Low Risk of Vertical Transmission of Hepatitis C Virus by Breast Milk

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To evaluate the risk of hepatitis C virus (HCV) transmission via breast milk, we collected 76 samples of breast milk from 73 chronically HCV-infected women and serum samples from their 76 perinatally HCV-exposed children. Enzyme immunoassay and strip immunoblot assay were used for detection of antibodies to HCV, and reverse transcriptase–polymerase chain reaction analysis was used for detection of HCV RNA. None of the 76 samples of breast milk contained HCV RNA, whereas 37 (59.7%) of 62 mothers tested for HCV RNA had HCV viremia. Only 1 of the 76 breast-fed infants had evidence of HCV infection. Because HCV infection in this child was detected 1 month after birth, it seems unlikely that it was transmitted by breast-feeding. These results indicate that HCV infection in pregnant women should not be a contra-indication for breast-feeding.

About 5% of children born to mothers with chronic hepatitis C virus (HCV) infection during pregnancy and delivery become infected with HCV [1], but the mechanism of transmission is still unknown. Some studies favor the transplacental route of transmission instead of infection by contact of the child to contaminated maternal body fluids during delivery [1, 2]. Controversial results have been reported on the detection of HCV RNA in breast milk. Although viral RNA was found in all colostrum samples tested in 1 study [3], none of the breast-fed children became infected with HCV. In other studies, no HCV RNA could be detected in any tested samples of breast milk [1, 4, 5]. To evaluate the risk of mother-to-infant transmission of HCV via breast-feeding, we conducted a prospective study of HCV-infected mothers, samples of breast milk from these women, and serum samples from their children.

Patients and Methods

Seventy-three mothers who had at least one test positive for antibody to HCV or reverse transcriptase (RT)–PCR analysis positive for HCV RNA during pregnancy were included in the study. They were recruited to our study from 1993–1998. None of the women were positive for HIV type 1 (HIV-1) or type 2. Most of the maternal HCV infections (65 cases) were diagnosed in our laboratory, although in 8 cases, HCV infection was confirmed by external laboratories. Samples of breast milk from all mothers were collected before and 73 days after delivery (mean, 6.2 days). Maternal serum samples were obtained between 59 days before and 73 days after delivery (mean, 2.1 days after delivery); in most cases (47), they were collected within 1 week of the samples of breast milk. The 73 women gave birth to 76 children; the first serum samples from the newborn infants were obtained during the first week of life (71 samples) or between the age of 1 and 3 months (5 samples). In 38 cases, follow-up serum samples obtained between the ages of 1 to 28 months (mean, 7.5 months) were also available.

Antibodies to HCV were determined with a second-generation EIA until March 1998 and then with a third-generation microparticle EIA (Abbott, Wiesbaden, Germany), both performed according to the manufacturer’s instructions. Confirmatory testing was done with an in-house strip immunoblot assay that uses recombinant antigens of NS5, NS4, NS3, and the core region of HCV, as described elsewhere [6].

For the determination of HCV RNA, fresh serum or breast milk was added to a ribonuclease (RNase)–inhibiting solution [7] and stored at –20°C; RNA was extracted, reverse transcribed, and amplified. PCR analysis was performed with primers of the highly conserved 5′-untranslated region, as described elsewhere [8]. To confirm their specificity, PCR products were blotted and hybridized [8]. Samples were designated negative when they were negative in 2 or 3 assays in which a positive control was clearly positive. Titters of HCV RNA were determined by comparing the PCR product’s signal with a serial dilution of a standardized serum obtained from the National Institute for Biological Standards and Controls (Potters Bar, U.K.); the lower detection limit was 10^2 copies/mL. Samples of breast milk were divided: 1 part was mixed with serum containing HCV RNA at a concentration of 10^4–10^6 copies/mL (1 part breast milk, 1 part HCV-containing serum, and 2 parts RNase-inhibiting solution), and the other part was not (1 part breast milk and 3 parts RNase-inhibiting solution). RT-PCR analysis was performed on both preparations.

Results

None of the 76 samples of breast milk were positive for HCV RNA. Breast milk did not interfere with RT-PCR analysis, since
Results of RT-PCR analysis for all 76 samples spiked with HCV RNA-containing sera were positive at an RNA concentration either as that of the native serum (51 samples) or one order of magnitude less (10⁻¹ copies/mL; 25 samples). All 63 maternal serum samples tested for antibodies to HCV were confirmed positive, and 37 (59.7%) of 62 mothers tested for HCV RNA had HCV viremia, with RNA titers ranging from 10⁶ to 10⁷ copies/mL (median, 10⁴ copies/mL). Seven mothers were considered highly viremic, with HCV RNA titers ranging from 10⁹ to 10¹⁰ copies/mL. All 76 children of the 73 HCV-infected mothers were breast-fed.

Results of initial PCR analyses for all of the children were negative. During follow-up, all but 1 child remained negative for HCV RNA. This child was negative for HCV RNA on the third day of life. When tested for the second time at the age of 27 days, PCR analysis was positive for HCV (level of viremia, 10⁴ copies/mL). No serum samples were obtained later. Twenty of the children were followed up until they lost maternal antibodies to HCV, which took 3–28 months (mean, 9.0 months).

Discussion

The rate of vertical transmission of HCV from infected mothers to their children is 3%–5% [1]. At least part of the children become infected with HCV in utero [9]. Conflicting results about the transmission of HCV via breast milk have been reported [3, 4, 10, 11]. Breast milk may be contaminated with viruses such as rubella virus, cytomegalovirus, and HIV-1 [12]. In HIV-1 infection, the relevance of viral presence in breast milk is controversial. HIV infection in mothers shortly after delivery led to high rates of transmission to their children probably because of high levels of maternal viremia, although a sufficient immune response of neutralizing antibodies in the breast milk is still lacking [13]. Despite these data, the World Health Organization still recommends breast-feeding for all mothers in developing countries regardless of their HIV-1 infection status [14].

Some studies found that HCV RNA was detectable in 10%-100% of samples of breast milk from HCV-infected women [3, 10, 11], but other studies failed to confirm this finding [1, 4, 5]. Even if HCV RNA is present in breast milk, there is little evidence for its relevance to HCV transmission to children. This lack of evidence might be due to a high blood-to-milk gradient ranging from 10⁶ to 10⁷ [3, 15], and low concentrations of viral particles may be inactivated in the gastrointestinal tract [9]. In addition, there may be a neutralizing effect of persisting maternal antibodies in nursed children [10].

It has been postulated that HCV may replicate in mononuclear cells [16]. HCV RNA was detected in up to 100% of colostrum samples [3], which contain significantly higher concentrations of mononuclear cells (especially macrophages) than does mature breast milk [13]. Despite this finding, none of the children who were fed with HCV RNA-containing breast milk became infected [3], reflecting either contamination with non-infectious viral particles or viral concentrations that were too low to result in transmission. Another possible but not proven reason for the low rates of transmission via breast milk might be the presence of lactoferrin in mammalian breast milk, which has been described to inhibit the replication of viruses such as cytomegalovirus and HIV-1 in vitro [17]. One of the largest studies including 71 breast-fed children found that none of them became infected, but examination of samples of breast milk was not included in this study [18].

Our results do not suggest that nursing enhances the risk of HCV transmission from infected mothers to their babies. None of the 76 samples of breast milk tested were positive for viral RNA, and only one of the breast-fed children became infected (PCR analysis for this child became positive during the first 4 weeks of life). This child most probably was infected in utero or during delivery. In utero transmission of HCV is discussed when PCR analysis for the child becomes positive within the first 3 months of life [9]. For chimpanzees, it has been shown that PCR analysis becomes positive ~1–2 weeks after intravenous challenge with HCV-positive material [19]. Seroconversion after enteral exposure, which so far has not been proven to exist, should take rather longer. Hence, although it cannot be completely excluded, in our study, transmission of HCV via breast milk was not proven; infection during pregnancy seems more probable.

The mother of the infected child in our study had an HCV RNA concentration of 10⁴ copies/mL at the time of delivery; the sample of breast milk obtained on the third day after delivery was negative for HCV RNA (i.e., if contaminated at all, the viral concentration in her breast milk was below the detection limit of our PCR assay). It is doubtful whether such a low viral load could lead to enterally transmitted HCV infection. In addition, in the other 37 children from whom follow-up serum samples were available, HCV transmission did not occur.

The results of our study show that HCV RNA is rarely detectable in mature breast milk, and only 1.3%–8.3% of breast-fed children are infected. This rate is within the range described for perinatally HCV-exposed children, regardless of whether they were breast-fed or formula-fed [1]. Thus there is no necessity to discourage chronically HCV-infected mothers from breast-feeding their children. Because of a higher level of viremia at a time when no neutralizing antibodies are present, mothers with acute HCV infection acquired after delivery might be more infectious to their children; therefore, different recommendations might be necessary in these cases.

References