

CONCISE COMMUNICATION

Prospective Reevaluation of Risk Factors in Mother-to-Child Transmission of Hepatitis C Virus: High Virus Load, Vaginal Delivery, and Negative Anti-NS4 Antibody

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Of 21,791 pregnant women screened in Tottori Prefecture, Japan, 127 (0.58%) were positive for anti-hepatitis C virus (HCV) antibody and 84 (0.39%) were positive for HCV RNA. Of 84 children followed up for at least 6 months, 7 (8%) were infected. All of them were born to 26 mothers with a high virus load (HVL; $\geq 2.5 \times 10^6$ RNA copies/mL [27%]), compared with 0 of 58 children born to non-HVL mothers ($P < .001$). Because all the infected children were vaginally delivered, the infection rate among 16 vaginally delivered children born to the HVL mothers was as high as 44%. The prevalence of anti-NS4 antibody in the mothers with an infectious HVL was significantly lower than that in the mothers with a noninfectious HVL ($P = .048$). Analysis of our results suggests that maternal HVL, vaginal delivery, and negative anti-NS4 antibody are significant risk factors for the mother-to-child transmission of HCV.

Screening of donors and heat treatment of blood products essentially ceased the transfusion-associated spread of hepatitis C virus (HCV) [1]. Several groups have observed the mother-to-child transmission of HCV in ~10% of HCV-positive mothers [2, 3], whereas others did not [4]. The true risk of mother-to-child transmission is still unclear, because carrier mothers were screened by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis, with varying sensitivities [5–7]. Nevertheless, a maternal high virus load (HVL) has been suggested as a major risk factor [2, 3]. Maternal coinfection with human immunodeficiency virus (HIV) complicated the matter further, because, in this population, HCV transmission occurs more often [3, 8].

The clinical significance of mother-to-child transmission of HCV has not been confirmed. Although some reports have indicated that most infected children developed chronic liver dysfunction [7], children remained asymptomatic in other studies [3]. In our experience, 4 of 6 patients, including a patient with 2-year persistence of HCV, possessed chronic elevation of

transaminase, and only 2 of 6 recovered from viremia within the 3-year observation period [9, 10].

The relative importance of mother-to-child transmission in developed countries is now significantly higher than would be expected because of the elimination of the blood-borne spread of HCV. We decided to look for a plausible measure to prevent mother-to-child transmission, so we prospectively reevaluated risk factors for the infection of children born to HCV-positive mothers.

Materials and Methods

Subjects. From June 1992 to December 1998, 21,791 pregnant women were screened for anti-HCV antibody in Tottori Prefecture, Japan. None of the mothers had risk factors for HIV infection, and the officially reported carrier rate in the prefecture is $<10^{-5}$. Seropositive (Ab^+) samples were subjected to RT-PCR analysis. The RT-PCR positive samples (RNA^+) were titrated by a branched DNA assay.

Serum samples of the children born to Ab^+ mothers were tested for anti-HCV antibody, HCV RNA, and liver function approximately every 3 months during the first year and biannually thereafter. Minimum follow-up period was 6 months. Breast-feeding was not discouraged.

Anti-HCV antibody. A second generation anti-HCV antibody assay kit based on passive hemagglutination (Dainabot, Tokyo, Japan) was used for screening. Positive samples were confirmed by a self-prepared Western blot that used a recombinant core antigen [9]. A third-generation enzyme immunoassay kit was used for epitope-specific antibodies (Sympep HCV-EIA II; Kyokuto Med, Tokyo, Japan).

Nested RT-PCR analysis. Methods for the nested RT-PCR

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Informed consent from each mother was obtained before and after the screening, and human experimentation guidelines of each participating institution were followed in the conduct of this research.

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analysis targeted on the 5' untranslated region were described elsewhere [9]. In brief, RNA was extracted from 50 μ L of the serum sample mixed with 20 μ g of glycogen (Boehringer-Mannheim, Mannheim, Germany) by the guanidinium method. After reverse transcription with 20 U of Moloney murine leukemia virus RT (Superscript; Gibco BRL, Gaithersburg, MD) at 45°C for 60 min, the whole product was used for the first PCR for 35 cycles at 94°C for 1 min (3 min for the first cycle), 55°C for 1 min, and 72°C for 2 min (7 min for the last cycle) for each cycle. A 2- μ L aliquot was used for the second PCR, with the inner primer pair under the same conditions. Reproducibility of each positive signal was confirmed by repeating the test on another day.

The minimum detection level of the RT-PCR was 10 HCV RNA copies per reaction, or 200 copies/mL of serum calibrated by in vitro synthesized HCV RNA from a deletion plasmid [9]. Two tubes containing 20 copies per reaction of the deletion plasmid DNA and a tube without a template were added to each run of the RT-PCR as the positive and negative controls, respectively. Data were discarded if both of the positive control tubes in a run were negative, and none of the negative controls became positive in any run.

Quantitative HCV-RNA assay and genotype. Serum HCV RNA was quantified by the branched DNA (bDNA) kit (Quantiplex version 1.0; Chiron, Emeryville, CA); the minimum detection level of HCV RNA was 0.5×10^6 copies/mL. The HCV genotype was determined by a nested RT-PCR kit (Sumai Test; Tokusyu Men'eki, Tokyo, Japan).

Potential maternal and perinatal risk factors. The following parameters were analyzed as potential risk factors for mother-to-child transmission: maternal HCV RNA level, mode of delivery, history of blood transfusion, history of hepatitis, HCV genotype, gestation period, intrapartum period, body weight at birth, placental weight, bleeding volume during delivery, feeding method, and epitope-specific maternal antibodies.

Statistical analysis. Unpaired Student's *t* test with Welch's correction was applied for numeric data after logarithmic conversion, and Fisher's exact test was used for dichotomized data.

Results

Sampling of mother-child pairs. Of the 21,791 pregnant women screened for anti-HCV antibody, 127 (0.58%) were Ab⁺ and 84 (0.39%) were RNA⁺; the latter represented 66% of Ab⁺ mothers. Of 43 dropouts, 41 were not referred to pediatricians who participated in this study. Those enrolled in this study consisted of 73 Ab⁺ and 50 RNA⁺ mothers, and 84 and 59 children born to these mothers, respectively. These mothers included 7 with 2 successive children, 1 with 3 successive children, and 1 with a pair of twins.

Children infected by mother-to-child transmission. Seven children born to 5 mothers were found to be positive for HCV RNA by RT-PCR analysis. The other children never tested positive within the follow-up period. Each possessed high titers of HCV RNA within 3 months of the age at the geometric average titer of 19.5×10^6 copies/mL (95% confidence interval [CI], 9.5×10^6 to 42.5×10^6 copies/mL). All 7 cord blood samples were negative by the bDNA assay (bDNA⁻), but 3 of 6

were found to be positive by RT-PCR analysis at titers $\leq 10^3$ copies/mL. In contrast, only 1 of 26 cord blood samples of uninfected children was found to be positive by RT-PCR analysis. The genotype of HCV in each mother-child pair was identical: genotype 1b in 4 pairs, including a pair of siblings, genotype 2a in one, and genotype 2b in a pair of siblings.

Maternal HCV titer and transmission. Of 77 Ab⁺ mothers without infected children (i.e., mothers who were noninfectious), 53 (69%) were RNA⁺, and 41 (53%) were bDNA⁺. Geometric average titer of the bDNA⁺ mothers was 2.7×10^6 copies/mL (95% CI, 1.9×10^6 to 3.8×10^6 copies/mL). In contrast, all the mothers with infected children (i.e., mothers who were infectious) were RNA⁺, with a geometric average titer of 8.0×10^6 copies/mL (95% CI, 3.8×10^6 to 16.7×10^6 copies/mL; *P* = .016). It must be noted that 43 (51%) of 84 Ab⁺ mothers were bDNA⁻ and therefore were not included in the comparison. By use of the average titer of noninfectious bDNA⁺ mothers, 26 mothers with a virus load $\geq 2.5 \times 10^6$ copies/mL were classified as HVL mothers. They consisted of 31%, 44%, and 54% of the Ab⁺, RNA⁺, and bDNA⁺ mothers, respectively. All the mothers who were infectious were classified with the HVL mothers. Relative risk of mother-to-child transmission for the HVL mothers versus the Ab⁺ mothers was 3.5 (95% CI, 1.1–10.9; *P* = .041), based on mothers, and 3.2 (CI, 1.3–8.4; *P* = .020), based on children.

Vaginal delivery as a risk factor. Of 84 deliveries, 28 babies were delivered by cesarean section (CS): 9 for precedent CS, 2 for breech presentation, 4 for cephalopelvic disproportion, 2 for twin pregnancy, 1 for gestosis, and 10 for unknown reasons (table 1). None were directly related to the HCV infection. All 7 infected children were delivered vaginally, and none of 18 CS children born to RNA⁺ mothers were infected. Among the children born to mothers with HVL, the vaginally delivered children had a significantly higher risk of infection than did the CS children (*P* = .023; table 1). Geometric average of the HCV RNA level in the 5 infectious mothers, 7.0×10^6 copies/mL, was significantly higher than that of the 31 noninfectious RNA⁺ mothers, which was 1.5×10^6 copies/mL (*P* < .001; table 2). Interestingly, an HVL mother had 2 successive children delivered vaginally who were infected and then bore a third CS child who was free from infection.

Effect of other maternal and perinatal factors on HCV transmission. We surveyed the possible role of maternal parameters in the mother-to-child transmission of HCV, including preceding history of blood transfusion, history of clinical hepatitis, and HCV genotype. None of these maternal parameters was significantly different between the mothers who were infectious and those who were not.

None of the perinatal parameters tested were significant between the infected and uninfected children, including gestation period, body weight at birth, placental weight, and bleeding volume during labor. The average intrapartum period was longer for the infected children, but the difference disappeared

Table 1. Mother-to-child transmission of hepatitis C virus (HCV) and the route of delivery.

Status, delivery	No. enrolled ^a	No. positive	Positive, %	P ^b
Ab ⁺ mothers				
Mothers				
Vaginal	45	5	11	.159
Cesarean	23	0	0	
Children				
Vaginal	50	7	14	.045
Cesarean	28	0	0	
RNA ⁺ mothers				
Mothers				
Vaginal	36	5	14	.304
Cesarean	14	0	0	
Children				
Vaginal	41	7	17	.089
Cesarean	18	0	0	
HVL mothers				
Mothers				
Vaginal	13	5	38	.111
Cesarean	8	0	0	
Children				
Vaginal	16	7	44	.023
Cesarean	10	0	0	

NOTE. Ab⁺, antibody positive, as assessed by second-generation assay and confirmed by Western blot test; HVL, high virus load $\geq 2.5 \times 10^6$ copies/mL, as determined by branched DNA assay; RNA⁺, HCV RNA positive by reverse transcriptase–polymerase chain reaction (≥ 200 copies/mL). All the mothers who were infectious had HVL.

^a Six deliveries without record of the delivery method were deleted.

^b Fisher's exact test.

when compared with the vaginally delivered children. The sample size was too small to test the effect of breast-feeding.

Epitope-specific antibodies and mother-to-child transmission.

All Ab⁺ mothers were positive for anti-core antibody. Anti-NS3 and -NS5 antibodies were positive in ~70% and ~40%, respectively, of mothers who were infectious and mothers who were noninfectious. Anti-NS4 antibody was positive in 12 (44%) of 27 RNA⁺ but non-HVL mothers and in 13 of 21 HVL mothers (62%; not significant). The positive rate for anti-NS4 antibody among the infectious mothers, 1 (20%) of 5, was significantly lower than that of the noninfectious HVL mothers, 12 (75%) of 16 ($P = .048$). However, the significance was lost when compared with the mothers who delivered vaginally, although a trend remained (5 [71%] of 7; $P = .242$).

Discussion

The most well documented risk factor for mother-to-child transmission of HCV is maternal HVL [2, 9, 11]. The risks of mother-to-child transmission were 8%, 12%, and 27% in children born to Ab⁺, RNA⁺, and HVL mothers, respectively. Analysis of our data suggests that the mother-to-child transmission of HCV among non-HIV mothers is, in fact, more frequent than generally thought, if the mothers were carefully selected. Importantly, no mothers who were infectious were found in the non-HVL group. However, the threshold of HVL used in this

study, $\geq 2.5 \times 10^6$ copies/mL, might be lower if the sample size were larger or for mothers coinfecting with HIV [3, 11].

All the infected children were vaginally delivered, and the risk of infection in vaginally delivered children born to HVL mothers was 44%. This high risk might be explained by intra-partum microtransfusion from mother to fetus, because it is well known that a prearranged CS can minimize the microtransfusion [12, 13]. These results, as well as the minimum amount of HCV RNA in the cord blood and the rapid increase of virus load within the first 1–3 months of life, are strong evidences for perinatal transmission. A previous study failed to find HCV infections in siblings of the index patients [14]. However, in this study, 2 of 3 HVL mothers who vaginally delivered >1 child each had 2 infected children, which suggests the presence of additional maternal risk factors.

Among HVL mothers, the prevalence of anti-NS4 in infectious mothers was marginally lower than that in noninfectious mothers ($P = .048$), which is consistent with findings in a previous report [15]. The finding may be related to a more recent acquisition of HCV infection among the mothers who were infectious, which explains the higher levels of viremia.

As maternal HVL and vaginal delivery are the major risk factors for the mother-to-child transmission of HCV, HVL at the time of delivery would be the critical factor for transmission. Approximately 40% of vaginally delivered children born to HVL mothers were infected with HCV, and more than half the infected children developed the chronic liver disease. Near-term titration of HCV would be recommended for Ab⁺ pregnant women and CS for those with HVL. The natural history of infected children is still uncertain. However, if it is strongly associated with chronic active hepatitis, liver cirrhosis, or even hepatocellular carcinoma, more intensive measures might be necessary. For example, interferon therapy might be considered to lower the HCV RNA titer at the time of delivery, if the treatment is proved safe and tolerable in the third trimester. A further study on a larger scale is required to substantiate this suggestion.

Table 2. Maternal hepatitis C virus (HCV) RNA titers of vaginally delivered children born to RNA⁺ mothers.

RNA ⁺ subjects	No. tested	HCV RNA, $\times 10^6$ copies/mL ^a		P ^b
		Geometric average	95% CI	
Mothers ^c				
Infectious	5	7.0	2.4–20.0	<.001
Noninfectious	31	1.5	0.9–2.3	
Children				
Infected	7	8.0	3.8–16.7	<.001
Uninfected	34	1.4	0.9–2.2	

NOTE. CI, confidence interval; RNA⁺, HCV RNA positive by reverse transcriptase–polymerase chain reaction (≥ 200 copies/mL).

^a HCV RNA titer determined by branched DNA assay. For the negative sample in branched DNA assay, 0.2×10^6 copies/mL was assigned.

^b Unpaired Student's *t* test with Welch's correction after logarithmic conversion.

^c For computation on 10 mothers with >1 child, data obtained from the first-order children were used.

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