MAJOR ARTICLE

Hepatitis C Virus (HCV) Vertical Transmission in 12-Month-Old Infants Born to HCV-Infected Women and Assessment of Maternal Risk Factors

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Background. Hepatitis C virus (HCV) is an underappreciated cause of pediatric liver disease, most frequently acquired by vertical transmission (VT). Current guidelines that include the option of screening infants for HCV RNA at 1–2 months are based on data prior to current real-time polymerase chain reaction (PCR)-based testing. Previous studies have demonstrated VT rates of 4%–15% and an association with high maternal viral load. We evaluated HCV RNA in infants with HCV VT and assessed maternal risk factors in a prospective cohort in Cairo, Egypt.

Methods. Pregnant women were screened for HCV from December 2012 to March 2014. For those with HCV viremia, their infants were tested at 12 months for HCV RNA using real-time PCR. Maternal risk factors assessed for HCV VT association included HCV RNA levels, mode of delivery, and maternal IL28B genotype.

Results. Of 2514 women screened, a total of 54 women were viremic (2.1%) and delivered 56 infants. Of those, 51 infants of 49 women were tested at 12 months of age. Only 7 infants were viremic, with an HCV VT rate of 14.3% (7 of 49). Median HCV RNA in the infants was 2100 IU/mL. None of the maternal risk factors analyzed were associated with transmission.

Conclusions. In Egypt where HCV is highly endemic, we observed an overall 12-month HCV VT rate of 14.3%. Further studies should focus on better identification of pregnant women more likely to vertically transmit HCV and earlier testing of infants to identify those likely to develop chronicity.

Keywords. Egypt; hepatitis C virus; infants; vertical transmission; viral load.

Hepatitis C virus (HCV) is a well known cause of adult liver morbidity and mortality, but its burden on children is often overlooked [1, 2]. Estimates of the pediatric burden of HCV in the United States include 25 000–50 000 existing cases and projected costs of $168–$404 million in the current decade before the approval of new and very expensive antiviral agents [3]. These figures are dwarfed by estimates from Egypt, where the overall HCV seroprevalence is almost 15 times higher than in the United States [4–9]. Although many children in the United States acquired HCV from infected blood or blood products prior to effective screening, the leading route of pediatric acquisition of HCV in most parts of the world is now vertical transmission (VT) [4, 10].

Previous studies of HCV VT demonstrated that approximately 4%–15% of infants become infected, but approximately half of those will spontaneously resolve their infection in the first 2 years of life [11–13]. These studies also described many infants with either episodic viremia or viremia that became detectable after several months of life. Hepatitis C virus RNA was detected in these HCV VT studies using older reverse transcriptase-polymerase chain reaction (RT-PCR) assays that yielded a qualitative result (positive or negative)

with less sensitivity. One study used a nested RT-PCR technique to increase the sensitivity of the assay [12]. Currently used methods for HCV RNA detection use real-time PCR technology, which offers increased sensitivity for low quantities of HCV RNA (25 IU/mL lower limit of detection) coupled with a broad dynamic range (up to 10^8 IU/mL) for quantitation when compared with older RT-PCR methods (~10^3 IU/mL) [14, 15]. Despite these meaningful improvements, there have been no studies that examined the performance of the current real-time assays in diagnosing HCV VT. Given that this is the leading route of pediatric acquisition of HCV and the assay is not currently being used for this purpose, one of our main study objectives was to fill this knowledge gap.

Prior studies of HCV VT have also examined whether certain maternal risk factors are associated with a higher rate of transmission [16]. Maternal HCV viral load has been implicated in several studies, but there is no defined level that is predictive of transmission [12, 17]. Mode of delivery has been examined, and Caesarean section has not been found protective of transmission [17–19]. Coinfection with human immunodeficiency virus (HIV) was demonstrated to increase the risk of HCV VT, but those studies were all done before highly active antiretroviral therapy [17, 20–22]. A recent study done in women with well controlled HIV demonstrated an HCV VT rate that was comparable to those with HCV monoinfection [23]. Given the association of IL28B rs12979860 genotype with a significantly enhanced rate of response to interferon-based therapy as well as with spontaneous resolution of HCV, 1 study investigated its association with HCV VT and found none [24–26].

Here, we describe the results of a 12-month follow up of infants born to HCV-infected pregnant women identified as part of a universal screening program we implemented in Cairo, Egypt [27]. We tested for the HCV RNA levels in their infants at 12 months of age and analyzed maternal risks for possible association with HCV VT.

METHODS

Detailed study procedures were described previously [27]. In brief, all pregnant women attending Cairo University Faculty of Medicine Obstetrics and Gynecology antenatal clinic were approached for HCV screening. After obtaining their informed consent, screening for HCV was performed on all women, and HCV RNA testing was performed on those who tested positive for antibodies to HCV. Prenatal care was conducted as per the usual standards. After delivery, the HCV-infected women were observed every 3 months after delivery for repeat testing and follow-up antenatal care. Infants were observed at 12 months of age for HCV RNA testing as part of the study. The study was independently approved by the Cairo University, University of North Carolina at Chapel Hill, and the University of Maryland School of Medicine institutional review boards. Each

pregnant woman signed a written informed consent form after all her questions were addressed, and for illiterate women, a literate companion attended the consenting process and cosigned the informed consent after all of their questions were addressed.

Laboratory Testing

Maternal blood samples were tested for HCV antibodies using a fully automated electrochemiluminescence immunoassay (Elecsys Anti-HCV immunoassay on the Cobas; Roche). Positive maternal samples and all infant samples were tested for quantitative HCV RNA using the Roche LightCycler 2.0 (real-time PCR) as described elsewhere [28]. Maternal IL28B assessment was performed using the TheLightMix IL28B allelic discrimination kit from Roche Diagnostics (Mannheim, Germany) for the detection of the human IL28B C/T polymorphism (rs12979860) in human nucleic acid extracts. This LightMix Kit was tested on the LightCycler 2.0 Instrument with the LightCycler FastStart DNA HybProbeMastermix.

Data Analysis

The prevalence of anti-HCV and HCV RNA was calculated using Microsoft Excel. The associations between maternal risk factors and VT were assessed and described, and given the small number of infected infants, nonparametric tests were used to compare between them: Fisher's Exact test was used for categorical variables, and the Mann-Whitney U test was used for continuous variables. Analyses were performed using the SPSS statistical software version 22.0 (IBM SPSS Statistics for Windows, Version 22.0; Armonk, NY: IBM Corp).

RESULTS

From December 2012 through March 2014, a total of 2514 pregnant women were screened at Cairo University. Of those, 98 (3.9%) were anti-HCV seropositive and 54 (2.1%) also were positive for HCV RNA. Based on previous published studies demonstrating VT only in women with viremia, we only performed follow-up studies on infants born to those women with viremia.

Fifty-six infants were born to the 54 viremic women, all of whom were tested for HCV viremia. There was 1 stillbirth and 2 infants who died at several hours of life (1 had multiple congenital anomalies and 1 had severe obstructive uropathy). Two infants have not yet completed 12 months of age and have not been tested yet. This left 51 infants (2 sets of twins) born to 49 mothers who were evaluated.

Hepatitis C Virus Vertical Transmission Rate

Of the 51 infants born to 49 mothers, 7 of 49 infants (14.3%) were HCV RNA-positive with real-time PCR testing. Results

Table 1. Summary of Maternal Risk Factors and Their Association With HCV VT[a]

	Infant RNA Negative	Infant RNA Positive	P Value
No. of HCV RNA-positive mothers	n = 42[a]	n = 7	
Infant HCV RNA			
Median (range)	N/A	2100 IU/mL (704–40 800)	N/A
Median log	N/A	3.32	N/A
Maternal Age	32 yrs (±5.2)	30.7 yrs (±5.3)	0.54
Mode of Delivery			
Cesarean section	21	5	0.42
NSVD	21	2	
IL28b Genotype			
CC	21 (50%)	5 (71%)	0.56
CT	14 (33%)	1 (14%)	
TT	5 (12%)	1 (14%)	
Refused	2 (5%)	0 (0%)	
Maternal HCV RNA			
Mean (range)	446 093 IU/mL (907–6 550 000)	145 612 IU/mL (134–459 000)	0.64
Median	99 750	85 400	0.64
Median log	4.99	4.93	0.62

Abbreviations: HCV, hepatitis C virus; N/A, not applicable; NSVD, normal spontaneous vaginal delivery; VT, vertical transmission.

[a] Forty-four HCV RNA-negative infants born to 42 HCV RNA-positive mothers, and 7 positive infants born to 7 positive mothers.

Figure 1. Distribution of maternal hepatitis C virus (HCV) RNA levels and VT. This figure shows the HCV RNA levels of mothers who did, and did not, vertically transmit HCV to their infants. Logarithmic values are presented on the y-axis and the 2 groups of mothers are plotted on the x-axis. HCV RNA distributions are presented using a "box and whisker" plot. The lower and upper borders of each box encompass the interquartile range (IQR), the horizontal line inside the box represents the median, the vertical lines from the ends of each box extend to the extreme data points, and the open circle is an outlier value.

are summarized in Table 1. Both sets of twins were negative for HCV RNA.

Viral IU/mL copies ranged from 704 to 40 800 (mean = 8643, standard deviation = 14 626). Median HCV RNA in the infants was 2100, and 5 of the HCV RNA-positive infants had viral quantities below 2500 IU/mL.

Analysis of Maternal Factors

We next analyzed maternal risk factors that have been reported to be associated with HCV VT in prior studies. Results are summarized in Table 1. Of the transmitting mothers, 2 had spontaneous vaginal deliveries and 5 underwent Cesarean section. All but 2 of the 7 women had prior Cesarean deliveries. Among the nontransmitting mothers, 21 had spontaneous vaginal deliveries and 21 had Cesarean sections (8 were first-time surgical deliveries, whereas 13 were repeat). There was no association with mode of delivery and HCV VT (P = 0.42).

We examined HCV RNA levels between transmitting mothers and nontransmitting mothers. Results are summarized in Table 1. Mean HCV RNA levels in transmitting mothers was 145 612 (median, 85 400) vs 446 093 IU/mL (median, 99 750) in the nontransmitting mothers. There was no association with HCV RNA and HCV VT in our cohort (P = 0.64). Based o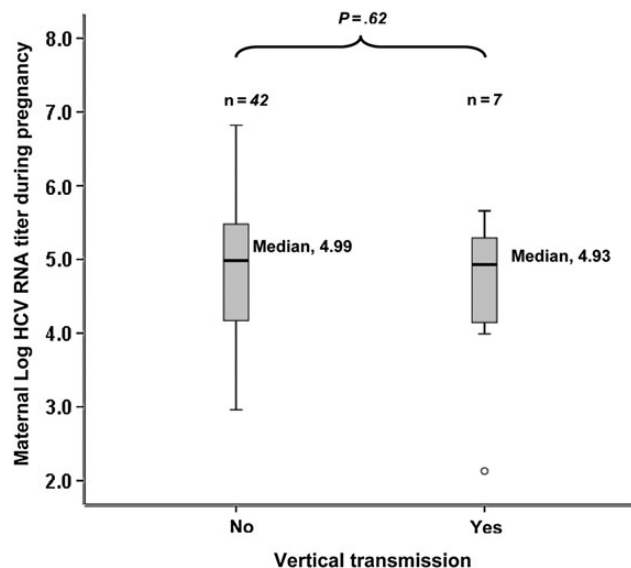n the distribution represented in Figure 1, one can see that there was no correlation with HCV RNA levels and transmission, and there was no cutoff that could be established below which VT did not occur.

We also examined the potential role of maternal IL28B genotype on HCV VT. When compared with the "TT" or "CT" polymorphism, the "CC" genetic polymorphism has been associated with significantly higher rates of spontaneous clearance and response to interferon-based therapy [24, 26]. Results are summarized in Table 1. Among the 7 mothers that transmitted HCV to their infants, 5 (71%) had the favorable CC genotype, 1 (14%) had the CT genotype, and 1 (14%) had TT. Among the nontransmitters, 21 (50%) were CC, 14 (33%) were CT, 5 (12%) were TT, and 2 (5%) refused to be tested. There was no association between IL28B genotype and HCV VT in our study (P = 0.56).

DISCUSSION

We conducted follow-up testing of infants born to Egyptian mothers identified with HCV as part of a universal screening program we implemented. We identified 7 infants with HCV VT using real-time PCR testing at 12 months of age for an estimated transmission rate of 14.3%. To put these numbers in additional perspective, one of the previous studies identified 9 infants with HCV VT born to 192 women with HCV RNA after screening more than 75 000 women over a period of 4 years at 2

sites in the United States [12]. A more recent study of HIV/HCV coinfected women identified 4 infants born to 47 women identified from 6 countries from 2002 to 2009 [23]. Given the high local seroprevalence in Egypt, our patient cohort is amongst the largest described with HCV VT.

Our findings are significant for several reasons. We conducted our follow-up studies using current real-time PCR assays. The prior large studies that looked at HCV VT used older assays that were less sensitive [11–13]. Those same studies demonstrated that some infants had episodic viremia at various time points in their first year of life. Real-time PCR-based testing for HCV allows for a broad dynamic range with excellent sensitivity down to 25 IU/mL [14]. The median HCV RNA of the infants we tested was 2100 IU/mL. It is possible that these infants may have tested negative with the prior assays. Current recommendations by the American Academy of Pediatrics Committee on Infectious Diseases (the *Red Book*) are that infants vertically exposed to HCV can be tested using molecular test between 1 and 2 months, or they can be tested with an antibody test after 18 months of age [29]. This recommendation is based on studies using the older PCR assays. As we stated before, the newer real-time PCR assays have never been studied in this population. It is entirely possible that several of the infants testing negative were positive at some point earlier in life. It is also possible that some of the infants testing positive were previously positive at a much higher level, and some may be on their way to spontaneous resolution. It is well known that infants can spontaneously resolve their viremia well into the 2nd year of life. It is also possible that these infants may experience a steady climb in their HCV RNA as chronic infection is established. What our results really indicate is the need for a systematic study of infants vertically exposed to HCV to determine when is the best time to use real-time PCR testing to accurately declare them infected or uninfected.

Our findings also reinforce the lack of a single maternal risk factor that is highly associated with HCV VT. Our analysis revealed that HCV RNA, mode of delivery, or maternal IL28B status were not associated with HCV VT. Older studies have suggested that high HCV RNA levels were associated with transmission, but we observed HCV VT even when maternal viral RNA levels were below 20 000 IU/mL [12, 17, 30, 31]. Because viral RNA levels do not correlate and because mode of delivery or IL28B does not influence VT, these observations seem to support one of our fundamental hypotheses about HCV VT, which is that it is most likely an in utero event that is due to a combination of unique viral and nonimmune-mediated host cellular factors. The presence of VT at low maternal RNA levels in our study does not support conclusions from prior studies that an overwhelming perinatal HCV exposure was responsible for infection of infants born to HCV-infected mothers with high viral loads. It may be that certain strains are better suited to cross the placenta but are less well suited to persist in the infant, where only half of infants progress to chronic infection.

Previous studies examining changes in maternal immune responses during and after pregnancy coupled with innate immune responses within the placenta and adaptive immunity in the children help to define the many layers of protection against HCV VT [32–34]. Again, these results suggest that ongoing detailed studies examining fundamental aspects of HCV VT are required.

Our findings are subject to some limitations. We acknowledge that our transmission rate is based on 1 follow-up test at 12 months and that we do not have liver function testing or HCV antibody results. This was based on resource limitations for testing at earlier time points. It is possible that further follow-up up to 2 years or longer would result in spontaneous clearance of some of these infections, as was observed in prior studies done by our group [13, 34]. We are in the process of planning a larger, more in depth study looking at the predictive value of testing at several time points to determine which one is optimal for outcome prediction. We made every effort to reduce the risk of contamination when processing samples, but it is possible that some of our low levels of RNA may be due to contamination, although we did not find such evidence in repeated maternal samples tested by the same laboratory. We also only studied patients at 1 site in Cairo, Egypt. Although we did not perform genotype testing on these patients, genotype 4 is seen almost exclusively in that region [35, 36]. Previous studies have shown that VT rates from Egypt mirror those observed in the United States and European cohorts [13]. We do allow that there may be inherent differences between the genotypes with respect to VT that are not yet fully appreciated. We did not actively screen pregnant women for HIV coinfection because Egypt is categorized by UNAIDS as having very low HIV seroprevalence (http://www.unaids.org/en/regionscountries/countries/egypt/). Although this cohort is relatively large for an HCV VT study, the number of infected children was small, and it is possible that the small sample size is a reason for the lack of significant association with maternal risk factors. We are continuing to screen, enroll, and observe women and their infants at this site, and we plan to retest the same infants at 2 years of life. We look forward to reporting our findings for a much larger cohort in the future.

CONCLUSIONS

We observed a high rate of HCV VT in our cohort when using real-time PCR for follow-up testing. Our findings suggest that there is the potential for generating a validated algorithm using real-time PCR to accurately segregate infected from uninfected infants. Larger studies should be performed with this goal in mind.

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