Prevalence of Antibody against Non-A,non-B Hepatitis Virus in Japanese Patients with Hepatocellular Carcinoma

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Antibodies against a possible causative agent of non-A,non-B hepatitis, hepatitis C virus (HCV), in Japanese patients with hepatocellular carcinoma were analyzed using the enzyme-linked immunosorbent assay (ELISA) system from Ortho Diagnostic Systems, Japan. Fifty of 58 cases of hepatitis B virus surface antigen (HBsAg)-negative hepatocellular carcinoma were positive for the antibody (86%) and 8 of 42 cases of HBsAg-positive hepatocellular carcinoma were positive (19%). Among patients with HBsAg-negative hepatocellular carcinoma, the prevalence of the antibody was greater among those who had received a blood transfusion (97%) than among those with no history of transfusion (70%). Only 3 of 54 patients with cancers other than hepatocellular carcinoma were found to be antibody-positive (5.6%) and all three patients had a history of blood transfusion. These results show a close relationship between the presence of anti-HCV antibody and HBsAg-negative hepatocellular carcinoma in Japan.

Key words: Non-A,non-B hepatitis virus — Hepatocellular carcinoma — Hepatitis C virus — Antibody

Hepatocellular carcinoma is a common neoplasm throughout the world, especially in Asia, the South Pacific and Africa.¹⁾ In Japan, there were more than 20,000 deaths from hepatocellular carcinoma in 1987 and this incidence was the third highest among deaths from cancer.²⁾ Epidemiological studies have shown that this neoplasm is associated with several possible causative factors including infectious agents, of which hepatitis B virus (HBV) is the most suspect.³⁻⁵⁾ One of the antigens, HBsAg, encoded by HBV and integration of viral DNA have been observed in the sera and liver, respectively, of patients with hepatocellular carcinoma, suggesting a close relationship between HBV infection and this neoplasm. However, in a recent epidemiological study in Japan, the incidence of hepatocellular carcinoma associated with HBV infection was found to have been constant over the last ten years, whereas the incidence of hepatocellular carcinoma has been increasing.⁶⁻⁸⁾ Carcinomas that are not associated with infection with HBV or hepatitis A virus (HAV) are thought to be associated with another infectious agent(s) named non-A,non-B hepatitis virus(s).⁶⁻⁹⁾ Non-A,non-B hepatitis is often observed in patients who have received blood transfusions and also in patients who have used blood products

such as clotting factors.¹⁰⁾ Since chronic liver diseases are thought to be the main causes of hepatocarcinogenesis, infection with non-A,non-B hepatitis virus seems a very important factor for development of hepatocellular carcinoma.⁹⁾

Recently genes of a causative agent of transfusion-associated non-A,non-B hepatitis have been isolated from the plasma of a chimpanzee containing a high titer of infectious non-A,non-B hepatitis¹¹⁾ and this causative agent has been named hepatitis C virus (HCV), although it is not yet proven that a living organism which contains this genetic material is really infectious or that the material causes liver disease. A viral protein expressed by one clone of the viral genes in yeast is used for detecting circulating antibody against HCV.¹²⁾ Patients with post-transfusion non-A,non-B hepatitis in Japan show a high prevalence of antibody against this protein, such as 78% in patients with chronic hepatitis and 15% in those with acute hepatitis.¹²⁾ Among blood donors in the Tokyo district, 1.2% were estimated to be positive for this antibody,¹³⁾ indicating an abnormally high incidence of the antibody in patients with hepatitis. Similar results have been reported from other countries.^{12, 14-19)}

Since post-transfusion chronic non-A,non-B hepatitis is often linked to liver cirrhosis, and may develop into hepatocellular carcinoma 20 to 30 years later, the inci-

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dence of the antibody is likely to be high in patients with hepatocellular carcinoma. To test this possibility, we compared the incidences of antibody-positive sera in patients with hepatocellular carcinoma and patients with cancers other than hepatocellular carcinoma.

We used the HCV antibody ELISA system supplied by Ortho Diagnostic Systems (Tokyo) to detect the antibody in these sera, and examined 100 sera from patients with hepatocellular carcinoma, of which 42 were positive for HBsAg. Of the 58 HBsAg-negative sera from cases of hepatocellular carcinoma, 15 were positive for both hepatitis B virus surface antibody (anti-HBs) and hepatitis B virus core antibody (anti-HBc) and 2 were positive only for anti-HBc. The ratios of males to females among the patients with hepatocellular carcinoma who were positive and negative for HBsAg were 14 to 5 and 11 to 4, respectively. Ten of the 42 HBsAg-positive cases and 31 of 56 HBsAg-negative cases (omitting 2 with unknown histories) had a history of blood transfusion. In addition, tests were made on 54 sera from patients with cancers other than hepatocellular carcinoma; namely, 20 with lung cancer, 9 with gastric cancer, 4 with colorectal cancer, 5 with pancreas carcinoma, 6 with gall bladder carcinoma, 2 each with cholangiocarcinoma, acute myeloblastic leukemia, multiple myeloma and malignant lymphoma and one each with ovarian cancer and ureter cancer, of which 30 were males and 24 were females. Three of these patients with cancer other than hepatocellular carcinoma had a history of blood transfusion. Samples of 20 μ l of 10-fold-diluted serum were tested for antibody by the method recommended by the supplier and values were measured as absorbance at 490 nm with a Microelisa Auto Reader (Dynatec Instrument, USA). As shown in Table I, 58% (58 of 100 cases) sera from patients with hepatocellular carcinoma were positive for

the antibody. The incidence of antibody in the HBsAg-negative sera was 86%, whereas that in the HBsAg-positive sera was only 19%. There was no difference in the incidences of HCV antibody in the anti-HBs and anti-HBc positive and negative sera in patients with HBsAg-negative hepatocellular carcinoma. The incidence of virus antibody in patients with other cancers was low (5.6%). These findings suggest a close association between the presence of the antibody, possibly indicating HCV infection, and liver cancer that is not associated with HBV infection. Similar results were reported by Kiyosawa *et al.*²⁰⁾

The higher incidence of anti HCV antibody in patients with other cancers (5.6%) than healthy carriers (1.5%) was thought to be due to blood transfusion. In fact, in our study all the "other cancer" patients with this antibody (3 of 48 cases) had a history of blood transfusion. Moreover, among patients with HBsAg-negative hepatocellular carcinoma, the incidence of anti-HCV antibody was higher in the group with a history of blood transfusion (97%) than in the group with no history of blood transfusion (70%) (Table II). On the other hand, in the group with HBsAg-positive hepatocellular carcinoma, the incidence of anti-HCV antibody was 40% (4 of 10) among recipients of blood transfusions. These results strongly suggest a close association between the incidence of virus infection, through blood transfusion or in community-acquired ways, and the pathogenesis of HBsAg-negative hepatocellular carcinoma.

There is evidence of a high rate of involvement of HCV in cases of hepatitis not associated with HBV infection. Among patients with blood transfusion non-A, non-B hepatitis in the United States, Italy, Japan and Spain, 71, 84, 78 and 85%, respectively, were found to have anti-HCV antibody.^{12, 14)} Since the incidences of asymptomatic carriers of HCV in these countries are estimated as 1–2% or less, these high values in patients with non-A, non-B hepatitis indicate significant involve-

Table I. Prevalence of Anti-HCV Antibody in Sera from Patients with Hepatocellular Carcinoma

Disease	Cases	Positive for anti-HCV antibody
Hepatocellular carcinoma		
HBsAg (+)	42	8
HBsAg (–)	58	50
anti-HBs anti-HBc		
(+) (+)	15	12
(–) (+)	1	1
(–) (–)	40	35
un un	2	2
Other cancers	54	3

un: unknown

Table II. Relation of Anti-HCV Antibody to History of Blood Transfusion in Patients with HBsAg-positive and -negative Hepatocellular Carcinoma

HBsAg	History of blood transfusion	Anti-HCV antibody	
		+	–
Positive	+	4	6
(42 cases)	–	4	28
Negative	+	30	1
(56 cases) ^{a)}	–	19	6

a) Two cases with no records about blood transfusion were not included.

ment of HCV infection in this disease. Application of systems other than that which we used in this study for detection of antibody against HCV will be important to get more accurate information.

The role of this virus in the development of liver cancer is unknown. Integration of HBV DNA into cancer cells has been observed in cases of hepatocellular carcinoma associated with HBV infection. For the detection of HCV gene, fractions of nucleic acid from cancerous and non-cancerous portions of liver obtained by surgical resection of hepatocellular carcinomas were subjected to reverse transcription followed by polymerase chain reaction using primers with nucleotide sequences adjusted to the Japanese type of HCV to detect a non-structural region of the virus genome.²¹⁾ In this way, 7 out of 10 liver specimens were found to be positive for HCV RNA. However, the copy number of viral RNA per cell in these specimens was estimated to be less than 0.01 (unpublished data) indicating that the virus genome

was not present in all the cancerous cells. This observation suggests that the role of HCV in maintenance of the malignant state of liver cancer, if any, is different from that of HBV.

Eight cases of HBsAg-negative hepatocellular carcinoma were shown to be negative for anti-HCV antibody. These cases may not have been infected with HCV or may have had too low a titer of antibody to detect by this method, but it is also possible that another causative agent(s) was responsible for those hepatocellular carcinomas.

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