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Hepatitis C Virus Infection in Pregnancy and the Risk of Mother-to-Child Transmission

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The risk of vertical transmission of the hepatitis C virus (HCV) from infected mothers to their children during pregnancy and delivery was determined in 120 children born to HCV-positive mothers. Methods included enzyme immunoassay and immunoblot for detection of HCV antibodies and reverse transcription polymerase chain reaction (RT-PCR) for detection of viral RNA. Six (5%) children were perinatally infected with HCV as shown by RT-PCR. None of the infected children had clinical signs of hepatitis. None of the pregnancies was complicated by abortion, stillbirth, premature birth, or malformation of the child. Special concern was given to the possibility of HCV transmission via breast milk. In no breast milk sample obtained from 34 HCV-infected mothers was HCV RNA detected. These observations indicate that HCV infection is not necessarily a contraindication for breast-feeding.

Infection with the hepatitis C virus (HCV) most often leads to persistent viremia and can result in chronic liver disease (1). In 1991 we reported finding a low prevalence of HCV antibodies in sexual or household contacts of HCV-infected patients (2), even though HCV RNA can usually be detected not only in the blood but also in the saliva and tears of infected patients (3, 4). Since then, we have given special attention to the possible transmission of HCV from infected mothers to their children during pregnancy and delivery. In previous studies on this subject, the numbers of patients included have been small (5–9). One group suggested that the risk of vertical transmission of HCV may be enhanced by coinfection with the human immunodeficiency virus (HIV), possibly due to increased HCV viremia caused by the immunosuppressing effect of HIV (10), but others failed to find such an association (11).

The present study, carried out between September 1991 and February 1996, was undertaken to assess the risk of mother-to-infant transmission of HCV in a large group of prospectively followed pre- and peri-natally exposed children. In addition, we collected samples of breast milk from HCV-infected mothers for determination of HCV RNA in order to estimate the risk of HCV transmission via breast-feeding. The time required for children to lose maternal HCV antibodies was assessed in follow-up studies.

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Patients and Methods

Patients. The prospective study included 120 children born to 117 mothers who had at least one positive HCV antibody test or reverse transcription polymerase chain reaction (RT-PCR) result during pregnancy; one mother gave birth to twins. Children were a median of 3 days old (range, 0–355 days) on entry into the study. They were retested for anti-HCV and HCV RNA at five-month intervals. None of the children had clinical signs of hepatitis. Most of the children (n=107) were tested immediately after birth; 13 were between 2 and 11 months old when tested for the first time. The majority of mothers had risk factors for acquiring hepatitis C, including intravenous drug use (n=71), blood transfusions (n=6), or receipt of clotting factor concentrates for treatment of Willebrand's disease (n=1); however, in 39 cases the route of HCV transmission could not be determined. In 19 cases no maternal serum sample was available for PCR testing or antibody determination in our laboratory. In these cases the HCV infection was confirmed in external laboratories. Samples of breast milk were obtained from 34 mothers one day to two months after delivery.

Hepatitis C Virus Antibody Assays. Antibodies were determined with a second-generation enzyme immunoassay (EIA) according to the manufacturer's instructions (Abbott, Ger-

Table 1: Vertical transmission of hepatitis C virus (HCV) in 120 perinatally exposed children.

		HCV antibodies		HCV PCR	
Age at first test	No. of children	No. tested	No. positive	No. tested	No. positive*
0–31 days	107	101	101	101	4
1–11 months	13	13	12	12	2

* Two children positive for HCV RNA immediately after birth but negative in follow-up examinations were designated non-HCV-infected and are not included among the PCR-positive results.
PCR, polymerase chain reaction.

many). Confirmatory testing was done with an in-house recombinant immunoblot assay described earlier using recombinant antigens of the NS5, NS4, NS3, and core region of HCV (12).

Reverse Transcription Polymerase Chain Reaction. Freshly drawn serum or milk was added to an RNAse-inhibiting solution (13) and stored at −20°C. RNA was extracted, reverse transcribed, and amplified. The PCR was performed with primers of the highly conserved 5' untranslated region, as described previously (14). To confirm their specificity, PCR products were blotted and hybridized (14). Samples were designated as negative when they were negative in two to three assays in which a positive control was clearly positive. Children were suspected to be HCV-infected if they were positive for HCV RNA. If the PCR result was positive during the first week of life but negative later, we considered the child noninfected. Milk samples were divided: one part was mixed with serum containing HCV RNA, and the other was not. Reverse transcription (RT) PCR was performed with both samples; the technicians were not aware which sample contained serum.

Follow-up. Twenty-five children were retested for anti-HCV and HCV RNA every five months until they became negative for HCV antibodies.

Biochemical Tests for Liver Function. All sera were tested for transaminases (alanine aminotransferase, ALT) and bilirubin levels.

Results

Diagnosis of Hepatitis C Virus Infection in Pregnant Women. Maternal serum from 98 women was available for testing in our laboratory. Maternal HCV infection was detected or confirmed in our laboratory before conception (n=39), during pregnancy (n=26), or at delivery (n=33). In 19 cases we did not receive maternal blood samples to confirm the diagnosis of HCV infection made by external laboratories. The RT-PCR was performed in 78 cases; HCV RNA was detected in 62 sera. In the 16 PCR-negative sera and the remaining 20 sera, HCV infection was confirmed by immunoblot. Fifty-three of 84 mothers tested had active hepatitis, shown by elevated ALT levels ranging from 20 to 117 U/l (median, 30 U/l; upper normal value, 19 U/l). There was no difference in ALT levels between mothers positive (median, 25 U/l; range, 15 to 90 U/l) or negative (median, 20 U/l; range, 15 to 117 U/l) by RT-PCR.

Course of Pregnancy and Delivery. None of the pregnancies was complicated by abortion, stillbirth, premature birth, or malformation of the child.

Vertical Transmission. Six (5%) of 120 perinatally exposed children were HCV-infected as demonstrated by serological investigation and RT-PCR (Table 1). Four of these children were monitored for, respectively, 18 months and three, four, and seven years; all remained positive for anti-HCV and HCV-RNA, thereby confirming perinatal transmission. In the remaining two children HCV infection was diagnosed immediately after birth, but no further samples were available; therefore, these results have not yet been confirmed.

Two other children were PCR-reactive immediately after birth, but follow-up examinations done at the age of 1, 2, and 9 months in one child and at 5 and 6 months in the other revealed a negative PCR result, indicating that there was no viral replication. The observed decline of maternal HCV antibodies also supports this assumption. Therefore, these children were considered noninfected.

Course of Maternal Antibodies in Noninfected Children. Twenty-four of the 107 newborns negative for HCV by RT-PCR were monitored until they lost maternal antibodies, which took from 3 to 22 months. In most cases (n=19) anti-HCV was lost during the first year of life. One child was still positive after 13 months, but at 22 months the child had become negative. The PCR result remained negative throughout the 22 months.

Hepatitis C Virus Transmission Via Breast Milk. None of the samples of breast milk obtained from 34 HCV-infected mothers was positive for HCV RNA, and none of the breast-fed children of these mothers became HCV-infected. None of 16 additional children who were breast-fed with-

out testing of a breast milk sample became positive for HCV RNA during the first year of life. In addition, all HCV-infected children who were tested immediately after birth had a positive PCR result with their first blood sample. In no case did we observe seroconversion afterward, which would have been the case had transmission occurred by breast-feeding. Breast milk did not interfere with RT-PCR, since sera containing HCV RNA and mixed with the milk samples remained positive for HCV RNA.

Influence of Coinfection with the Human Immunodeficiency Virus. In our study HIV status was known for only 41 of the 117 mothers; five of these were positive for HIV type 1. These mothers gave birth to six children; one became perinatally infected with HCV, with no transmission of HIV-1.

Immunotolerance in Hepatitis C Virus Infection. Sera of 14 children who were RT-PCR negative and anti-HCV positive at birth were retested for the presence of HCV RNA after they had lost HCV antibodies: all remained negative. In addition, all HCV-infected children were positive for both HCV antibodies and HCV RNA.

Clinical Course of Perinatally Transmitted Hepatitis C Virus Infection. To date, none of the six HCV-infected children in our study have developed clinical evidence of hepatitis or liver disease, as demonstrated by their normal ALT (6 to 12 U/l) and bilirubin levels and their clinical status immediately after birth and on at least two to three occasions during the first year of life.

Discussion

In our study of 120 children born to 117 HCV-infected mothers, eight children were positive by RT-PCR during the first year of life. Of these, four were shown to be HCV-infected by subsequent tests. Two became negative during follow-up and did not develop antibodies; therefore, they were considered noninfected. Because follow-up sera could not be obtained from two children, these cases must be considered indeterminate. Overall, the rate of perinatal transmission was between 3 and 5%.

Previous studies yielded controversial results, especially when they involved small numbers of children (5, 6). In larger study populations the reported transmission rates ranged from 2.3 to 6.9% (10, 11, 15, 16). Levels of maternal viremia may influence transmission rates, as suggested by Moriya et al. (15) and Ohto et al. (16). However, Zanetti et al. (10) reported that although mothers coinfected with HCV and HIV had higher mean titers of HCV RNA than those with HCV alone, no significant difference was seen between coinfected mothers who transmitted the HCV infection and those who did not. In our study, one of the six children born to five HIV-infected women was infected with HCV, while none of the children were positive for HIV. However, this number is too small to draw any conclusions.

In another study from our laboratory, tear fluid of HCV-infected persons was positive for HCV RNA by RT-PCR (3). Therefore, it was of interest whether mother-to-infant transmission of HCV might be possible by breast milk. Since not one of 34 samples of mother's milk was positive for HCV RNA by RT-PCR, we did not find evidence for HCV transmission via breast-feeding. This finding was supported by the fact that none of 16 additional breast-fed children became infected. Breast milk obviously did not interfere with RT-PCR, since sera positive for HCV RNA mixed with breast milk remained RT-PCR-positive. Because of these results, we do not advise HCV-infected mothers to stop breast-feeding their children. In previous studies, results of examination of breast milk for HCV RNA and the role of HCV transmission by this route have been controversial. Although HCV RNA could be detected in all colostrum samples tested, this route of HCV transmission was not considered important because no transmission occurred; the authors postulated that the samples might have been contaminated by small amounts of blood (17).

It is not yet known whether HCV infection prior to delivery is possible, since the rate of infection occurring in vaginal deliveries has not been compared with that in caesarian sections. An infection in utero might lead to an immunotolerance, as has been described for hepatitis B (18); transplacental infection may lead to a lack of antibodies to HBcAg and persistent antigenemia with HBsAg and HBeAg. In these cases most of the children were asymptomatic (18). In our study 14 children were tested for HCV RNA after they had become negative for HCV antibodies; not one was positive. Since all of the HCV-infected children in our study are positive for anti-HCV, there was no indication of immunotolerance. This finding is in accordance with the results of others (11, 19). The children with perinatally acquired HCV infection in our study have thus far shown no clinical signs of hepatitis.

In two children in this study, umbilical cord blood and blood samples drawn on the fourth day of life were of low diagnostic value because they gave positive PCR results that later became negative. It is possible that viral RNA was transferred to these children without transmission of infective viral particles. Therefore, sera for RT-PCR should be drawn after the first week of life to avoid contamination with RNA from maternal blood.

In conclusion, to detect or exclude HCV infection in children born to HCV-infected mothers, serum samples should be tested by antibody assays and RT-PCR. We usually repeat testing for HCV antibodies and RT-PCR at the ages of 3 and 6 months. In our study maternal HCV antibodies disappeared during the first year of life. The loss of HCV antibodies was indicative of no infection, and all children who lost maternal antibodies were consistently negative by PCR. Although children who became HCV-infected have not demonstrated the clinical or biochemical signs of hepatitis, they will be observed further to determine their long-term outcome.

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