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A Study of Patient with Chronic Non-B, Non-C Hepatitis who Developed Liver Cirrhosis and Hepatocellular Carcinoma Over 14 Years Observation

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Summary. **In 1986, a 58-year-old male was diagnosed as having chronic non-A, non-B hepatitis by a liver biopsy showing fibrous enlargement and lymphocyte infiltration in the periportal area. Since then, he continued to be followed at our outpatient clinics as a case of chronic hepatitis of unknown etiology and was found to be negative for the antibody to hepatitis C virus. During the follow-up, elevation of alanine aminotransferase (ALT) levels continued and his plasma albumin levels gradually decreased, leading to a diagnosis of liver cirrhosis. In 2000, multiple nodular lesions developed in his liver and a biopsy revealed well differentiated hepatocellular carcinoma with cirrhosis. We could not identify any etiological agent which might have caused his disease progression and the occurrence of hepatocellular carcinoma. However, the histological findings and the similarity of the clinical course to chronic hepatitis B and hepatitis C suggested that his disease might be due to chronic infection with another, as yet unidentified, virus.**

Key words—histological evolution, non-B, non-C hepatitis, hepatocellular carcinoma.

INTRODUCTION

Although liver cirrhosis is a hypercarcinogenic stage, hepatocarcinogenesis also depends on the etiology of the cirrhosis and on secondary risk factors. Chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are the greatest risk factors while excessive alcohol intake may be a moderate risk factor. Histological progression from chronic hepatitis to liver cirrhosis is closely associated with the risk of development of HCC[1,2].

The incidence of hepatocellular carcinoma (HCC) varies between different countries[3,4]. HBV infection is the most common etiological agent in China and Southern Africa[5,6]. In countries with a low prevalence of HCC, a fairly high proportion of HCC cases is of non-viral origin[7-9]. In Japan, more than 90% of the cases are due to either HBV or HCV infection[10,11].

Here we describe a patient with chronic non-B, non-C hepatitis, who developed liver cirrhosis and multiple HCCs during a follow-up period of 14 years.

CASE REPORT

A 52-year-old male habitual drinker of alcohol was found to have a mild elevation of alanine aminotransferase (ALT) during health screening in 1981. He was again shown to have liver dysfunction, together with cholecystolithiasis, mild diabetes mellitus, and hyperlipidemia at a further health screening in 1986. He had a past history of jaundice when he was 20 years old but did not have a history of blood transfusion. He was referred to our hospital in 1986 for specific examination of his liver disease.

Laboratory data at admission are shown in Table

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Abbreviations—ALT, alanine aminotransferase, HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus

Table 1. Laboratory tests at the first and latest admission

Peripheral blood	1986	2000	Autoantibodies	1986	2000
RBC (/μl)	487×10^4	394×10^4	ANA*	(−)	(+) (23.1)
Hb (g/dl)	15.2	13.0	ASMA	(−)	(−)
Ht (%)	45.6	37.1	AMA	(−)	(−)
WBC (/μl)	9700	8420			
Plt (/μl)	15.3×10^4	11.4×10^4			
Biochemistry			Viral markers		
T.Bil (mg/dl)	0.8	1.6	HBsAg	(−)	(−)
D.B (mg/dl)	0.2	0.3	anti-HBc ×1 dilution	(+)	(+)
AST (IU/l)	304	43	×200 dilution	(−)	(−)
ALT (IU/l)	496	23	anti-HBs	(+)	(+)
LDH (IU/l)	683	768	HBeAg	(−)	(−)
ALP (IU/l)	284	389	Anti-HBe	(−)	(−)
γ-GTP (IU/l)	148	70	HBV DNA		(−)
T.P (g/dl)	7.7	6.0	anti-HCV		(−)
Alb (g/dl)	4.7	2.4	HCV RNA		(−)
T.Chol (mg/dl)	291	154			
BUN (mg/dl)	14	15	Tumor markers		
Cr (mg/dl)	1.2	0.8	AFP (ng/ml)	8	42
FBS (mg/dl)	114	84	PIVKAII (mAU/ml)	NT#	23
IgG (mg/dl)	1214	1829			
IgA (mg/dl)	370	955			
IgM (mg/dl)	84	104			
γglb (g/dl)	1.3	2.1			

*ANA had been tested with an Immunofluorescence method until 1998, and has been changed to ELISA since then. Normal range with ELISA is less than 12.0 (COI).
#Not tested.

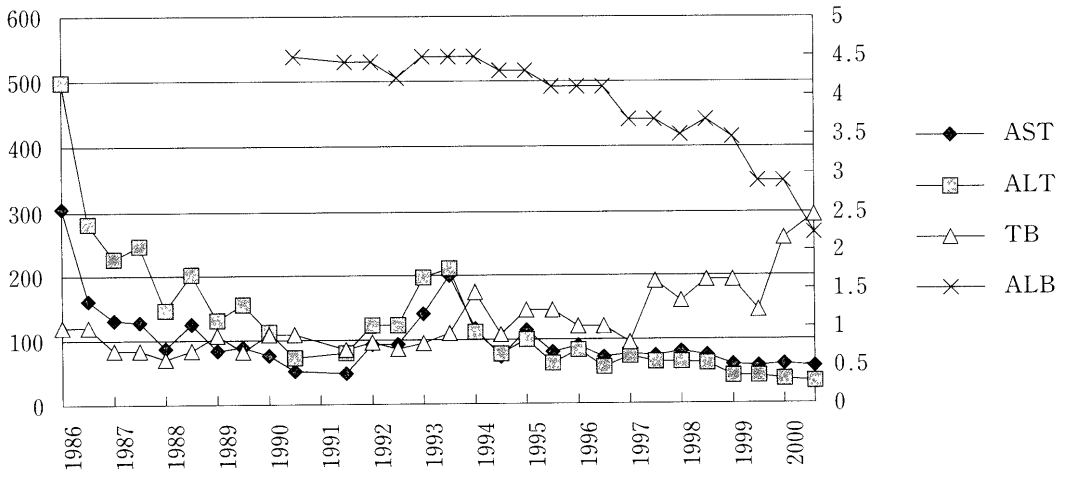

Fig. 1. Clinical course of the patient's laboratory data.

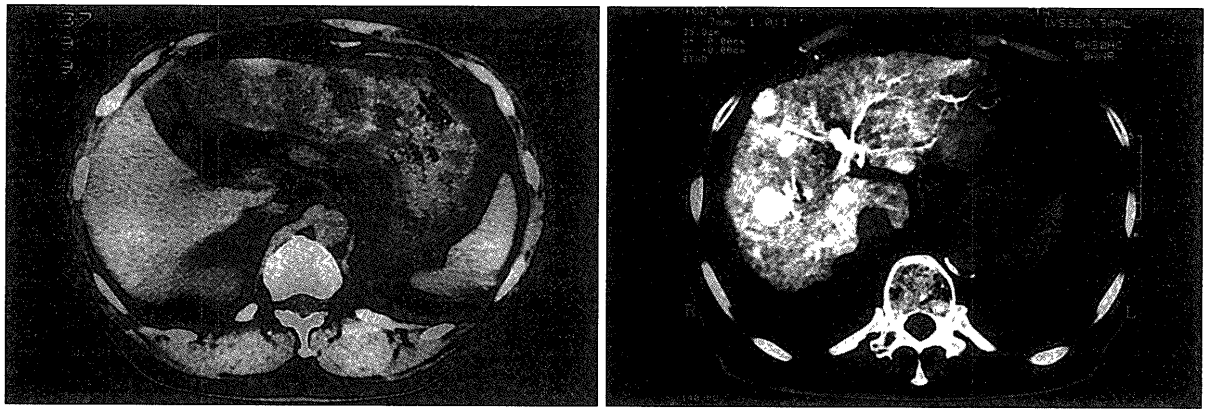

Fig. 2. CT scans in 1986 **a** and in 2000 **b**. An atrophic liver with multiple hypervascular legions in the early phase of CT angiography is shown in **b**.

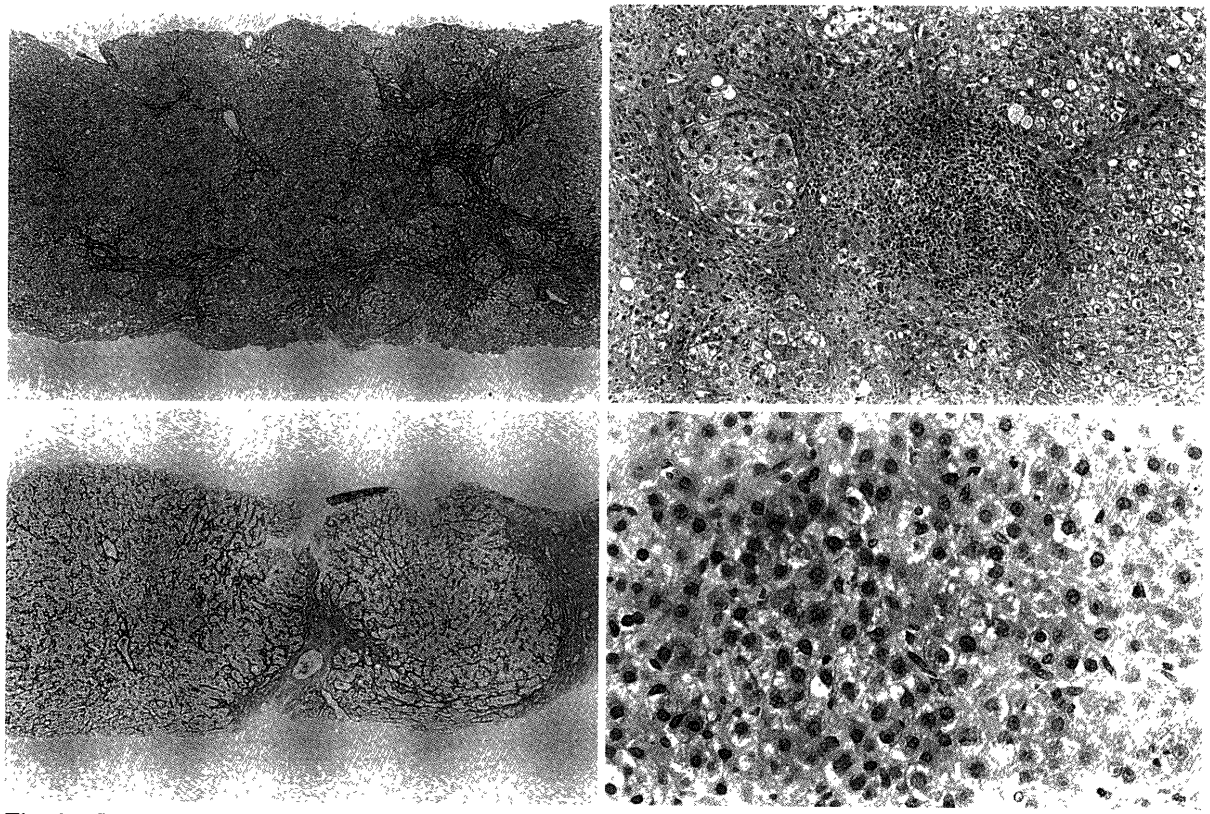

Fig. 3. Liver biopsies. **a.** 1st liver biopsy, 1986: Fibrous enlargement of the periportal area and bridging fibrosis between adjacent periportal areas. **b.** Lymphocyte infiltration and piecemeal necrosis. **c.** 2nd liver biopsy, 2000: nodule formation in the non- tumor portion which suggested liver cirrhosis. **d.** well differentiated HCC.

1 and longitudinal data are shown in Fig. 1. He was negative for HBsAg, but positive for anti-HBs and positive for anti-HBc at low titer. Computed Tomography (CT) did not show any particular abnormality in the liver (Fig. 2a). The CT density of the liver was almost same as that of the spleen. A liver biopsy was obtained by laparoscopy. The gross appearance of the liver was not particularly abnormal except for mild diffuse redness. Histological examination showed a fibrous enlargement of portal area and infiltration of lymphocytes with piecemeal necrosis (Fig. 3a and b), which suggested that his liver injury

was attributable to a chronic viral infection. It did not show much evidence of alcoholic liver injury, such as pericellular fibrosis or alcoholic hyaline bodies. Because this was prior to the availability of diagnostic tests for HCV, he was diagnosed as having chronic non-A, non-B hepatitis.

Subsequently, he was treated with ursodeoxycholic acid to lower his ALT level. He remains negative for anti-HCV, even according to the latest version of the assay. His serum obtained in 1995 was negative for HBV DNA by nested-PCR using HBsAg region primers. Abnormality of ALT values continued. His plasma albumin levels decreased gradually and gamma globulin levels increased. Periodic screening by ultrasonography revealed liver atrophy and a rough internal echo pattern in 1994. An upper gastrointestinal endoscopy showed an esophageal varix in 1998. According to these findings, he was diagnosed with liver cirrhosis. Small hypervascular nodular legions in the liver were found by magnetic resonance imaging (MRI) in January 2000. He was admitted to our hospital again on February 8, 2000 for further examination of the liver tumor. Physiological examination showed mild emaciation and edema. The liver was atrophic and he had an ascites. Laboratory test results are shown in Table 1. Hematological findings showed a low platelet count. A normal level of ALT showed that he was in the terminal stage of long-lasting inflammation. He had a hypoalbuminemia and polyclonal hypergammaglobulinemia due to liver cirrhosis. He remained negative for anti-HCV and for HCV RNA using PCR (Amplicore, Roche). His stored sera obtained at the recent admission and in 1990 were negative for HBV DNA by using PCR test (Amplicore). His serum in 1997 was negative for hepatitis G virus (HGV/GBV-C) RNA[12] but positive for TTV DNA[13] by PCR. The alpha-fetoprotein level was elevated. Ultrasonography showed a highly atrophic liver with multiple small nodules and splenomegaly. A dynamic CT scan showed multiple hypervascular nodular regions that were compatible with HCC (Fig. 2b). A biopsy was taken from one of the nodules and the non-tumor portion after obtaining informed consent. Histological findings from the non-tumor portion showed nodule formation (Fig. 3c). The tumor portion revealed HCC (Fig. 3d). Multiple HCC prevented him from receiving any active treatment.

We could not detect HBV DNA in the formalin-embedded liver tissue (Fig. 4, lane 1).

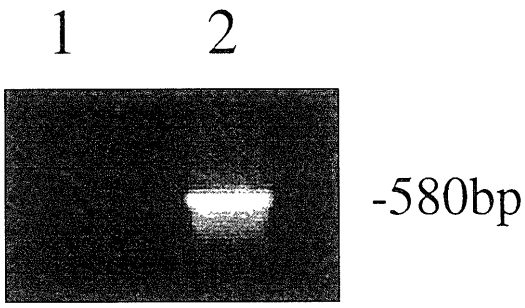

Fig. 4. PCR analysis for HBV DNA in the paraffin-embedded liver tissue of the patient (lane 1) and the patient positive for HBsAg (lane 2). Primers that amplify 580 bp, from nucleotide 1250 to 1830 (1230 to 1900 for the first round) of HBV DNA, were used for the nested-PCR analysis.

DISCUSSION

Considering the pathogenesis of the patient's disease, involvement of several etiological factors should be discussed. The first concern is the possible involvement of his alcohol consumption with the disease progression. In order to clarify the precise role of his alcohol consumption on the pathogenesis, we obtained detailed information on his alcohol history. He had been an heavy drinker of about 80 grams ethanol daily until 1985. The first liver biopsy, taken in 1986, one year after he ceased consuming alcohol, did not show much evidence of alcoholic liver injury, such as pericellular fibrosis and alcoholic hyaline bodies. He remained abstinent until 1993. Although he resumed drinking alcohol in 1993 and continued until the latest admission, the amount was low (about 28 grams ethanol per day). Thus, alcohol does not seem to be the main etiological factor in the pathogenesis, although it may have accelerated the disease progression and been involved in the development of HCC as in chronic hepatitis B and C[14]. The diagnosis of non-alcoholic steatohepatitis (NASH) also should be ruled out. Although he had a mild diabetes mellitus, the level of fatty infiltration was too low to be diagnosed as NASH (Fig. 3a). That the CT density of the liver was not any lower than that of spleen (Fig. 2a) also does not suggest a high concentration of fat in the liver.

The possible involvement of HBV in the pathogenesis also should be discussed. The patient has been negative for HBsAg, but positive for anti-HBs throughout the disease progression. HBV DNA was negative both by PCR using primers for the surface region and by the commercial PCR test in several serum samples obtained during his clinical course. In

addition, we could not detect HBV DNA in the formalin-embedded tissue (Fig. 4, lane 1). Thus, we could not obtain any evidence of clinically overt infection with HBV. The possibility remains that a small amount of HBV DNA in the liver, which was under the detection limit of PCR, might play some role in the hepatocarcinogenesis. However, generally speaking, the negativity of HBV DNA in the sera by these sensitive tests may rules out the possibility that HBV is the main cause of long-standing hepatic inflammation and further hepatocarcinogenesis in this patient. In addition, recent reports deny a role in hepatocarcinogenesis for GBV-C and TTV[15-18]. Thus, despite the positivity of this patient for TTV, we are not able to identify any specific factor that caused the disease progression.

Omagaki et al.[19] analyzed 40 patients with chronic liver disease who were negative for both HBsAg and anti-HCV and reported that 9.7% of the patients with liver cirrhosis developed HCC annually. Twenty-seven (67.5%) of the patients showed a mild elevation of ALT levels. This patient might have the same etiology as some of those patients.

Because liver cirrhosis is a premalignant stage, there was a high probability that HCC would develop and may not have been related to any specific etiology. However, in this case, HCC arose from liver cirrhosis which had evolved from chronic hepatitis of which the histological findings were similar to those of a viral origin. In addition, the patient's clinical course was similar to chronic hepatitis B and C. For these reasons, we suspect a viral etiology for the pathogenesis, a virus which can not be detected by any currently available any serological tests.

REFERENCES

1) Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Koida I, Arase Y, Fukuda M, Chayama K, Murashima N, Kumada H: Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. *J Hepatol* **28:** 930-938, 1998.
2) Takano S, Yokosuka O, Imazeki F, Tagawa M, Omata M: Incidence of hepatocellular carcinoma in chronic hepatitis B and C: A prospective study of 251 patients. *Hepatology* **21:** 650-655, 1995.
3) Okuda K: Hepatocellular carcinoma. *J Hepatol* **32: (Suppl. 1)** 225-237, 2000.
4) Bosch X, Ribes J, Borras J: Epidemiology of primary liver cancer. *Semin Liver Dis* **19:** 271-285, 1999.
5) Yuan J-M, Govindarajan S, Henderson BE, Yu MC: Low prevalence of hepatitis C infection in hepatocellular carcinoma cases and population controls in Guanxi, a hyperendemic region for hepatocellular carcinoma in the People's Republic of China. *Br J Cancer* **74:** 491-493, 1996.
6) Kew MC, Yu MC, Kedda MA, Coppin A, Sarkin A, Hodkinson J: The relative roles of hepatitis B and C viruses in the etiology of hepatocellular carcinoma in Sourthern African Blacks. *Gastroenterology* **112:** 184-187, 1997.
7) Di Bisceglie AM: Hepatitis C and hepatocellular carcinoma. *Hepatology* **26 (Suppl. 1):** 34S-38S, 1997.
8) Kubicka S, Rudolph KL, Hanke M, Tietze MK, Tillmann HL, Trautwein C, Manns M: Hepatocellular carcinoma in Germany: a retrospective epidemiological study from a low-endemic area. *Liver* **20:** 312-318, 2000.
9) Widell A, Verbaan H, Wejstal R, Kaczynski J, Kidd-Ljunggren K, Wallerstedt S: Hepatocellular carcinoma in Sweden: its association with viral hepatitis especially with hepatitis C viral genotypes. *Scand J Inf Dis* **32:** 147-152, 2000.
10) Suga M, Senota A, Arima K, Kodama T, Ikuta S, Sakamoto H, Ohe Y, Sugai S, Yachi A: Prevalence of HBV and HCV infection in Japanese patients with hepatocellular carcinoma. *Hepatogastroenterology* **41:** 438-441, 1994.
11) Hamasaki K, Nagata K, Tsutsumi T, Tsuruta S, Nakao K, Kato Y, Shima M, Koji T, Nagataki S: Changes in the prevalence of hepatitis B and C infection in patients with hepatocellular carcinoma in the Nagasaki Prefecture in Japan. *J Med Virol* **40:** 146-149, 1993.
12) Shimizu M, Osada K, Okamoto H: Transfusion-transmitted hepatitis G virus following open heart surgery. *Transfusion* **36:** 937, 1998.
13) Nishizawa T, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M: A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. *Biochem Biophys Res Commun* **241:** 92-97, 1997.
14) Tagger A, Donato F, Ribero ML, Chiesa R, Portea G, Gelatti U, Albertini A, Fasola M, Boffetta P, Nardi G: Case-control study on hepatitis C virus (HCV) as a risk factor for hepatocellular carcinoma: the role of HCV genotypes and the synergism with hepatitis B virus and alcohol. Brescia HCC study. *Int J Cancer* **81:** 695-699, 1999.
15) Abe K, Edamoto Y, Park YN, Nomura AM, Taltavull TC, Tani M, Thung SN: In situ detection of hepatitis B, C, and G virus nucleic acids in human hepatocellular carcinoma tissues from different geographic regions. *Hepatology* **26:** 1653-1657, 1997.
16) Bréchot C, Jaffredo F, Lagorce D, Gerken G, Büs-

chenfelde KM, Papakonstontinou A, Hadziyannis S, Romeo R, Colombo M, Rodes J, Bruix J, Williams R, Naoumov N: Impact of HBV, HCV, and GBV-C/HGV on hepatocellular carcinomas in Europe: results of a European concerted action. *J Hepatol* **29:** 173-183, 1998.

17) Tagger A, Donato F, Ribero ML, Binelli G, Gelatti U, Portera G, Albertini A, Fasola M, Chiesa R, Nardi G: A case-control study on a novel DNA virus (TT virus) infection and hepatocellular carcinoma. The Brescia HCC study. *Hepatology* **30:** 294-299, 1999.

18) Kim SR, Hayashi Y, Kudo M, Imoto S, Song KB, Ando K, Shintani S, Koterazawa T, Kim KI, Taniguchi M: TTV positivity and transfusion history in non-B, non-C hepatocellular carcinoma compared with HBV- and HCV- positive cases. *Intervirology* **43:** 13-15, 2000.

19) Omagari K, Komatsu K, Kato Y, Nakata K, Kusumoto Y, Mori I, Furukawa R, Tanioka H, Tajima H, Koga M, Yano M: Clinical manifestations of HBsAg and anti-HCV negative chronic liver disease in Nagasaki Prefecture in Japan. *Intern Med J* **35:** 600-604, 1996.