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Increased Prevalence of HTLV-I Infection in Patients with Hepatocellular Carcinoma Associated with Hepatitis C Virus

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The progression from chronic hepatitis C virus (HCV) infection to hepatocellular carcinoma (HCC) has been reported. We evaluated whether co-infection with the human T-lymphotropic virus type I (HTLV-I) might be associated with this transition in a cross-sectional analysis of 127 patients with HCV-chronic hepatitis (mean age=51.7) and 43 patients with HCV-associated HCC (mean age=62.4); the seroprevalence of anti-HTLV-I was 9.5% and 30.2%, respectively. For subjects 50 years or older, the seroprevalence of anti-HTLV-I in HCC patients was 13/41 (31.7%) which was significantly higher than that in chronic hepatitis patients (6/82, 7.3%) ($P=0.001$). The relative risk (RR) of association was 12.8 ($P=0.0004$) among the males, however, no association was evident among the females, RR=1.3 ($P=0.80$). The increased prevalence of HTLV-I positivity among the HCC cases could not be attributed to a higher rate of prior transfusion. These data suggest that co-infection with HTLV-I may contribute to the development of HCC among patients with HCV-induced chronic liver diseases in a highly HTLV-I-endemic area.

Key words: Human T-lymphotropic virus — Hepatitis C virus — Hepatocellular carcinoma

A slow progression from chronic hepatitis due to hepatitis C virus (HCV) through liver cirrhosis to hepatocellular carcinoma (HCC) has been well recognized.[1,2] Whether other factors contribute to this progression is unknown. On the other hand, human T-lymphotropic virus (HTLV-I) is known to be a causal factor of adult T-cell leukemia.[3] In the south-western part of Japan, where HTLV-I is endemic, the HTLV-I seroprevalence in patients with various malignancies is higher than expected.[4–6] To analyze the effect of HTLV-I co-infection on the oncogenesis in HCC associated with HCV, we examined anti-HTLV-I antibody in patients with chronic hepatitis due to HCV and in patients with HCC positive for anti-HCV antibody in a cross-sectional survey. We found that the HTLV-I seroprevalence in patients with HCC was significantly higher than in patients of comparable age with chronic hepatitis.

Serum samples were obtained from a sequential series of 127 patients seropositive for anti-HCV antibody (mean age=51.7, 17–81 years; 86 males and 41 females) with chronic hepatitis and a sequential series of 43 HCV-seropositive patients (mean age=62.4, 39–76 years; 33 males and 10 females) with HCC who were admitted to our hospital between 1982 and 1994. All patients were positive for HCV antibody measured using a second-generation enzyme-linked immunosorbent assay (Immunocheck-HCV Ab, Kokusaishiyaku, Kobe) and were persistently negative for hepatitis B surface antigen. The diagnosis of chronic hepatitis was confirmed by the pathological examination of liver biopsy specimens. The diagnosis of HCC in 23 patients was based on the examination of biopsy, surgery or autopsy specimens and that in the remaining patients on evaluation of the results of blood biochemistry and several imaging examinations such as ultrasonography, computed tomography and celiac angiography. All of these patients resided in Miyazaki Prefecture, which is one of the most highly HTLV-I-endemic areas in Japan.[7] Anti-HTLV-I antibody was measured using a particle agglutination assay (Serodia-HTLV, Fujirebio, Tokyo) and, in all serum samples positive for HTLV-I antibody, the presence of anti-HTLV-I antibody was confirmed by western blot analysis (Problot HTLV-I, Fujirebio or Eitest-ATL, Eisai, Tokyo). The significance of differences between groups was assessed by chi-square test. The relative risk (RR) of association between HTLV-I seropositivity and HCV-associated HCC was estimated in terms of the odds ratio (OR) with 95% confidence interval (CI), using the Mantel-Haenszel procedure with adjustment for age group (50–59, 60–89).

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Table I. HTLV-I Seropositivity in Patients with Chronic Hepatitis C and Hepatocellular Carcinoma Associated with HCV

Age (years)	Male		Female		Total	
	Chronic hepatitis	Hepatocellular carcinoma	Chronic hepatitis	Hepatocellular carcinoma	Chronic hepatitis	Hepatocellular carcinoma
10–19	0/1[a] (0.0%)	none	none	none	0/1 (0.0%)	none
20–29	1/4 (25.0%)	none	0/2 (0.0%)	none	1/6 (16.7%)	none
30–39	4/16 (25.0%)	0/1 (0.0%)	0/2 (0.0%)	none	4/18 (22.2%)	0/1 (0.0%)
40–49	1/14 (7.1%)	0/1 (0.0%)	0/6 (0.0%)	none	1/20 (5.0%)	0/1 (0.0%)
50–59	1/28 (3.6%)	3/10 (30.0%)	1/16 (6.3%)	0/3 (0.0%)	2/44 (4.6%)	3/13 (23.1%)
60–69	1/22 (4.6%)	6/15 (40.0%)	3/14 (21.4%)	2/5 (40.0%)	4/36 (11.1%)	8/20 (40.0%)
70–79	0/1 (0.0%)	2/6 (33.3%)	none	0/2 (0.0%)	0/1 (0.0%)	2/8 (25.0%)
80–89	none	none	0/1 (0.0%)	none	0/1 (0.0%)	none
Total	8/86 (9.3%)	11/33 (33.3%)	4/41 (9.8%)	2/10 (20.0%)	12/127 (9.5%)	13/43 (30.2%)

a) Number of patients who tested positive for anti-HTLV-I antibody/Number of patients examined.

Table II. Relationship between HTLV-I Infection and Blood Transfusion in Patients with Chronic Hepatitis C and Hepatocellular Carcinoma Associated with HCV

HTLV-I infection	Chronic hepatitis	Hepatocellular carcinoma	Total
Positive	6/12[a] (50.0%)	6/12 (50.0%)	12/24 (50.0%)
Negative	39/108 (36.1%)	12/30 (40.0%)	51/138 (37.0%)
Total	45/120 (37.5%)	18/42 (42.9%)	63/162 (38.9%)

a) Number of patients who had a history of blood transfusion/Number of patients examined.

Table I shows the HTLV-I seropositivity in each group. The overall rates of HTLV-I seropositivity in patients with chronic hepatitis with HCC were 9.5% and 30.2%, respectively. In chronic hepatitis, HTLV-I seropositivity peaked among those in their thirties and declined thereafter. In contrast, the seropositivity in the general population of Miyazaki Prefecture is known to increase with age, especially for women, reaching a maximum of about 15%.[7] As expected, the HCV-positive HCC patients were older than those with HCV-positive chronic hepatitis.

To evaluate whether co-infection with HTLV-I contributes to the apparent evolution of chronic hepatitis to HCC, we compared the prevalence of HTLV-I antibody by sex and age-group among patients aged 50 and older, using the chronic hepatitis group as the referent. The overall seroprevalences of anti-HTLV-I in patients with HCC and chronic hepatitis patients were 13/41 (31.7%) and 6/82 (7.3%), respectively, and the difference between them was significant ($P=0.001$). The HTLV-I seropositivity in male patients with chronic hepatitis and with HCC was 3.9% (2/51) and 35.5% (11/31), respectively. There was a strong association between the prevalence of HTLV-I and HCV-associated HCC among men, OR=12.8 (95% CI, 3.3–52.3), but not among women, OR=1.3 (0.17–10.1). The lack of an association for women in these data is difficult to explain. Because the numbers are small, additional studies on this question should allow a better evaluation of risk for both sexes.

Other groups are also of interest for comparison. We screened 23 patients with cirrhosis associated with HCV and 3 (13.3%) were positive for anti-HTLV-I antibody (data not shown); this prevalence was slightly higher than that in patients with chronic hepatitis but was not as high as that in patients with HCC. This group may possibly include some HCC cases, as it is very difficult to differentiate completely between HCC and cirrhosis. We also examined the prevalence of HTLV-I seropositivity in 40 patients with CH due to hepatitis B virus (HBV) and in 18 patients with HCC associated with HBV (data not shown). The prevalence of anti-HTLV-I antibody positivity in these two groups was 5.0% and 16.7%, respectively. However, we could not control for age in this comparison, because the age distributions were quite different.

Because blood transfusion is a transmission route for both HTLV-I and HCV, we examined the relationship

between the anti-HTLV-I antibody status and the history of blood transfusion in the patients (Table II). Forty-five (38.0%) of 120 patients with chronic hepatitis and 18 (42.9%) of 42 patients with HCC had a history of at least one blood transfusion. Although the transfusion rates were somewhat higher for the HTLV-I-positive subjects in both groups, the differences were not significant. Nor was there evidence of a difference in transfusion rates and HTLV-I seroprevalence between the two groups. Moreover, the time of blood transfusion, which was known for 5 of the 6 HCC patients positive for anti-HTLV-I antibody, was more than 10 years prior to the date of diagnosis of HCC in 4 of these 5 patients, suggesting that the HCC developed long after blood transfusion. These results suggest that the high prevalence of HTLV-I antibody positivity among HCC patients is not related to the blood transfusion status.

Our results suggest that HTLV-I infection may play some role in the progression from chronic liver diseases to HCC. It is difficult to specify which step of the progression from chronic hepatitis to HCC through cirrhosis might be affected by HTLV-I infection. Although HCC is closely related with HCV infection, the mechanism of hepatocarcinogenesis due to HCV is not clear. We also cannot account for the high HTLV-I seropositivity in patients with HCC associated with HCV. It is very unlikely that the HTLV-I infection directly causes HCC. However, the altered immune surveillance system in HTLV-I carriers is probably related to their development of malignancies.[4–6]

Furthermore, HCV RNA was detected in peripheral blood mononuclear cells of patients with chronic hepatitis C.[8] HCV was also demonstrated to infect a human T-cell line *in vitro*.[9] CD4 T-cells are known to be the target cells of HTLV-I and these cells are observed around the portal area and around piecemeal necrosis in liver tissue specimens in patients with chronic hepatitis.[10] The functions of the CD4 T-cells, such as cytokine production, are reported to be altered in HTLV-I carriers,[11] and this alteration might be involved in the progression of hepatitis. This might result in the development of cirrhosis, which is closely related to HCC. Alternatively, co-infection of HCV with HTLV-I may result in a diminished ability to clear chronic HCV infection, since HTLV-I carriers have significantly reduced cellular immunity.[12]

We have shown here that, in a highly HTLV-I-endemic area, co-infection with HTLV-I is significantly more frequent among patients with HCV-induced HCC than among patients with HCV-induced chronic hepatitis, although the numbers of patients involved in this study were limited. Additional large-scale prospective studies are necessary to clarify whether HTLV-I co-infection is a risk factor for carcinogenesis in patients with HCV-induced chronic liver diseases in HTLV-I-endemic areas.

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