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A Pregnant Case of Severe Acute Hepatitis Type C Successfully Treated with Natural Interferon-Alpha and Showing No Evidence of a Maternal Transmission of HCV to the Newborn

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Summary. **We report here a case of a 21-year-old Japanese woman suffering from severe acute liver injury due to hepatitis C virus (HCV) infection in the third trimester of her first pregnancy. When a second-generation antibody reactivity to HCV had not yet appeared, HCV RNA was detected in her serum using a polymerase chain reaction. Subsequent natural interferon-alpha therapy played a key role in the recovery from severe hepatic injury, and the disappearance of HCV RNA in her serum. Consequently, HCV RNA has not been detected for over 17 months after the cessation of interferon administration. On the other hand HCV RNA was not detected in her cord or the baby serum. Therefore, maternal transmission of HCV was not evidenced. This is a rare case in which a severe form of acute hepatitis C was successfully treated with natural interferon-alpha, and no indication of a maternal transmission of HCV in acute hepatitis C could be demonstrated with polymerase chain reaction analysis.**

Key words—acute hepatitis type C, interferon, pregnancy, vertical transmission.

INTRODUCTION

Hepatitis C virus (HCV), a main causative agent for chronic hepatitis, is responsible for a high incidence of hepatocellular carcinoma in the world.[1,2] Unfortunately it is not fully understood how HCV can be transmitted and maintained in a community at a high rate.[3] It is necessary for a reduction of the contraction rate to clarify the transmission route and establish reasonable therapy inhibiting sustained viral infection. We report here on an informative case regarding interferon therapy on acute hepatitis C and vertical transmission of HCV.

MATERIALS AND METHODS

Fresh serum from the case was subjected to immunological study. For the investigation of HCV RNA, the serum from the case, her baby and cord, parents, and husband was collected and stocked at −20℃ until use. All samples were collected under informed consent along the line of the 1975 Helsinki Declaration. The IgM class of antibodies was evaluated by radioimmunoassay for hepatitis A virus and a core antigen of hepatitis B virus, enzyme immunoassay for cytomegalovirus (CMV), and fluorescence antibody method (FA) for a viral capsid antigen of Epstein-Barr virus (EBV VCA). The IgA class of antibody was analyzed by FA for an early antigen of EBV. The IgG class of antibodies for CMV and EBV VCA was also analyzed by the same methods used for evaluation of the IgM class for each virus. Immunoreactivity against HCV was analyzed using a second generation of enzyme immunoassay for HCV (Anti-HCV II, Dainabott, Tokyo, Japan).

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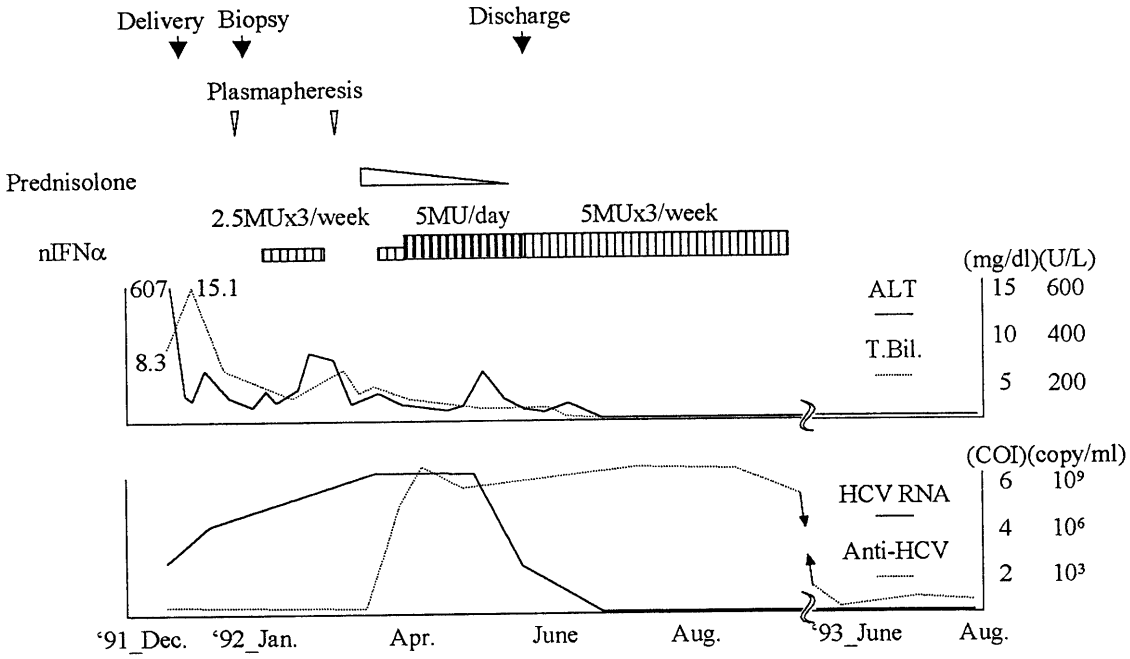

Fig. 1. Clinical course. Serial alanine aminotransferase and total bilirubin levels are represented with the COI of anti-HCV II and the genome number of HCV RNA. The quantity of HCV RNA was assessed by multicyclic PCR analysis. nIFNα, natural interferon alpha; MU, million units; COI, cut off index.

Table 1. Laboratory findings on admission

WBC	11000 /μl	T.P.	5.8 g/dl
RBC	394 $\times 10^4$/μl	Alb	2.5 g/dl
Hb	12 g/dl	γ-glb	0.9 g/dl
Ht	34.5 %		
PLT	28.4 $\times 10^4$/μl	APTT	53.5 %
		PT	38.6 %
Na	139 mEq/l	HPT	28 %
K	3.7 mEq/l	TTO	24 %
CI	106 mEq/l	ANA	(-)
		ASMA	(-)
CRP	0.5 mg/dl	anti-PDH	(-)
ESR	25/62 mm		
Crt	0.5 mg/dl	Fisher ratio	1.2
		Cu	111 μg/dl
		Cp	22 mg/dl
ALT	607 IU/I		
AST	682 IU/I	anti-HA(IgM)	(-)
ALP	340 IU/I	anti-HBc (IgM)	(-)
LDH	611 IU/I	HBeAg	(-)
γ-GTP	32 IU/I	CMV (IgM)	(-)
ChE	3633 IU/I	EBV EA (IgA)	(-)
T.Bil	8.3 mg/dl	EBV VCA (IgM)	(-)
D.Bil	7.3 mg/dl	anti-HCV II	(-)

Cp, ceruloplasmin; ANA, anti-nuclear antibody; ASMA, anti-smooth muscle antibody; anti-PDH, anti-pyrvate dehydrogenase complex; CMV, cytomegalovirus; EBV, Epstein-Barr virusantibody; EA, early antigen; VCA, viral capsid antigen; APTT, activated partial thromboplastin time; PT, prothrombin time; HPT, heraplastin time; TTO, thrombo-test.

HCV RNA was detected in the serum by the reverse transcription coupled nested polymerase chain reaction (RT-PCR). In brief, RNA was extracted from 100μl of serum by acid guanidinium thiocyanate-phenol-chloroform method. cDNA was elongated from the same primer, CR, used in the first PCR amplification. Nested-PCR was performed using two sets of primers locating 5' non-coding region of HCV: CR, 5'-CCATAGATCACTCCCCTGTG-3' and 196, 5'-GGGAACTTAACGTCCTGTGG-3' for the first; and C, 5'-CCATGGCGTTAGTATGAGTG-3' and 197R, 5'-GTGCTCATGATGCACGGTCTAC-3' for the second PCR. A reaction mixture for each PCR consisted of 10mM Tris-HCl [pH 8.3], 2mM $MgCl_2$, 50mM KCl, 1pmol of each primer, 2nmol of deoxynucleotide triphosphates, 10μl of cDNA, and 2.5U *Taq* polymerase (Takara, Shiga, Japan) in a volume of 50μl. PCR was performed by initial denaturation at 90℃ for 5 min, followed by 33 cycles of denaturation at 90℃ for 1 min, annealing at 55℃ for 45 sec, and extension at 72℃ for 2 min, with a final extension at 72℃ for 5 min. The genotype of HCV was determined by PCR using a mixture of four genotype-specific primers[4].

CASE

Clinical course and laboratory findings

A 21-year-old Japanese woman was transferred to our hospital in the 31st week of pregnancy from a local obstetrician with a diagnosis of impending miscarriage. The patient had had abnormal genital bleeding and dark urine 3 days earlier with gradually increasing general fatigue. She was a cashier at a shopping center, and this was her first pregnancy. She had been in good health without previous hospital admissions, surgical procedures, blood transfusions, injecting drug use, tattoos, acupuncture, alcohol abuse, trip abroad, other illnesses or use of antibiotics during the pregnancy. Her family history was not notable for liver diseases. The physical status on admission showed severe jaundice. Hepatic encephalopathy was not observed. An external monitor revealed moderate uterine contractions. The results of the admission laboratory are summarized in Table 1. The deviation of blood cell counts can be explained as a physiological change in pregnancy. There were no signs which suggested autoimmune hepatitis or Wilson's disease. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations were both elevated. Liver function tests revealed that she was suffering from severe hepatic injury, i. e. a low concentration of choline esterase, an elongated prothrombin time, and high concentrations

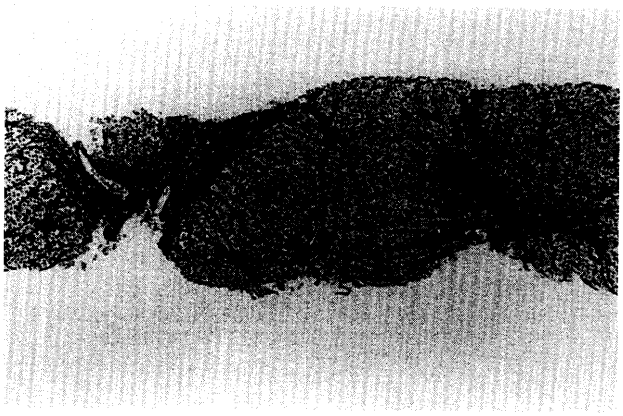

Fig. 2. Needle-biopsy specimen of the liver. Marked bridging necrosis is observed with thickened cell plates. (Silver stain; original magnification 2.5×4)

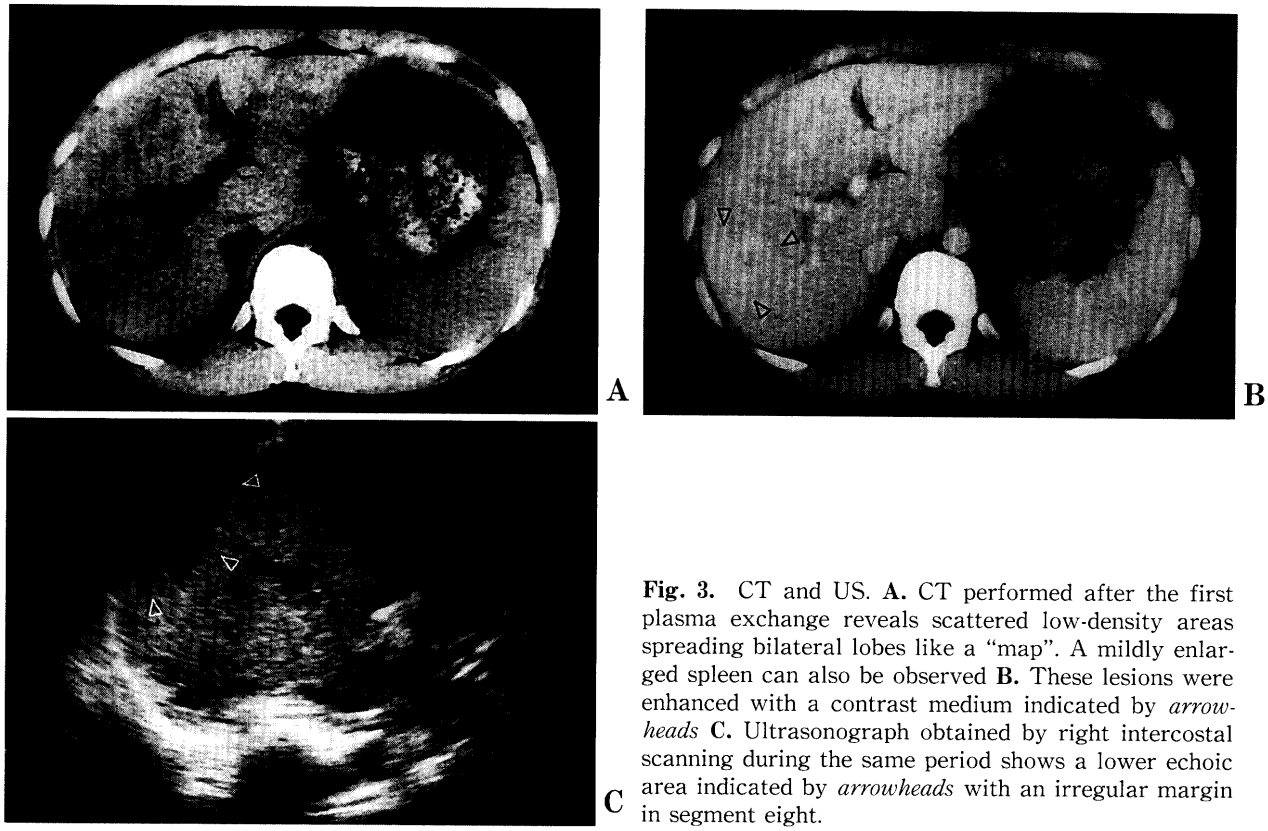

Fig. 3. CT and US. **A.** CT performed after the first plasma exchange reveals scattered low-density areas spreading bilateral lobes like a "map". A mildly enlarged spleen can also be observed **B.** These lesions were enhanced with a contrast medium indicated by *arrowheads* **C.** Ultrasonograph obtained by right intercostal scanning during the same period shows a lower echoic area indicated by *arrowheads* with an irregular margin in segment eight.

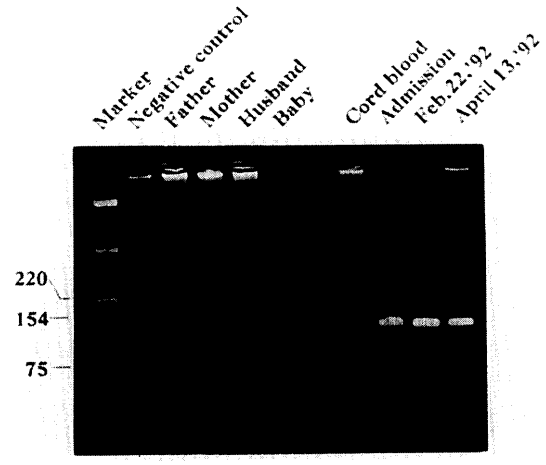

Fig. 4. Electrophoretic pattern of PCR products. PCR products compatible for the expected size (144bp) were not generated in the serum from either the family members or the cord, but the patient on admission, February 22 and April 13, 1992. Corresponding cDNA was amplified with primers deduced from 5' non-coding region of HCV genome. PCR products were separated through 4% agarose gel and visualized with ethidium bromide staining. Migration positions and the size (bp) of pBR322 digested with HinfI are indicated.

of total bilirubin and ammonia. On the third day, the pregnancy ended by normal vaginal delivery without any complications. Liver damage did not improve, however, even two weeks after delivery. Laboratory tests also exhibited a sustained lower activity of hepaplastin time around 15% and a higher concentration of total bilirubin at 5.1 mg/dl. Prolonged liver dysfunction made us perform a plasma exchange on 22 day (Fig. 1). We were afraid that the slowly progressing couse might prove fatal to her. Because we could not define a cause for her liver injury by immunoreactivity against conventional viruses, we performed nested PCR analysis with two pairs of primers deduced from 5' non-coding region of HCV genome. There was a possibility that acute hepatitis C was in the window phase in which immune reactivity would not yet appear. From the results, we diagnosed that she had been suffering from acute hepatitis C on the basis of viremia of HCV RNA, no notable past history for liver diseases, or no histologic evidence indicating chronic liver diseases and characteristic clinical features including the findings of CT and US. Finally, Natural interferon-alpha (nIFNα) administration was employed and resulted in the normalization of ALT and AST levels. All laboratory findings concerning liver function appeared to normalize with the sustained disappearance of HCV genome from her serum. Even after cessation of IFN, no deterioration of her liver function was observed over the next 17 months.

Interferon therapy

nIFNα administration was started as an anti-viral agent on day 40 subcutaneously at a dose of 2.5×10^6 IU three times a week. Because ALT, AST and total bilirubin concentrations began increasing 14 days after starting this therapy, we once suspended the administration. After the improvement of laboratory findings, nIFNα was employed again on day 77 with the same schedule as the previous one but covered by prednisolone (PSL) administration, which had been started 7 days before beginning IFN therapy at 30 mg every day with a gradual decrease (Fig. 1). When we confirmed the absence of any deterioration of her liver function under IFN therapy, the dose and frequency were increased to 5×10^6 IU every day from day 84. After she became an outpatient on day 142, the same amount was continued three times a week. In total, 5×10^8 IU was administered over a period of 6 months.

Viruses

There was no positive immunoreaction for acute viral infection of HAV, HBV, CMV and EBV. Only the IgG class of antibodies for EBV VCA and CMV was positive at titers of $\times160$ and over 2816, respectively. Anti-HCV II was also negative. HCV RNA was, however, detected in the serum obtained upon her admission as a type II of HCV. On the other hand, HCV RNA could not be detected in her cord serum nor in her family members—her mother, father, husband or baby (Fig. 4). On day 81, four days after beginning the second IFN therapy, anti-HCV II reactivity appeared at the cut off index (COI) of 1.6 compared with 0.2 on day 78 (Fig. 1). The value increased rapidly and persisted at over 6 for 5 months. Finally, the COI gradually decreased and fluctuated around a value of 1, even 14 months after the cessation of nIFNα administration. On the other hand, the quantity of HCV RNA in her serum quickly responded to this therapy. The number of HCV RNA at 10^9 genome/ml just before beginning the second IFN therapy decreased to 10^3 genome/ml within 2 months. Finally, HCV RNA became undetectable by PCR within 3 months. Since then, HCV RNA has been undetectable for over 17 months without IFN therapy.

Ultrasonography and Computed tomography

Ultrasonography (US) performed on the first hospital day showed a slightly reduced liver volume with no gall stone, no focal lesions in the liver and normal bile ducts appearance. Computed tomography (CT) on day 25 of hospitalization showed scattered low-density areas of a so-called map sign (Fig. 3a), which indicated severe liver cell necrosis, in bilateral lobes that were enhanced with contrast medium (Fig. 3b). US performed the same day revealed similar regions as lower echoic lesions (Fig. 3c).

Histological findings

US guided liver biopsy specimens obtained after plasma exchange on the day 29 showed obvious bridging necrosis with thickened cell plates suggestive of regeneration from massive liver cell necrosis as seen in Fig. 2. Neither significant plasma cell nor fatty infiltration was observed.

DISCUSSION

Although an interferon (IFN) therapy is approved mainly for a patient with chronic HCV or HBV infection, the literature contains a number of studies which show that there is a better response to interferon in symptomatic acute hepatitis C.[5,6] The effect of IFN on HCV infection must vary depending on concentrations and subtypes of the virus,[7,8] the dose and schedule administered,[9] and host immunity.[10] Unfortunately, most of these factors could not be operative except the one concerning treatment. In this context, the acute phase of HCV infection could be suitable for this therapy because the number of HCV in the body must be smaller than that in chronic state. Consistently, HCV was successfully cleared in this case even HCV was type Ib, which was reported to be a resistant subtype to IFN.[11] The optimum regime should be determined through further study.

In this case, we once suspended IFN therapy because we were afraid of possible adverse effects on her liver dysfunction. We speculated that the effect of IFN on her immunity might indirectly bring on the aggravation as reported in chronic hepatitis B,[12] because there have been few reports about apparent severe direct cytotoxicity of IFN to the liver. Therefore, we employed nIFNα again with PSL aiming for the effects of the cytoprotection and immunosuppression. Fortunately, the second administration resulted in a dramatic improvement of all data with a decreasing number of HCV genome in the blood. The efficacy of steroid usage should be determined with further study.

The transmission route of HCV in this case was not defined. She had no possible history of inducing HCV infection, and HCV RNA was not detected in her family members. In fact, it has not yet been well established how HCV transmits and spreads into a community at a high rate. Maternal transmission is one of the ways regarded as the main route for hepatitis B virus to establish a new carrier state.[13] Although some seroepidemiological findings suggest the existence of familial clustering in HCV infection,[14] other observations suggest that maternal transmission is not common except in HIV coinfected cases.[15] HCV viremia, vaginal delivery, and female offspring are also reported to show trends toward higher risk.[16] In this case, the baby was born by normal vaginal delivery and is female, but the number of HCV in mother's blood at the delivery was low, 10^3 genome/ml. From the results, mother-to-infant transmission was not evidenced, because HCV RNA was detected neither in the baby nor in the cord serum, and liver cell damage has get to be clinically detected in the baby. Thus, it appears likely that the concentration of the virus in the blood of mother at delivery is the most important determinant for the establishment of vertical transmission. Consistent observations have been reported in cases with HIV coinfection.[17]

This paper has reported a pregnant case of severe acute hepatitis due to HCV, which was diagnosed in the early stage with PCR analysis, and successfully eradicated by nIFNα administration. In addition, no vertical transmission was confirmed by PCR analysis for the baby and cord serum. We have to appreciate the effects of PCR analysis and IFN therapy in acute hepatitis C to avoid risks for missing curable diseases and periods.

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