Limited Evidence of HCV Transmission in Stable Heterosexual Couples from Bahia, Brazil

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HCV infected patients frequently ask their physician about the risk of transmission to their partners. Although it is easy to answer that the risk does exist, it is difficult to quantify. We studied the transmission of HCV infection in stable heterosexual couples: anti-HCV positive patients in hemodialytic therapy and their partners. Thirty-four couples were tested by third generation ELISA and RIBA. Blood samples of anti-HCV positive patients were evaluated by RT-PCR and detected sequences were genotyped by restriction fragment length polymorphism. Concordance of infection was observed in only one couple in which both subjects were in dialytic therapy. One other partner had two positive ELISA tests and an indeterminate RIBA, with negative RT-PCR, which may suggest a false positive or a previous resolved infection. Either sexual relations, sharing of personal items and history of parenteral exposure (hemodialysis, blood transfusion) could explain transmission in the only couple with concordant infection. We observed, in accordance with previous reports, that this risk is minimal or negligible in stable heterosexual couples.
Key-Words: Hepatitis C, renal dialysis, sexual partners, sexually transmitted diseases.

The estimated global prevalence of Hepatitis C virus (HCV) infection is 2.2%, which represents 130 million people. There are marked geographic variations as the overall prevalence is < 1% in Northern Europe and reaches 15-20% in Egypt [1]. HCV infection is implicated in 27% of all cases of cirrhosis and 25% of all hepatocellular carcinomas worldwide [2]. Salvador is a large coastal urban metropolitan area where 60% of the 2.6 million inhabitants live in slum communities [3]. It is the capital and largest city of Bahia state, Brazil. The second most populous city of the state is Feira de Santana, with an estimated population of 530,000 inhabitants. A recent population based study in Salvador detected an overall HCV prevalence of 1.5% with the genotype 3 being the commonest identified (53%) followed by genotype 1b (40%) and 2 (7%) [4]. In hemodialysis patients of Salvador, the HCV seroprevalence dropped from 23.8% (1994) to 10.5% (2006) in the last decade. In these patients, the commonest identified genotype is 1b (78%) followed by 3 (11%) and 2 (5%) [5].

The clearest risk factor to acquire HCV infection is parental exposition such as blood and blood derivatives transfusion, use of illegal injecting drug use, hemodialysis, tattoos, acupuncture and organ transplants. It is generally accepted that sexual transmission of HCV does occur, however, the magnitude of its contribution to HCV epidemiology is disputed. In United States, the Center for Disease Control and Prevention (CDC) estimates that sexual contact is implicated in 15-20% of all cases [6]. A historical series of risk factors identified for acute HCV hepatitis in the United States from 1986 to 1996 clearly show a marked drop of transfusions and an elevated curve of sexual exposure in the absence of other known risk factors [7]. Most studies focusing on stable heterosexual couples, however, found a 0-3% risk of transmission from an infected partner to the other [8-12]. Therefore, many authors argument that sexual contact brings a minimal or negligible risk of transmission. On the other hand, studies focusing on sex transmission among drug abusers, homosexuals and sex workers have found higher rates [7, 13]. HCV infected patients frequently ask their physician about the risk of transmission to their partners. Although it is easy to answer that the risk does exist, it is difficult to quantify it. Few studies to date have adequately addressed this issue by selecting couples living together in sexual activity, controlling other potential sources of infection and providing genetic evidence of high degree of similarity between the viruses isolated in each partner [14, 15]. The aim of this study was to evaluate the prevalence of HCV infection in stable heterosexual partners of hemodialysis HCV infected patients.

Materials and Methods

Patients

The study enrolled patients from two hemodialysis clinics: one from Salvador and one from Feira de Santana. The Brazilian Ministry of Health requires routine screening for HCV in hemodialysis centers. In Salvador, 22 (9%) of the 241 hemodialysis patients had already a third generation ELISA positive test. Twelve patients had stable sexual partners for more than six months and nine of them agreed to enter in the study. In Feira de Santana, 54 (15%) of the 355 hemodialysis patients had already a second generation ELISA positive test. Thirty-two patients had stable sexual partners for more than six months and twenty-six of them agreed to enter in the study. One couple was made by two patients, thus, 25 couples agreed to enter in the study. All patients and partners gave written informed consent to participate in the present study. They answered a standard questionnaire that included demographic information, sexual history and data on other potential exposures to HCV. The interviews and first samples collection

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occurred in June 2003. All volunteers were informed as to the nature of the research and the samples were collected with the written consent of the subjects, according to a protocol approved by the Human Ethical Committee of the Oswaldo Cruz Foundation (CPqGM/FIOCRUZ 21/2002).

Serological Tests

All HCV infected hemodialysis patients had already a previous serological evidence of infection. For methodological standardization, all cases were confirmed by a third generation ELISA performed in *Central Laboratory for the State of Bahia* (LACEN-BA). The same test was also performed in serum samples of their partners. When a partner had a positive ELISA, the same test was repeated in a second sample and subsequently confirmed by RIBA 3.0 (Chiron Co, USA).

RT-PCR and Genotyping

Two hundred microliters of serum was used for HCV-RNA extraction using Trisol LS reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to manufacturer instructions, precipitated with ethanol, and dried [16]. HCV-RNA was immediately transcribed into cDNA using random primers (Amersham Biosciences, Piscataway, NJ, USA). cDNA was targeted by a nested-PCR directed at the 5' untranslated region using specific primers 939, 209, 940, and 211, as previously described [17]. The 251-bp (unlabeled) second PCR product was submitted to electrophoresis using a 1.5% routine agarose gel in 1X Tris borate buffer and visualized by ethidium bromide staining under ultraviolet light. Positive samples were genotyped by restriction fragment length polymorphism (RFLP) according to Davidson et al. [18]. Briefly, restriction digestions were carried out for 4-16 h after adjustment with 10X enzyme reaction buffer as appropriate. Reactions were carried out at 37ºC in the presence of 10 units each of a) RsaI and HaeIII, and b) HinfI and MvaI. Digestion products were visualized under ultraviolet light after 4% Metaphor agarose gel electrophoresis (BMA, Rockland, ME, USA) in 1X Tris borate buffer containing 0.5 µg/mL ethidium bromide. Previously genotyped samples from our laboratory were used as positive controls for genotypes 1 and 3. Samples with undetectable HCV-RNA by nested-PCR were extracted at least twice in different experiments. Even when confirmed to be negative, all patients and partners were instructed to repeat blood collection within 12 months after the first exam to avoid false-negative results.

Statistical Analyses

Statistical analyses were performed using non-parametric Mann-Whitney test (for numeric variables) and Fisher´s exact test (for categoric variables) using GraphPad Prism 4.0 software (GraphPad Software, San Diego, CA, EUA), p-values <0.05 were considered significant in this study.

Results

Median age of patients and partners enrolled in this study was 39 and 38 years, respectively. Mean ± standard deviation age was 43.4 ± 12.3 and 41.2 ± 12.8, respectively. Males accounted for 18/34 patients (52.9%). In regard to formal education status, the subjects had the following frequencies: 15 patients and 15 partners were illiterate, 52 and 58 had incomplete primary education, 18 and 15 had complete primary education, 12 and 12 had secondary education, and 3 and zero had a graduation degree. Among 34 patients in dialysis, the median period of therapy was 93 months (mean ± SD: 95.8 ± 40.1) ranging from 22 to 192 months.

The mean period of living together was 16.6 ± 13.7 years (median: 10 years) ranging from 3 to 52 years. Couples reported their sexual activity as occurring in the following intervals: daily (n=2 or 6%), within 2-3 days (n=12 or 54%), weekly (n=5 or 15%), biweekly (n=5 or 15%), monthly (n=4 or 12%) and occasionally (n=6 or 18%). Previous history of sex partners was: less than six partners (76% of patients and 73% of partners), between six and ten (15 and 23%) and more than 10 (9 and 4%). Three patients and three partners reported sex history with sex workers. Two patients and one partner reported previous sexually transmitted diseases. History of anal sex and male homosexual partner were reported by one patient.

In regard to other potential exposures to HCV, 30 (88.3%) of all patients reported previous blood transfusions, and 12 had a history of more than 10 blood or blood derivatives transfused units. Importantly, 10 of them had these transfusions before 1993 when there was no obligatory screening for HCV in blood banks. The only partner with previous history of blood transfusion was the one who also was also in dialytic therapy. Two patients and one partner reported illegal injecting drug use. None of the subjects reported tattoos, previous acupuncture treatment, needle accidents in health-care settings or symptomatic hepatitis.

In regard to potential sexual and non-sexual risk factors involved in transmission of HCV between patients and partners, the following data was collected. The use of condom was reported to be regular by one couple, sporadic by 11 couples and 23 couples reported that they have never used it. Sharing personal items was common in the population studied: 4 (11.8%) shared tooth brushers, 11 (32.4%) shared razor blades and 24 (70.6%) shared nail clippers and manicure pliers. Seven couples (20.6%) shared all these items and 2 (5.9%) did not share any of them. All demographic and risk quantification data are summarized in Table 1.

Third generation ELISA confirmed all previous ELISA positive patients including the one partner who was also in dialytic therapy and had a previous diagnosis of HCV infection. Among serum samples of all other 33 partners with no previous anti-HCV test, five (15%) were reagent at first evaluation. All these samples were from partners of patients with detectable viremia. The same test was repeated after 14 months when only one partner had a positive serum sample. RIBA confirmed that these four samples were non reagent and the sample from the partner with two positive ELISAs was RIBA indeterminate.

Table 1. Demographic and risk quantification information of enrolled patients and partners.

	Mean ± SD or n (%)
Sex of partners	
Male	16 (47)
Female	18 (53)
Mean age ± standard deviation (in years)	
Patients	43.4 ± 12.3
Partners	41.2 ± 12.8
No. of years of steady relationship	16.6 ± 13.7
No. of months in hemodialysis treatment	95.8 ± 40.1
Sexual risk exposure of partners	
Frequency of intercourse with HCV infected patients	
Daily	2 (06)
2-3/week	12 (54)
Weekly	5 (15)
Biweekly	5 (15)
Monthly	4 (12)
Occasionally	6 (18)
Use of condom	1 (03)
Men who have sex with Men (MSM)	1 (03)
History of sex with sex-worker	1 (03)
Other risk exposures of partners	
Blood transfusion	1 (03)
Dialysis	1 (03)
Sharing tooth brushers with patients	4 (12)
Sharing razor blades with patients	11 (32)
Sharing nailed clippers and manicure pliers with patients	24 (71)
Illegal injecting drug use	1 (03)
Tattoos	0
History of acupuncture sessions	0
Needle accidents in health-care settings	0

The viral RNA was detected in 26/34 (76.5%) of the anti-HCV positive patients. RT-PCR was repeated after 12 months in 6 patients with undetectable HCV, and two additional cases of viremia were detected. Viremia was not detected in the 33 partners who were not in dialysis. Genotyping identified genotypes 3a and 3 in the couple in which both subjects were in dialysis. Among the other 24 patients with detectable viremia, the following genotypes were observed: 1(n=2), 1a (n=4), 1b(n=13), 1a/b (n=2), 3 (n=1) and 3a (n= 2).

Discussion

HCV sequences can be detected in semen, cervical fluid and saliva [19-21] thus suggesting that sex or even intimate contact between family relatives may be a risk. Importantly, 20-50% of all HCV infected patients do not have an identifiable risk factor [22, 23]. A recent cohort study conducted in Egypt identified that the strongest risk factor for HCV infection was having a anti-HCV positive family member. The highest risk was observed for children younger than 10 living in the same household of an anti-HCV parent [24].

Our results mirror those of other authors that suggest HCV transmission between partners of stable heterosexual couples is minimal or negligible [25]. Indeed, we could not identify a case were this transmission occurred. The HCV positive couple shared other risk factors such hemodialytic therapy and history of blood transfusion. The other case in which positive ELISA results could not be confirmed by RIBA may have two different explanations. First, it could be a false positive. In cases sharing similarities with the present one, it is considered difficult to interpret RIBA indeterminate samples especially in low risk patients [26]. Second, it could be a case of previously resolved HCV infection with residual antibodies. This was a 27 year old female partner and, according to previous data, young females more frequently resolve episodes of acute HCV infections maintaining negative virus tests in blood [27, 28].

Few studies specifically focused on intrafamily transmission in hemodialysis HCV infected patients. In Taiwan, a study enrolling 84 hemodialysis patients, 50 spouses and other relatives observed that the 5.4% infection rate of family members was not different from the healthy control population. Among family members, there was a trend for higher infection rate in spouses when compared to other relatives (15% vs 3%) albeit not significant [29]. In Italy, none of ten patients in dialytic therapy had a HCV infected partner [30].

In Sao Paulo, phylogenetic analysis of 24 couples in whom both partners had viremia revealed 98.3% sequence similarity in 22 using NS5b region for analysis. A high frequency of sharing personal items was also observed and that precluded the distinction of sexual or non-sexual forms of transmission [25].

Some long-term hemodialysis patients, due to co-morbidities, may not be as active sexually as the general population and this could be a drawback of the present study design. However, a recent investigation of sex activity among Brazilians observed a similar rate (mean of one sex relation between 2-3 day intervals) which is similar to that observed in the present series [31].

In conclusion, we provide further evidence that HCV transmission between partners of stable heterosexual couples is minimal or negligible. Although the present study was hampered by a relatively small sample, the results are in agreement with similar reports, particularly those enrolling hemodialysis patients with a comparable casuistics. Our study population had a relatively long period of living together, sexual activity and sharing of personal items. These factors did not increase the risk of HCV transmission in the population studied.

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