorial diagnosis of HCV infection were evaluated. Of these, 24 met the inclusion criteria. Baseline data are detailed in a recent publication.[9] Nine couples of this population were selected for this study. The patients were recruited from the Hepatitis Outpatient Clinics of the Division of Infectious Diseases, Clinical Hospital, University of São Paulo, and of the Guilherme Álvaro Hospital, Santos, São Paulo. Both hospitals are referral centers for patients with a diagnosis of hepatitis.

Inclusion criteria were the detection of HCV RNA by PCR in both spouses and the absence of co-infection with HIV and HBV.

The patients were interviewed privately using a standardized questionnaire for the collection of demographic data and risk factors for the acquisition of HCV. The variables analyzed were age, time spent living together (in years), number of sexual relations maintained by the couple per month, number of extramarital relations, and STDs, as well as risk factors for the acquisition of the disease such as blood transfusion, use of intravenous and inhaled drugs, acupuncture and tattooing. The couples were also asked about behavioral factors at home, especially about the shared use of personal hygiene items such as toothbrushes, razor blades, nail clippers and manicure cutters.

Blood was collected from the patients by vacuum venipuncture using a dry 10-mL tube. The serum was separated, centrifuged, aliquoted and stored at -85°C until the time of use.

All patients recruited for the study signed a free informed consent form.

Virological methods

TRUGENE HCV 5’NC genotyping kit: The specimens were sequenced with the TRUGENE HVC 5’NC genotyping kit according to manufacturer instructions (Bayer Health Care Diagnostics, Berkeley, CA, USA). The test uses 244-bp fragments from the 5’NC region of the HCV genome previously amplified by RT-PCR with the COBAS AMPLICOR Hepatitis C test (Roche Molecular Systems, Inc., Branchburg, NJ, USA). The amplification products were purified with the QIAquick PCR purification kit (Qiagen, Inc., Valencia, CA, USA) before use in the TRUGENE assay. HCV sequences were collected and analyzed with the OpenGene DNA sequencing system using Gene Object sequence analysis software (GL 3.1.2).[10]

HCV NS5b sequencing assay: All HCV samples were further analyzed using the TRUGENE HCV NS5B sequencing assay (Bayer Health Care Diagnostics). Prior to use, all specimens were processed with the Epicentre RNA Masterpure purification kit (Epicentre Technologies, Madison, WI, USA). A portion of the HCV NS5b region was amplified using the Titan One Step RT-PCR kit (Roche Applied Science, Mannheim, Germany) and used directly in the TRUGENE NS5B sequencing assay. HCV NS5b sequences were analyzed with the OpenGene DNA sequencing system using GeneObject DNA analysis software in the HCV NS5B GeneLibrarian module.[10,11,12]

Phylogenetic analysis: The 156-bp sequences of the HCV NS5B region produced by the HCV NS5B sequencing assay (nt 8353 to 8508) were analyzed with the PHYLIP software package, version 3.5c. Distances between pairs of sequences were estimated with the DNA DIST program. All phylogenetic trees were constructed by the unweighted pair group method using arithmetic averages on the previous sets of pairwise distance. Significance of the clustering was insured by bootstrap resampling (1,000 replicates). The following GenBank sequences were included in the analysis. Previously reported GenBank sequences were included in the analysis. All patients are mentioned in terms of Arabic numbers and letters in the Phylogenetic tree and the patients (couples) analyzed were 2a/2b, 4a/4b, 12a/12b, 18a/18b, 20a/20b, 23a/23b, 24a/24b, 26a/26b and 31a/31b.[11,12]

RESULTS

The mean age of the nine heterosexual couples was 43.7 years (women: 39.7, men: 47.7). When interviewed, all of the women (2a, 4b, 12a, 18b, 20a, 23a, 24b, 26b and 31b) denied the presence of risk factors for acquisition of the disease, whereas the cause of infection in the nine husbands could be attributed to blood transfusions in two (22.2%), use of intravenous and inhaled drugs in six (66.7%), acupuncture in one (11.1%), and tattooing in one (11.1%). The mean time spent living together was 17.9 years (range: 5 to 31) and the mean annual income was US$ 2,000. The reported number of sexual relations per month was 10.6.

The couples reported no STDs acquired over the past year but there were reports of STDs acquired before that time. Eight of the nine men and only one woman reported to have had STDs during their lives. The only woman reported herpes infection and the men reported syphilis in three (33.3%), chancre in two (22.2%), gonorrhea in five (55.5%), condyloma in one (11.1%), crabs in two (22.2%) and trichomonas in one (11.1%).

In the present study, casual relationships were defined as sporadic sexual relations (one or more times) or relationships (involving sexual intercourse) lasting less than 6 months. Female and male prostitutes were not considered to be "casual partners". In the present study, all men reported relationships with prostitutes and only two had no casual partners during their life. All women denied relationships involving paid sex and only one had two or three casual partners during her life (Table 1).

The frequency of sharing personal hygiene items was high. Among the nine couples, seven were part of a group in which at least one of the spouses admitted the shared use of razor blades, eight shared nail clippers, six shared manicure cutters, and three shared toothbrushes.

The mean homology score (NS5b- HCV) was 98.4%. Among the nine couples with matching subtypes, one (11.1%) was infected with subtype 1a, three (33.3%) with subtype 1b, and five (55.5%) with subtype 3a. The percentages of homology were 99.4%, 98.3% and 97.5% for the subtypes respectively.

The phylogenetic tree was constructed based on the sequencing of the NS5b region of the HCV genome. The sequences (156 bp) were aligned using the Clustal W program (Clustal W Multiple Sequence Alignment Program, v1.7, June 1997) and the phylogenetic tree was generated based on this alignment by the neighbor joining method included in the PHILIP 3.5c program (Figure 1).

Table 1. Number of sexual relations with casual partners and of paid sex throughout life reported by the nine MALE partners

Number of partners	Casual partner		Paid sex	
	n	%	n	%
None	2	22.2	0	0
2 to 3	2	22.2	3	33.3
4 to 10	2	22.2	5	55.5
11 to 20	2	22.2	0	0
21 to 50	1	11.1	1	11.1
Total	9	100	9	100

Figure 1: Phylogenetic tree constructed based on the sequencing of 156 bp corresponding to the NS5b region of the HCV genome.

DISCUSSION

Epidemiology

In the population studied, the women were on average 8 years younger than their partners and practically had no extramarital sexual experience. Only one woman had another partner, whereas all men reported extramarital relations and/or relations with sex professionals (Table 1). Eight of the nine men but only one woman reported STDs. Parenteral risk factors were analyzed based on the questionnaires and none of the nine women reported any risk situation for the acquisition of the disease, whereas their respective husbands presented one or more risk factors. These findings support the hypothesis of male-to-female transmission of HCV in this group.

Classically, parenteral exposure involves risk factors that are relevant for the transmission of HCV.[1,3,7,13-15] Sexual transmission of viral diseases mediated by sexual secretions suggests a greater potential of male-to-female transmission. Certainly, trauma to the mucosa during sexual intercourse increases the risk of viral transmission, as well as the high viremia levels and the presence of viral particles in semen. Male-to-female transmission seems to be more common than female-to-male.[16-18]

Various researches have been able to detected HCV in semen but studies analyzing semen by molecular biology techniques have reported difficulty in eliminating inhibitors present in the sample, which frequently leads to false-negative results. Cavalheiro *et al.* studied semen and blood samples of the 13 HCV men infected and the semen and seminal fractions (52 samples) revealed 86.5% of the results to be inhibited. This preliminary study indicates the need for new research protocols in order to obtain more reliable results.[19]

Sexual promiscuity and the use of illicit drugs before having stable relations with their current partners was a strong risk factor among men, increasing the possibility that these men might have acquired HCV infection before their partners. The women, in turn, presented sexual and intrafamilial contact as the only risk factor, which was aggravated by the sharing of personal hygiene items with their partners.

The prevalence of HCV infection is higher among prostitutes, male homosexuals, and partners of intravenous drug abusers. There seems to be a positive correlation between the HCV infection and the number of sexual partners, sexual activities involving a trauma or anal intercourse, history of other STDs, and those coinfection with HIV.[20]

Minola *et al.*, studying HCV transmission among sexual partners, reported that the risk of HCV transmission increases by 13.2% when the partners used illicit drugs.[21] Hershow *et al.* identified sexual promiscuity, intravenous drug users as partners, associated STD, paid sex and a long time (> 10 years) spent with an HCV-positive partner as significant risk factors for infection with HCV.[22]

In the present study, the couples reported a mean time spent living together of 17.9 years, an average annual income of US$ 2,000 and a mean number of 10.6 sexual relations per month.

In a review, Rooney and Gilson reported an estimated risk of HCV infection of 1.5 (confidence interval: 1.05-2.2) per decade of marriage.[4] In addition, women with HCV-positive partners presented a 3.7-fold higher chance of contracting the disease. Capelli *et al.* reported a case of asymptomatic HCV infection acquired by a repeat blood donor from her sexual partner. She admitted a sexual partnership with a man with chronic HCV infection.[23] According to Weisbord *et al.*, having sexual contact with an HCV-positive person was independently associated with being anti-HCV positive.[15]

In the case of HCV-positive patients, it is still not possible to clearly distinguish between those who present a potential of sexually transmitting HCV and those who do not. In addition, for long-lasting relationships in which the index case presents low viremia and a discordant partner, the possibility of transmission of the infection in the future cannot be ruled out.[7]

Interestingly, in the present study the frequency of sharing personal hygiene items was high. This fact is important and a matter of concern since separate questioning of the spouses showed that, if one of them is careful not to share hygiene items to prevent the risk of transmission, the other does not necessarily take the same care. It was common in the present population that one spouse omitted to the other the shared use of razor blades and toothbrushes. We believe that this finding serves as an alert and indicates the need that in households where one HCV index case is present the whole family should be made aware of not sharing cutting items.

Seven of the nine women were part of a group in which at least one of the spouses admitted the shared use of razor blades. The other two women reported the shared use of nail clippers and manicure cutters.

These high rates might be explained by cultural factors. The sharing of personal hygiene items, especially razor blades, leads to the discussion of intrafamilial transmission of HCV. A cautious attitude leads us to consider the habits that involve family contact as a confounding factor when discussing sexual transmission between these partners, preventing a categorical statement about the occurrence of sexual transmission of HCV.

In the literature, sharing personal hygiene items is commonly cited in the discussion of sexual and intrafamilial transmission of HCV. Among other investigators, Rooney and Gilson showed that, in addition to family contact and sexual behavior, the shared use of personal items such as razor blades, toothbrushes, nail clippers and manicure cutters might be an important factor for the intrafamilial transmission of HCV.[4,14,21]

This opinion does not agree with other recent Brazilian studies, where the authors concluded that the risk of transmission of HCV did not increase with a relative long periods of living together, sexual activity and sharing of personal items.[24]

Minola *et al.* stated that the risk of sexual transmission of HCV should not be underestimated and that other non-sexual factors of intrafamilial contact such as sharing personal hygiene items might be relevant for transmission of the infection.[21] Cavalheiro *et al.*, supports the hypothesis that HCV transmission occurs between partners and the shared use of personal hygiene items makes it difficult to interpret the data in terms of sexual transmission of HCV and suggests that intrafamilial transmission is a likely infection route. Comparison between sex partners and other family members residing with the same household, having a HCV carrier as a reference, shows that intrafamilial contact is an important factor in the transmission of HCV. Cultural habits differ from one population to another and can interfere in the results of epidemiological studies that involve HCV transmission.[9]

Virology

In a phylogenetically study of the NS5b region, Norder *et al.* and Zylberberg *et al.* reported this region to be a powerful tool for the investigation of nosocomial transmission of HCV, emphasizing the fact that many of the analyzed samples belong to the same viral subtype.[11,25]

The distribution of HCV types observed in the present study was compatible to that reported by others authors for the Brazilian population.[26,27]

The high percentage of similarity observed between couples, with a mean homology score of 98.4% (Figure 1), suggests that these spouses were infected with the same virus or that the infection was acquired from a common source, indicating transmission from one spouse to the other.

The Brazilian researchers group combined the NS3 and NS5 regions of HCV and suggests that couples infected with viral strains that showed monophyletic relationships, indicated a common source of contamination, yet they had no means to assess whether these contaminations were due to sexual or other forms of close contact.[28]

Morsica *et al.* and Capelli *et al.* reported acute infection of only one female patient, with similarities of 94.9% and 93.4%, respectively, with an HCV-positive sexual partner being the only risk factor in the two studies.[7,23] Chayama *et al.* reported similarity ranging from 91.4% to 99.8% in eight cases seen at a hepatology service.[29]

Williams and Alter, researchers at the Viral Hepatitis Division of the Centers for Disease Control and Prevention, followed up 263 cases of acute hepatitis C and 13% of these patients reported sexual exposure as the only source of risk for the acquisition of the disease. Serum samples from seven of 24 positive partners were analyzed and five presented genotype concordance and genome sequence identity ranging from 97.8% to 99.3%. HVR 1 was analyzed. The relationships found between these spouses support sexual activity as a route of HCV transmission in the United States.[30]

Homology between sequences only suggests the same source of infection in both partners, but does not permit to determine where or how the infection was contracted, i.e., from an external source such as sharing personal hygiene items or during sexual intercourse.[31,32]

According to Terrault, the comparison of genetic sequences between concordant couples has been used as evidence of the occurrence of transmission, but does not always support the confirmation of the route of transmission. However, this approach strongly suggests sexual transmission when no other associated risk factor is present.[31]

The phylogenetic study of HCV is a powerful and precise tool for virological analysis, especially for the evaluation of viral transmission, but if the study is based solely on virological interpretation, without support from epidemiological data, it might well reach very different conclusions. Furthermore, a study based only on epidemiological findings runs the risk of being incomplete.[9]

The high similarity between the HCV genomes supports the hypothesis of transmission of the virus between these couples. The shared use of personal hygiene items impairs the interpretation of the data in terms of sexual and intrafamilial HCV transmission. The present study supports the hypothesis of male-to-female transmission of HCV.

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