Interspousal transmission of hepatitis C in Thailand

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Background. Previous studies evaluating the possibility of interspousal sexual transmission of hepatitis C virus (HCV) have yielded many conflicting results. Our study was carried out to determine the exact potential and risk factors of interspousal HCV transmission. Methods. The spouses (54 men and 106 women; mean age ±SD, 48 ± 8 years) of 160 patients with HCV infection (106 men and 54 women) were serologically tested for HCV using a third-generation enzyme-linked immunosorbent assay (ELISA). Positive results were confirmed by reverse transcriptase polymerase chain reaction (RT-PCR). For positive couples, the cluster nucleotides of the HCV gene and genotypes were compared on the basis of restriction fragment length polymorphism (RFLP), Innogenetic Line Probe Assay (INNO-LiPA), and direct sequencing. Similarly, phylogenetic tree and sequence homology analysis was performed in order to precisely verify interspousal transmission. Risk factors promoting interspousal HCV transmission were also identified. Results. Throughout a mean duration of exposure of 23 5 years, most of the 160 partners had their usual and unprotected sexual relationships with the index patients. HCV-associated antibodies and HCV-RNA were detected in only 3 (1.88%) of the 160 spouses. Furthermore, homology and phylogenetic tree analysis could not clearly demonstrate that any one of these 3 positive spouses was infected with the same strain of HCV as that identified in the index cases. Because a positive group remained elusive, risk factors of interspousal HCV transmission could not be determined in this study. Conclusions. According to this study, interspousal transmission of HCV seems to be very rare. HCV-positive spouses should be firmly reassured that they can maintain their normal marital life.

Key words: hepatitis C virus, transmission, RFLP, risk factors

Introduction

Hepatitis C virus (HCV), the principal cause of non-A, non-B hepatitis,1 is an RNA virus belonging to the family of flaviviridae; an estimated 170 million people, or about 3% of the global population, have been infected with HCV.2 The virus can be grouped into at least six genotypes in different geographic areas.3 Infection with different genotypes may affect the clinical outcome and response to treatment.4–7

Hepatitis C virus is transmitted by direct percutaneous exposure to infected blood products, such as the transfusion of various derivatives of blood products, and by intravenous drug abuse, which are well-established causes.8,9

To prove the mode of transmission, the identification of common strains of HCV can be performed by various methods, e.g., genotyping and polymorphism analysis,10 direct sequencing of the genome, and phylogenetic analysis.11

Although sexual contact has been implicated as a route of transmission, the results are still controversial.12–18 Infrequent sexual transmission of HCV has been shown in studies performed in Western countries, but many studies originating from Asia suggest that interspousal transmission may be crucial for the interfamilial spread of HCV, with a longer duration of marriage as the most evident risk factor.17,18 Sexual transmission was documented in the presence of coexistent HIV infection, and this suggested the co-transmission of HCV and HIV to be more efficient than HCV transmission alone.19

To determine more accurately the epidemiology of interspousal HCV-transmission and possible risk
factors, spouses of HCV-infected index patients were enrolled in this study. We tested for HCV infection by screening for anti-HCV and HCV-RNA. Furthermore, we applied molecular biological analysis, including genotyping, direct nucleotide sequencing, homology, and phylogenetic tree analysis to precisely document interspousal transmission, if it indeed existed. These issues are essential for providing accurate counseling to HCV-positive spouses, about how to maintain their marital life and whether any preventive measures should be routinely recommended.

Subjects and methods

The study proposal was approved by the Ethics Committee, Ministry of Public Health. Prior to enrolment, all patients were informed about the objective of the study and written consents were obtained from the patients and their spouses.

Population study

The spouses of 160 HCV-infected patients who attended Phramongkutklao, Siriraj, and Vichaiyut hospitals, Bangkok, from January to December 2001 were consecutively recruited into the study. All of the HCV-infected patients (index cases) were negative for HIV infection, based on serology. Before blood sampling, the couples were asked to complete a questionnaire addressing issues such as the duration of their marriage, the history and timing of any parenteral exposure, sexual behavior before and after recognition of HCV infection, based on serology.

Laboratory tests

Serology
The spouses, 54 men and 106 women (mean age, 48 ± 8 years), were tested for HCV-associated anti-bodies, using a third-generation enzyme-linked immuno sorbent assay (United Biochemical, Beijing, China) according to the manufacturer’s recommendations.

HCV-RNA detection
The anti-HCV-positive plasma samples were screened for HCV-RNA. All target specimens were tested for HCV-RNA by reverse transcriptase polymerase chain reaction (RT-PCR), using primers specific for the core region. Having obtained the plasma samples, we extracted RNA by employing the guanidinium method, and we performed RT-PCR as previously described,21,22 reverse transcribing it into cDNA, using primer 410, representing the core region. We used nested primer pairs 954 and 410, and 953 and 951, respectively, for amplification.23 The nucleotide (nt) sequences of these primers were: primer 954, 5′ AGGTCTCGTAGAC GGGGTGCTTGCGAC 3′ (nt –30–nt –51) as an outer sense primer; and primer 410, 5′ GCCGATCTCAT GGGTATAT 3′ (nt 350–nt 332) as an outer anti-sense primer; primer 953, 5′ AGGTCTCGTAGAC CGTGC ACCATG 3′ (nt –16–nt 3) as an inner sense primer; and primer 951, 5′ TCA TCGATAC CCTTA CATG 3′ (nt 324–nt 306) as an inner anti-sense primer. Accordingly, we modified the annealing temperature to 48°C. The details of the amplification steps have been presented elsewhere. Upon completing the second PCR round, we analyzed the 405-bp product between positions –21 and 383 by electrophoresis on a 1.5% Nu-Sieve agarose gel (FMC, Rockland, ME, USA) stained with ethidium bromide. Under UV light, the amplified core region became visible.

HCV genotyping
Genotyping was performed applying restriction fragment length polymorphism (RFLP)23,24 and the Innogenetic Line Probe Assay (INNO-LiPA) HCV II line probe assay.

For RFLP, we employed a modified method, as described by Mellor et al.,24 using the restriction enzymes Ava I and Smal. This method is intended to use these enzymes to discriminate genotype 6a from genotype 1 by conventional 5′ noncoding region (NCR) determination (INNO-LiPA test). The digested products were visualized under UV light after electrophoresis on a 3% Nu-Sieve agarose gel containing ethidium bromide (FMC) in 1× Tris Buffer EDTA (TBE) buffer.

For the INNO-LiPA assay, labeled PCR products obtained from the 5′ NCR (INNO-LiPA HCV Amplification kit; Innogenetic, Ghent, Belgium) were hybridized to immobilized oligonucleotide probes specific for the six major genotypes and capable of identifying most subtypes.

Sequencing
The target PCR products on the agarose gel were purified for sequencing, using a Perfectprep Gel Cleanup Kit (Eppendorf; Westbury, NY, USA), according to the manufacturer’s specifications, and subjected to 1.5% agarose gel electrophoresis in order to ascertain their purity.