Increased Risk of Mother-to-Infant Transmission of Hepatitis C Virus by Intrapartum Infantile Exposure to Maternal Blood

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Virological and clinical data from 73 hepatitis C virus (HCV)–infected pregnant women who gave birth to 75 children were merged retrospectively, by logistic regression analysis, to investigate risk factors for vertical transmission of HCV. Eighty-two percent of the HCV-infected mothers were HCV-RNA–positive during pregnancy, and 10% were coinfected with human immunodeficiency virus (HIV). Nine children were HCV infected, 1 was HIV infected, but none was HIV-HCV coinfected. Among vaginal deliveries, the mean HCV load of mothers who transmitted HCV to their infants was higher than that of those who did not (vs. copies/mL; ). A reduction in umbilical cord–blood pH (relative risk, 3.9; ) or the occurrence of perineal or vaginal laceration (relative risk, 6.4; ) during vaginal delivery significantly increased the risk of vertical HCV transmission. In conclusion, high maternal viremia, infantile hypoxia, and intrapartum exposure to virus-contaminated maternal blood increased the risk of HCV transmission during vaginal deliveries. Consequently, cesarean section may reduce the risk of vertical HCV transmission in selected cases.

In developed countries, the majority of new hepatitis C virus (HCV) infections are acquired through injection drug use (IDU) [1]. Although vertical transmission of HCV from mother to child during delivery is a rare route of infection, with reported average transmission rates of 5%–10% [2], it is the predominant mode of infection among children [3–6].

Until now, only a few risk factors influencing vertical HCV transmission during the perinatal period have been identified (i.e., human immunodeficiency virus [HIV] type 1 coinfection and the presence of HCV RNA in maternal blood). However, it is still controversial whether high maternal virus load also poses a higher risk for transmission [7–9]. Furthermore, the timing of perinatal transmission is uncertain, and understanding of the obstetrical factors that influence vertical transmission of HCV is still limited. Although several studies did not find an increased risk of transmission during vaginal delivery [9, 10], a recent investigation of a large cohort found evidence that elective cesarean delivery results in a reduction in mother-to-child transmission rates [6]. Because the factors influencing transmission are still unclear, screening of pregnant women for HCV infection is currently not recommended, and preventive measures during delivery are not available [11, 12]. This uncertainty about risk factors that influence vertical transmission of HCV stands in contrast to mother-to-infant transmission of HIV-1, where risk factors for transmission have been clearly identified (i.e., plasma HIV-1 RNA level and vaginal delivery). As a consequence, medical interventions that significantly reduced vertical transmission rates of HIV-1, including antiviral
The aim of the present study was to identify preventable risk factors associated with HCV transmission and to provide a basis for the prevention of HCV infection in newborns. For this purpose, we retrospectively evaluated virological and clinical parameters (e.g., mode and course of delivery) within a large, well-defined cohort of HCV-infected pregnant women. Although vaginal delivery itself was not a risk factor for transmission in the present study, we found that perinatal infantile hypoxia and vaginal or perineal laceration that occurred during vaginal delivery significantly increased the risk for HCV transmission.

PATIENTS AND METHODS

Study population. The study population included HCV-infected women who were being studied at the Institute of Virology (University of Vienna, Vienna) and who gave birth between 1994 and 1999. HCV infection was identified in these women, before or during pregnancy, by serologic detection of HCV-specific antibodies by use of an ELISA and was confirmed by detection of HCV RNA in serum by use of a reverse-transcriptase polymerase chain reaction (RT-PCR). For the RT-PCR–positive women, HCV load was determined with a quantitative PCR. For the RT-PCR–negative women, the specificity of the antibodies detected by ELISA was confirmed by use of an immunoblot. Furthermore, available serum samples were tested for maternal HIV coinfection according to World Health Organization guidelines [15]. For women without information on HCV load or HIV infection, data were completed by retrospective testing of available serum samples (clinical specimens were stored at −80°C).

The children of these mothers were considered to be HCV uninfected if an HCV-specific RT-PCR done ≥1 month after birth or an HCV-specific antibody test done ≥12 months after birth or both were negative [6]. They were considered to be HCV infected if HCV RNA was detected in at least one of their blood samples (umbilical cord blood was not tested).

Children were considered to be HIV-1 uninfected if they were antibody negative on ≥1 occasion or had at least 2 PCR-negative samples, with at least 1 test done after age 6 months. They were considered to be HIV-1 infected if HIV-1 RNA was detected in at least one of their blood samples. Children with inadequate HIV-1 laboratory data were classified as indeterminate.

Virological investigation. Antibodies to HCV were determined in plasma by ELISA (Monolisa anti-HCV PLUS; Sanofi Diagnostics Pasteur), as recommended by the manufacturer. Before testing of serum samples by PCR, HCV-RNA was extracted with a QIAamp Viral RNA kit (Qiagen). HCV RNA positivity was determined by a qualitative RT-PCR (AmpliCor HCV Detection kit [detection limit, 20–1000 copies/mL]; Roche Diagnostic Systems), and HCV load was measured using a quantitative RT-PCR (AmpliCor Monitor HCV Assay; Roche Diagnostic Systems). All HCV-PCR–positive results were confirmed by testing a different aliquot of the original sample, and the sensitivity of each qualitative RT-PCR was demonstrated in routine testing by coextraction and codetection of a positive control with ∼1000 copies/mL. For PCR-negative samples, the specificity of ELISA-positive results was confirmed by a recombinant immunoblot (Riba HCV 3.0; Chiron).

Testing for HIV-1 antibodies was done with a commercially available HIV-specific ELISA (Abbot HIV 1/2 gO; Abott Diagnostika). If the antibody-ELISA result was positive, it was confirmed by a Western blot (New LAV-BLOT I; Sanofi Diagnostics Pasteur). HIV-1–specific PCRs were done using a commercially available assay (AmpliCor HIV-1 Monitor Test; Roche Diagnostic Systems), as recommended by the manufacturer.

Clinical investigation. Clinical data on HCV-infected mothers were assessed by questionnaires sent to mothers and their gynecologists immediately after delivery and by review of case histories and obstetric notes. The questionnaires asked for the number of previous deliveries, maternal age, and illicit drug use. The case histories and obstetric notes were reviewed for parity of the women, illicit drug use during pregnancy, gestational age, premature rupture of membranes, mode and course of delivery (elective vs. emergency cesarean section and perineal or vaginal laceration), medical interventions during delivery (e.g., episiotomy), umbilical cord–blood pH, APGAR (activity, pulse, grimace, appearance, and respiration) score, and birth weight of the child. Rupture of membranes was considered to be premature if it occurred before the first regular uterine contraction.

Drug use was assessed by self-reports and toxicologic testing of urine samples, as described elsewhere [16]. A woman was classified as positive for drug use during pregnancy if she had a positive urine toxicology result or a positive self-report. She was classified as negative for drug use if she had either a negative self-report and a negative urine screen or a negative self-report, if urine toxicology results were unavailable. HCV was reported to have been acquired through IDU for 41 (56%) women and from infected blood or blood products for 6 (8%) women. Other or unknown routes of acquisition were reported for 26 (36%) women. For 4 women with a history of IDU, urine toxicology results were negative during pregnancy, which is indicative of discontinuation of IDU.

Data analysis. The effects of the different risk factors for perinatal HCV transmission were evaluated by unconditional logistic regression analysis. For the analysis of HCV load as a risk factor for vertical transmission, HCV-infected women with HCV RNA levels below the limits of detection were assigned a level of 10 copies/mL for logistic regression analysis. Because HCV load may change over time, only quantitative and qualitative HCV-PCR results obtained in samples that were taken...
Table 1. Estimated risk of mother-to-infant transmission of hepatitis C virus (HCV) in relation to virological and clinical risk factors of the mother.

<table>
<thead>
<tr>
<th>Maternal risk factor</th>
<th>No. of mother-child pairs</th>
<th>Mother-to-infant transmission of HCV</th>
<th>Estimated RR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virological parameters</td>
<td></td>
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<tr>
<td>HCV RNA positive, no. (%)</td>
<td>73</td>
<td>9 (100)</td>
<td>51 (80)</td>
<td>NC</td>
</tr>
<tr>
<td>HCV load for all deliveries, geometric mean copies/mL (SF)</td>
<td>65</td>
<td>7.3 × 10^4 (2.3 × 10^4)</td>
<td>2.5 × 10^4 (1.0 × 10^4)</td>
<td>4.1 (0.78–21.52)</td>
</tr>
<tr>
<td>HCV load for vaginal deliveries, geometric mean copies/mL (SF)</td>
<td>38</td>
<td>8.1 × 10^4 (2.1 × 10^4)</td>
<td>1.4 × 10^4 (1.3 × 10^4)</td>
<td>9.9 (0.94–103.35)</td>
</tr>
<tr>
<td>Maternal HIV coinfection, no. (%)</td>
<td>68</td>
<td>1 (13)</td>
<td>6 (10)</td>
<td>1.3 (0.13–12.31)</td>
</tr>
<tr>
<td>Clinical parameters</td>
<td></td>
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<tr>
<td>Maternal age, mean years (SD)</td>
<td>75</td>
<td>30.9 (6.1)</td>
<td>27.4 (5.6)</td>
<td>1.1 (0.98–1.25)</td>
</tr>
<tr>
<td>Primipara, no. (%)</td>
<td>53</td>
<td>5 (71)</td>
<td>27 (59)</td>
<td>1.7 (0.31–10.0)</td>
</tr>
<tr>
<td>Gestational age, mean weeks (SD)</td>
<td>65</td>
<td>39 (1.9)</td>
<td>37 (3.3)</td>
<td>1.3 (0.90–1.74)</td>
</tr>
<tr>
<td>Premature rupture of membranes, no. (%)</td>
<td>62</td>
<td>2 (22)</td>
<td>10 (19)</td>
<td>1.2 (0.22–6.83)</td>
</tr>
<tr>
<td>Cesarean section, no. (%)</td>
<td>66</td>
<td>1 (11)</td>
<td>22 (39)</td>
<td>0.2 (0.23–1.70)</td>
</tr>
<tr>
<td>Elective cesarean section, no. (%)</td>
<td>22</td>
<td>1 (100)</td>
<td>13 (62)</td>
<td>NC</td>
</tr>
<tr>
<td>Episiotomy in vaginal deliveries, no. (%)</td>
<td>42</td>
<td>2 (25)</td>
<td>9 (27)</td>
<td>1.1 (0.18–6.36)</td>
</tr>
<tr>
<td>Perineal or vaginal laceration in vaginal deliveries, no. (%)</td>
<td>42</td>
<td>5 (63)</td>
<td>7 (21)</td>
<td>6.4 (1.23–33.65)</td>
</tr>
<tr>
<td>Child’s birth weight for vaginal deliveries, mean grams (SD)</td>
<td>42</td>
<td>3153 (382)</td>
<td>2778 (676)</td>
<td>2.2 (0.37–13.28)</td>
</tr>
<tr>
<td>Umbilical cord-blood pH, mean (SD)</td>
<td>48</td>
<td>7.2 (0.08)</td>
<td>7.3 (0.06)</td>
<td>3.9 (1.06–13.96)</td>
</tr>
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NOTE. CI, confidence interval; HIV, human immunodeficiency virus; NC, not computable; RR, relative risk; SF, scatter factor.

* a Univariate logistic regression.

* b Based on logarithmic transformation; risk increases per 10-fold increase of virus load.

* c Premature rupture was defined as rupture before onset of labor.

* d RR was estimated for a reduction of standard cord blood pH (a standard value of 7.27 was assumed) and with respect to a change of pH of 0.1.

Results

Analysis of virological parameters. For the evaluation of possible risk factors for the vertical transmission of HCV, 73 pregnant, HCV-infected women were identified between 1994 and 1999. Serum samples of 71 women (97%) were tested by RT-PCR; 58 women (82%) were HCV RNA positive. Four mothers had been HCV RNA positive before pregnancy but were negative during pregnancy. For 11 mothers, HCV infection was confirmed by the identification of HCV-specific antibodies by use of an immunoblot.

The 73 women gave birth to 75 children, including 2 sets of twins; 9 of the children were HCV infected. Seven (10%) mothers were HIV-HCV–coinfected, 59 (81%) were HIV seronegative, and, for 7 (10%), HIV status could not be determined. For 6 of the 7 HIV-HCV–coinfected mothers, HCV- and HIV-PCRs were done, and both HCV RNA and HIV DNA were detected. Of the 7 mothers with HIV coinfection, 1 transmitted HCV to her offspring and 1 transmitted HIV, but none transmitted both viruses to her child. One of these 7 children (the HIV-infected one) was delivered vaginally, 5 (including the HCV-infected child) were delivered by elective cesarean section, and the mode of delivery was unknown for 1 child.

Analysis of risk factors for mother-to-infant transmission of HCV. Estimation of the likelihood of mother-to-infant transmission of HCV for individual virological and clinical parameters was done by unconditioned logistic regression analysis (table 1). The sample size varied, depending on the number of women for whom data were available for a particular variable.

Detectable HCV viremia in the mother was a prerequisite for transmission, since only children from mothers with detectable viral RNA became HCV infected. The mothers of infected children had a higher mean HCV load than did mothers of uninfected children (7.3 × 10^4 vs. 2.5 × 10^4 copies/mL), and this difference in HCV load was even more pronounced among mothers who underwent vaginal delivery (8.1 × 10^4 vs. 1.4 × 10^4 copies/mL). However, no statistically significant as-
Figure 1. Follow-up investigation of hepatitis C virus (HCV)–infected children by HCV polymerase chain reaction. +, Positive result; −, negative result.

Association could be found between maternal HCV RNA level and the risk of mother-to-infant transmission of HCV, although a trend toward a higher risk of transmission with increasing levels of maternal viremia was noted (table 1).

In mothers with HIV-HCV co-infection, no increased risk for mother-to-infant transmission of HCV was observed in the present study, although the number investigated was small. In the 7 HIV-HCV–coinfected mothers for whom a quantitative HCV-PCR result was available, the mean HCV load was not significantly higher than that in mothers without HIV-HCV coinfection (1.7 × 10^3 vs. 3.2 × 10^3 HCV RNA copies/mL; n = 53; P = .113, Mann-Whitney U test).

In the present study, vaginal delivery itself did not increase the risk for transmission, compared with cesarean section. However, children who were delivered vaginally and whose mothers sustained a perineal or vaginal laceration had a 6-fold higher risk of becoming HCV-infected than did vaginally-delivered children whose mothers had no laceration (table 1). In contrast, no significantly increased risk was observed when an episiotomy was performed. The transmission rate of HCV did not differ between emergency and elective cesarean sections, although the number observed was small.

In addition, every reduction in umbilical cord–blood pH by 0.1 increased the risk of mother-to-infant transmission of HCV by 4-fold (assuming a pH standard value of 7.27). An APGAR score of 9/10 at 1 and 5 min was noted for all these HCV-infected newborns.

Birth weight of the newborn and gestational age did not increase the risk of mother-to-infant transmission, but we observed a trend for a higher birth weight of the child in women who sustained a perineal or vaginal laceration (mean weight, 2.93 vs. 3.18 kg; estimated relative risk [RR], 3.0; P = .082, logistic regression analysis). No increased risk of mother-to-infant transmission of HCV was observed in primipara or in mothers who experienced a premature rupture of membranes. Six (10%) of 58 mothers did breast-feed, but none of their children was HCV infected.

Interactive effects of the risk factors investigated were subsequently evaluated by a multivariate analysis. Results of this analysis were similar to those obtained by univariate logistic regression, except for perineal or vaginal laceration (RR, 2.99; 95% confidence interval, 0.32–27.70) and umbilical cord–blood pH (RR, 5.0; 95% confidence interval, 1.06–23.80).

Follow-up investigation of children. To evaluate the course of the HCV infection in infected children and the clearance of maternal HCV antibodies in uninfected children, the 75 children were monitored for as long as possible. All 9 HCV-infected infants were singleton births and were surveyed for a minimum follow-up period of 11 weeks (median, 54 weeks; interquartile range, 22–89 weeks; figure 1). The earliest time that 1 newborn was tested and HCV RNA could be detected was at the age of 9 days. Two other children were HCV RNA positive already at the age of 15 and 22 days, respectively. Eight of the 9 HCV-infected children had consistently positive RT-PCR results in the samples collected after the age of 1 month (in 2 children, HCV RNA could be still detected at the age of 5 years). One infected child with detectable HCV RNA became
HCV RNA negative at the age of 15 months and remained negative thereafter.

All 66 HCV-uninfected children were at least 5 weeks old when last tested for HCV viremia by PCR (median age, 28 weeks; interquartile range, 10–35 weeks). Altogether, 114 samples were collected from these HCV-uninfected children and tested for HCV-specific antibodies by ELISA. When ELISA results were analyzed in relation to the age of the children, a 50% probability of an HCV antibody-negative result was attained at the age of 9.6 months.

**DISCUSSION**

Vertical transmission from infected mothers has become the most important mode of HCV infection among children [3]. Although numerous studies have addressed this issue [3, 6–8, 17–21], the mechanisms of vertical HCV transmission, including the timing of infection, remain largely unknown [3].

It is generally agreed that the risk of vertical HCV infection in mothers without detectable HCV viremia is exceedingly low [18, 19, 22, 23]. We also found HCV viremia to be a prerequisite for transmission, because none of the HCV RNA–negative mothers transmitted the virus to her child.

However, there is no general consensus that the risk of transmission is higher in mothers with high HCV load than in mothers with low HCV load [7, 8, 19–21]. In the present study, the mean maternal HCV load was higher in mothers whose children were HCV infected than in those whose children were not infected, but high viremia was not a statistically significant risk factor for transmission. However, when we included the mode of delivery into our analysis, it became apparent that, in the case of a vaginal delivery, this difference in mean virus load was even more pronounced. In addition, except for 1 HCV–HCV–coinfected woman, none of the 23 mothers who had a cesarean section transmitted HCV to her offspring.

Previous reports found strong evidence that mother-to-child transmission rates and substantial intrapartum transmission of HCV may possibly be reduced by elective cesarean delivery [6, 24, 25]. However, some studies were criticized for testing only a minority of women for HCV RNA [26] and for not addressing possible mechanisms involved in the reduction of vertical transmission of HCV by elective cesarean section [27]. We not only tested 97% of the mothers for HCV RNA but also identified factors that may increase the risk of HCV transmission in vaginal deliveries.

We observed a significantly increased risk of HCV infection in children with a low umbilical cord–blood pH, which is indicative of infantile hypoxia. Whether this intrapartum hypoxia may have led to aspiration of HCV-contaminated maternal fluids or to other hypoxia-related mechanisms of virus transmission can only be speculated.

Moreover, a significantly increased risk of mother-to-infant transmission of HCV was observed in the case of leakage of maternal blood into the birth canal by tears of the cervix or vagina. In contrast, when an episiotomy, which also is associated with maternal bleeding, was performed, it did not increase the risk for vertical transmission of HCV. This apparent contradiction may suggest a more extensive, and possibly longer, exposure of children to virus-contaminated maternal fluids during vaginal deliveries that involve vaginal or perineal lacerations than during those that involve an episiotomy. Although it may be theorized that passage through the birth canal may be prolonged in mothers with relatively large children or that, in women with vaginal or cervical tears, the canal first had to be dilated by the child before passing through it, which allowed for more extensive exposure to virus-contaminated maternal blood, these details will have to be elucidated in prospective clinical trials. Still, since an episiotomy is, in general, performed at the end of labor, intrapartum exposure to maternal blood is very likely shorter in this incidence than in the case of vaginal or perineal lacerations.

Evidence for the significance of labor-associated factors for the transmission of blood-borne pathogens was already found in investigations of vertical HIV transmission in twin births [28, 29]. First-born twins were more likely to be infected with HIV-1 than their second-born siblings, especially when the former was of greater birth weight. It was suggested in these studies that the first-born twin would have dilated and, to some extent, mechanically cleansed the birth canal, thereby reducing the duration of exposure of the second twin and, consequently, the risk of transmission.

Nevertheless, these observations on the transmission of HCV or HIV represent only indirect evidence for an increased risk in vaginal deliveries with prolonged passage through the birth canal. Data on the exact time required for passage of the birth canal, however, are not routinely recorded during delivery and will have to be collected in future prospective clinical trials.

Results of studies on HIV infection as a risk factor for vertical HCV transmission also have been ambiguous [6, 30, 31]. Nevertheless, HIV-HCV coinfection reportedly accelerates HCV disease progression by increasing HCV load [32, 33], and a higher HCV load in HCV-HIV–coinfected patients may be explained by the observed inverse relation between baseline CD4+ cell count and HCV load [32]. In addition, the risk for transmission is exceedingly low in women who are HIV-HCV coinfected but who have little or no detectable HCV RNA [18, 34]. Consequently, we propose that the more important predictive factor for HCV transmission is very likely HCV load, and not HIV coinfection. In the present study, which included only a small number of HIV-HCV–coinfected mothers, no increased risk for vertical HCV transmission was observed in mothers with HIV coinfection. This finding may be explained by the fact that HCV load in our group of HIV-HCV–coinfected
mothers did not significantly differ from that in HIV-uninfected mothers.

Reported average mother-to-infant transmission rates of HCV are 5%–10% [2, 5, 6]; we observed a somewhat higher transmission rate of 12%. However, because of the retrospective design of the present study and the fact that HCV infection status could not be assessed for all children, a selection bias cannot be excluded for this parameter. In addition, our laboratory is the major reference laboratory for the geographic region covered by this study; therefore, preselection of patients possibly occurred.

In conclusion, vaginal delivery itself does not appear to be a significant risk factor for mother-to-infant transmission of HCV, but the risk of transmission increased with increasing maternal HCV load and with the occurrence of infantile hypoxia or vaginal or perineal lacerations during vaginal delivery. Although it would be premature to recommend routine cesarean section for HCV-infected women, elective cesarean section may reduce the risk of vertical transmission of HCV among mothers with high HCV viremia, those who are at risk for birth injuries during vaginal delivery, or those whose children are at risk for intrapartum hypoxia.

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References


