Endogenous Interferon-α Level is Increased in Hepatitis C Virus (HCV)-Positive Pregnant Women

Delia Maria Paternoster, MD, Anna Belligoli, MD,* Nanhornguè Kimta Ngaradoumbe, MD,* Silvia Visentin, MD,* Riccardo Franco, MD,* Stefano Fagiuoli, MD,† Caterina Boldrin, MD,‡ Giorgio Palù, MD,‡ Vincenzo Baldo, MD,§ and Annarosa Floreani, MD†*

Background: Mother-to-child transmission of hepatitis C virus (HCV) has been reported in around 5% of cases, and is much more likely to occur in case of coinfection with HIV. However, other cofactors influencing the vertical transmission are still debated.

Aim: To assess the serum concentration of endogenous interferon (IFN) during pregnancy, and its eventual role on the vertical transmission of HCV.

Methods: Forty-seven HCV-infected pregnant women, and 3 control groups: (1) 75 HCV-negative pregnant women; (2) 29 HCV-positive nonpregnant women; (3) 29 HCV-negative nonpregnant women entered into the study. Endogenous IFN was assayed by enzyme-linked immunosorbent assay. The following parameters were also analyzed: viral load, HIV infection, risk factors for acquiring HCV, parity, gestational age, mode and course of delivery.

Results: Vertical transmission of HCV was observed in 2 cases (4.3%). Plasma levels of IFN were significantly higher in HCV-positive pregnant women compared with either HCV-positive and HCV-negative nonpregnant women. The 2 mothers who transmitted the infection had IFN levels within the same range as the women who did not transmit the infection.

Conclusions: In HCV-positive pregnant women, there is an increased production of endogenous IFN-α. Further studies are warranted for clarifying the mechanisms of this cytokine in the prevention of HCV transmission.

Key Words: hepatitis C, HCV, vertical transmission, interferon, HIV

(*J Clin Gastroenterol* 2008;42:204–207)

Received for publication July 13, 2006; accepted September 13, 2006.
From the Departments of *Gynecological Science and Human Reproduction; †Surgical and Gastroenterological Sciences; ‡Histology, Microbiology and Medical Biotechnologies, Section of Microbiology and Virology; and §Hygiene and Public Health, University of Padua, Padua, Italy.
The authors declare no conflict of interest.
This study was partially supported by a Ministerial grant.
Reprints: Prof Annarosa Floreani, MD, Department of Surgical and Gastroenterological Sciences, Via Giustiniani, 2-35128 Padova, Italy (e-mail: annarosa.floreani@unipd.it).

Hepatitis C virus (HCV) infection is still a worldwide problem. It is estimated that about 170 million people, with a prevalence of 3% of the world's population, are infected with HCV.[1,2] A number of epidemiologic studies have focused on the risk of transmission of the infection and the role of vertical transmission. The average transmission rate is around 5%, but is higher in HIV-coinfected women.[3,4] A number of cofactors of infection have been explored, including viral load, intravenous drug abuse, long duration between membrane rupture and delivery, mode of delivery.[5,6] However, none of these conditions seems to have a pivot role in vertical transmission. Recently, it has also been suggested that at least one third and up to one half of infected children acquire infection in utero.[7] Nevertheless, it seems that elective cesarean delivery will not protect against neonatal HCV.[8]

In a previous paper, we hypothesized that endogenous interferon-α (IFN-α) produced by placenta might protect the infant from acquiring HCV infection.[9] This endogenous production might also explain the reduction in transaminases serum levels which is common during pregnancy.[10]

The aim of the present study was therefore to assess the serum concentration of endogenous IFN during pregnancy, and its eventual role in vertical transmission of HCV.

MATERIAL AND METHODS

Study Population

The study was carried out on pregnant women attending the antenatal clinic (Department of Obstetrics and Gynecology) of the University of Padua, Italy. The study involved 47 HCV-positive/HCV-RNA-positive women consecutively seen from January 1999 to January 2004. Twenty-seven of them had a past or actual history of intravenous drug use (IDU) (78.7%); 3 had blood transfusion as a risk factor of HCV transmission (6.4%), and the remaining 17 women did not have apparent risk factors for HCV infection.

As controls 3 groups of subjects were chosen:

1. 75 hepatitis C-negative pregnant women;
2. 29 HCV-positive/HCV-RNA-positive nonpregnant women;
3. 29 HCV-negative nonpregnant women.

Women of the control groups were HBs-Ag and anti-HIV-negative and were matched for age to the subjects of the study group. Informed written consent was obtained from all participants.

Clinical Investigation

In pregnant women, the following details were recorded: risk factors for acquiring HCV, HCV genotype, parity, gestational age, mode and course of delivery, weight of placenta, and birth weight of the child.

All the newborn of the HCV-positive mothers were tested at birth (on cord blood samples) and after 6 months for HCV-RNA.

Serologic Investigation

HCV infection was identified by serologic detection of HCV-specific antibodies by use of an enzyme-linked immunosorbent assay test. Positive subjects were further tested for: (i) qualitative polymerase chain reaction (PCR) for HCV-RNA (Roche Diagnostic System, USA); (ii) HCV genotyping using the Typing C kit (INNO-LiPA); (iii) quantitative PCR for viral load (in every trimester) by the bDNA assay (PCRb-DNA, Bayer, detection limit 3200 copies/mL; Quantiplex 2.0, Chiron Diagnostics, Lyon, France). All samples were run in duplicate.

Anti-HIV was determined by EIA (Abbott Labs, USA) and all tests were carried out in duplicate and confirmed by Western blot (Sorin-Biomedica, Italy).

In both HCV-positive and negative pregnant women, we also checked hepatitis B surface antigen (HBs-Ag) and antibodies to human immunodeficiency virus (anti-HIV).

To evaluate IFN production, we sampled venous blood 3 times in pregnant, whereas only once in nonpregnant women. Samples were immediately centrifuged at 3500*g* for 5 minutes and stored at -80°C. Human IFN-α was measured using a sandwich enzyme-linked immunosorbent assay kit (HyCult biotechnology, Uden, The Netherlands), according to the manufacturer's instructions. IFN-α concentrations in the samples were calculated using standard curves generated from the recombinant IFN-α-2b standard, and the results were expressed in picograms per milliliter. The lower limit of detection for IFN-α is 25 pg/mL IFN-α. The intra-assay coefficient of variation was 8.6%, and the interassay coefficient was 9.4%.

Statistical Analysis

The Fisher exact test was used to compare categorical variables and the Mann-Whitney test and Student paired *t* test were used when appropriate to compare continuous variables. The Pearson correlation test was used to evaluate and quantify association between variables.

All statistical analyses were performed with the Statistical Package for the Social Sciences 11.0 (SPSS, Chicago, IL).

RESULTS

All the 47 anti-HCV-positive mothers were found HCV-RNA-positive. Coinfection with HIV was found in 4 HCV-positive mothers (8.5%). Genotyping was performed in 39 subjects: 10 had genotype 1 (25.6%), 6 had 2 (15.3%), 7 had 3 (17.9%), and 9 had 4 (23%). The details of this study group are summarized in Table 1, in comparison with the HCV-negative pregnant mothers. No significant differences were observed between the 2 groups, except the rate of preterm delivery, which was higher in the study group compared with the group of the HCV-negative pregnant women.

Among the study group, the mean ± SD of IFN serum concentration was 11.334 ± 11.5556 pg/mL in the first trimester, 8.265 ± 7.639 pg/mL in the second, then increased to 13.47 ± 11.650 pg/mL in the third (Fig. 1). Among the hepatitis C-negative pregnant women the mean ± SD of IFN serum concentration was 783.1 ± 523 pg/mL in the first trimester, 1.292.7 ± 873 pg/mL in the second, then increased to 3.798.7 ± 1.074 pg/mL in the third. The difference between the 2 groups was statistically significant ($P < 0.001$). The mean ± SD of IFN serum levels in nonpregnant women was respectively 15.3 ± 5 pg/mL in the group of HCV-positive and 27.6 ± 9 in the group of HCV-negative women. The difference between pregnant and nonpregnant women was statistically significant ($P < 0.001$).

Among the study group, HCV-RNA levels were $5.2 \pm 12 \times 10^6$ copies/mL in the first trimester, $8.4 \pm 13 \times 10^6$ in the second, then increased to $12 \pm 21 \times 10^6$ in the third. The changes in viral load recorded at the above-mentioned intervals failed to reach any statistical significance. No statistical correlation was found between viral load and IFN serum levels. However, a negative correlation between IFN serum levels and aspartate aminotransferase in the study group ($P < 0.01$).

TABLE 1. Characteristic of the HCV-positive and HCV-negative Pregnant Women

Characteristics	HCV-positive (n = 47)	HCV-negative (n = 75)
Mean age (y)	32.3 ± 4.6	29.0 ± 5.5
Gestational age	38.2 ± 2.2	37.9 ± 2.0
Parity		
Nulliparous	23 (48.93%)	36 (48%)
Pluriparous	24 (51.06%)	39 (52%)
Maternal HIV coinfection		
Preterm delivery (< 37 g)	4 (8.5%)	—
Mode of delivery	11 (23.4%)	4 (5.3%)*
Vaginal	26 (55.3%)	39 (52%)
Cesarean section	5 (10.7%)	—
Elective cesarean section	16 (34%)	36 (48%)
Placental weight (mean ± SD, g)	536.3 ± 134.9	564.4 ± 136.5
Birth weight of the baby (mean ± SD, g)	3153.9 ± 592.8	3213.4 ± 507.8

*$P < 0.05$.

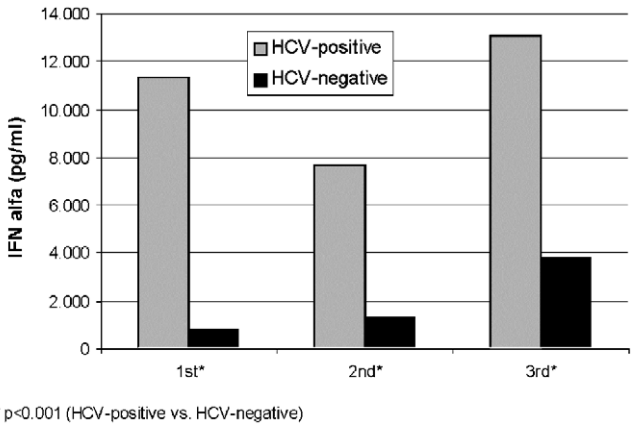

FIGURE 1. Endogenous IFN concentration in HCV-positive and HCV-negative pregnant women.

Moreover, no statistical correlation was found between HCV-positive women with history of drug abuse and those without history of drug abuse in either IFN serum levels or viral load.

The overall rate of vertical transmission was 4.2% (2/47). Table 2 shows the clinical characteristics of these 2 mothers. None of these was coinfected with HIV. Patient 1 was infected with genotype 4 and had past IVD abuse; her viral load was 4.5×10^6 copies/mL. Patient 2 was infected with genotype 1, her viral load was relatively high (58×10^6 copies/mL), and the risk for acquiring HCV was unknown. Both underwent cesarean delivery.

DISCUSSION

In our prospective study, we have demonstrated that HCV-positive mothers have a significantly higher production of endogenous IFN compared with either HCV-negative pregnant mothers or HCV-positive nonpregnant mothers. Such endogenous production does not correlate with viral load. These preliminary results should be interpreted as encouraging from several points of view. First of all, the 2 groups of pregnant women were homogeneous and similar regarding their obstetric characteristics. We only observed a higher rate of preterm delivery in the HCV-positive compared with the HCV-negative group. This difference could be explained with the risk factors related to HCV transmission, namely the IDU. In fact, 5 out of the 11 women who experienced preterm delivery were active intravenous drug users, and 4 had a past history of IDU.

TABLE 2. Characteristics of the 2 Mothers Who Transmitted the HCV to Their Babies

Characteristics	Mother No. 1	Mother No. 2
Age (y)	40	22
Viral load (copies/mL)	4.5×106	58.7×106
Genotype	4c/4d	1b
Coinfection with HIV	Absent	Absent
Risk factor for acquiring HCV	Past history of IDU	Unknown
Serum IFN at the third trimester (pg/mL)	18.500	1200
Parity	Primiparous	Primiparous
Gestational week	38	39
Mode of delivery	Cesarean	Cesarean

Up to now, the most well-documented risk factor for mother-to-child transmission of HCV is the maternal high viral load.[11–13] Moreover, the highest risk is reached in the case of HIV coinfection[14–17] or drug abuse.[15,16,18,19] However, factors that promote mother-to-infant transmission have not been completely clarified, including the maternal immune state. In general, comparing vertical transmission rate of HCV (almost 5%) with HIV infection (30% in absence of therapy),[20] we can consider this event quite infrequent. The high IFN levels in pregnant in comparison with nonpregnant women could likely be due to the existence of a placental production. In fact, during pregnancy, placenta is a source of IFN that can be detected in maternal and fetal blood. In particular, it has been demonstrated that human trophoblast produces different levels of IFN[21] and it seems that a high correlation is present between IFN levels in maternal blood and in trophoblast.[22]

The main limitation of our study is the small sample size, which is inadequately powered to detect whether IFN levels have an impact on maternal transmission of HCV. on the basis of our data, the most encouraging result is the increased production of endogenous IFN during pregnancy in HCV-infected women. Further studies are needed to explore the in situ placental production of IFN in HCV-infected women and the role exerted by IFN in the prevention of trans-placental spread of HCV, in similar way as in different viral infections.[23] Because trophoblast is the first fetal cell layer that an invading agent has to traverse from mother to fetus and in light of the role of IFN in innate immune system, it is probable that trophoblast can produce this cytochine. IFN play a role in the protection from virus infection in utero.[24] This concept seems to be supported by in vitro findings of Paradowska et al[25] concerning vesicular stomatitis virus infection. In addition, the population-based studies of vertical transmission of herpes simplex virus[23] and of HIV,[26] support the notion that placental IFN have antiviral functions.[24] On this point of view, we suggest to perform a multicenter collaboration and large case-control collections to explore this interesting field.

As far as the effect of pregnancy on liver damage in concerned, it has been confirmed a tendency toward a reduction in serum transaminase during pregnancy.[10,27] This is also confirmed by the negative correlation between serum IFN and aspartate aminotransferase in our study.

In conclusion, endogenous IFN production might be responsible for the reduction of serum transaminases during pregnancy. Further studies are warranted for clarifying the mechanisms of this cytokine in the prevention of HCV transmission and the immunologic role of the placenta in the regulation of mother-to-child transmission of HCV.

REFERENCES

1. Memon MI, Memon MA. Hepatitis C: an epidemiological review. *J Viral Hepatitis*. 2002;9:84–100.
2. Poynard T, Yuen M-F, Ratziu V, et al. Viral hepatitis C. *Lancet*. 2003;362:2095–2100.
3. Resti M, Azzari C, Galli L, et al. Maternal drug use is a preeminent risk factor for mother-to-child hepatitis C virus transmission: results from a multicenter study of 1372 mother-infant pairs. *J Infect Dis*. 2002;185:567–572.
4. Tovo P, Paloma E, Ferraris G, et al. Increased risk of maternal infant hepatitis C virus transmission for women coinfected with human immunodeficiency virus type 1. Italian Study Group for HCV infection in Children. *Clin Infect Dis*. 1997;25:1121–1124.
5. Newell ML, Pembrey L. Mother-to-child transmission of hepatitis C virus infection. *Drugs Today (Barc)*. 2002;38:321–337.
6. Roberts EA, Yaung L. Maternal-infant transmission of hepatitis C virus infection. *Hepatology*. 2002;36(5 suppl 1):S106–S113.
7. Mok J, Pembrey L, Tovo P-A, et al., and the European Paediatric Hepatitis C virus network. When does mother to child transmission of hepatitis C virus occur? *Arch Dis Child Fetal Neonatal Ed*. 2005;90:F156–F160.
8. Mast EE. Mother-to-infant hepatitis C virus transmission and breastfeeding. *Adv Exp Med Biol*. 2004;554:211–216.
9. Floreani A, Paternoster DM, Zappala F, et al. Hepatits C virus infection in pregnancy. *Br J Obst Gynaecol*. 1996;103:325–329.
10. Paternoster DM, Santarossa C, Grella P, et al. Viral load in HCV RNA positive pregnant women. *Am J Gastroenterol*. 2001;96: 2751–2754.
11. Batallan A, Faucher P, Poncelet C, et al. La transmission materno-fcetale du virus de l'hépatite C: actualités sur l'intérêt de la césarienne. *Gynècologie Obstétrique Fertilté*. 2003;31:964–968.
12. Okamoto M, Nagata I, Murakami J, et al. Prospective reevaluation of risk factors in mother to child transmission of hepatitis C virus: high virus load, vaginal delivery, and anti-negative anti-NS4 antibody. *J Infect Dis*. 2000;182:1511–1514.
13. Ranger-Rogez S, Alain S, Denis F. Virus des hépatites: transmission mère-enfant. *Pathologie Biologie*. 2002;50:568–575.
14. Gibb DM, Goodall RL, Dunn DT, et al. Mother to child transmission of hepatitis C virus: evidence for preventable peripartum transmission. *The Lancet*. 2000;356:904–907.
15. Granovski MO, Minkoff HL, Tess BH, et al. Hepatitis C virus infection in the mothers and infants cohort study. *Pediatrics*. 1998;102:355–359.
16. Mazza C, Ravaggi A, Rodella A, et al. Prospective study of mother to infant transmission of hepatitis C virus (HCV) infection. Study Group for Vertical Transmission. *J Med Virol*. 1998; 55:328.
17. Paccagnini S, Principi N, Massironi E, et al. Perinatal transmission and manifestation of hepatitis C virus infection in a high risk population. *Pediatr Infect Dis*. 1995;14:195–199.
18. Resti M, Azzari C, Mannelli F, et al. Mother to child transmission of hepatitis C virus: prospective study of risks factors and timing of infection in children born to women seronegative for HIV-1. Tuscany study Group on Hepatitis C Virus Infection. *Br Med J*. 1998;317:437–441.
19. Zanetti AR, Tanzi E, Romano L, et al. A prospective study on mother to infant transmission of hepatitis C virus. *Intervirology*. 1998;41:208–212.
20. Ahmad N. The vertical transmission of human immunodeficiency virus type 1: molecular and biological properties of the virus. *Crit Rev Clin Lab Sci*. 2005;42:1–34.
21. Aboagye-Mathiesen G, Tóth FD, Zdravkovic M, et al. Functionl characteristics of human trophoblast interferons. *Am J Reproduct Immunol*. 1996;35:309–317.
22. Ebbesen P, Hager H, Norskov-Lauritsen N, et al. Concurrence of high levels of interferons α and β in cord and maternal blood and simultaneous presence of interferon in trophoblast in an African population. *J Interferon Cytokine Res*. 1995;15:123–128.
23. Zdravkovic M, Knudsen HJ, Liu X, et al. High interferon alpha levels in placenta, maternal, and cord blood suggest a protective effect against intrauterine herpes simples virus infection. *J Med Virol*. 1997;51:210–213.
24. Fink T, Zachar V, Ebbesen P. Biological characterisation of three novel variant of IFN-α 13 produces by human placental trophoblast. *Placenta*. 2001;22:673–680.
25. Paradowska E, Blach-Olszewska Z, Sender J, et al. Antiviral nonspecific immunity of human placenta at term: possible role of endogenous tumor necrosis factor and interferons. *J Interferon Cytokine Res*. 1996;16:941–948.
26. Zachar V, Fink T, Koppelhus U, et al. Role of placental cytockines in transcriptional modulation of HIV type 1 in isolated villous trophoblast. *Aids Res Human Retroviruses*. 2002;18: 839–847.
27. Gervais A, Bacq Y, Bernuau J, et al. Decrease in serum ALT and increase in serum HCV RNA during pregnancy in women with chronic hepatitis C. *J Hepatol*. 2000;32:293–299.