

# High prevalence of anti-hepatitis B virus serological markers in patients with hepatitis C virus related chronic liver disease in Japan

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Accepted for publication 17 February 1999

## Abstract

**Background/Aims**—Evidence is accumulating that hepatitis B virus (HBV) is present in patients who are hepatitis B surface antigen negative but have antibody to hepatitis B core antigen (anti-HBc). Furthermore, recent studies have shown that patients with hepatocellular carcinoma who have antibody to hepatitis C virus (HCV) often possess HBV related serological markers. Data on the seroprevalence of HBV infection in patients with HCV related chronic liver disease were collected to evaluate the significance of the presence of antibodies to HBV.

**Methods**—The prevalence of HBV related serological markers was analysed in a total of 2014 Japanese patients with HCV infection. The control group comprised 352 subjects without liver disorder.

**Results**—A large number of patients (49.9%) with HCV related chronic liver disease including hepatocellular carcinoma were positive for anti-HBc. In addition, the prevalence of anti-HBc closely correlated with the clinical stage of the liver disease. There was no relation between a past history of blood transfusion and the prevalence of anti-HBc. Notably, anti-HBc was the only serological marker for HBV infection in a significant number of patients with HCV related chronic liver disease (24.1%).

**Conclusions**—Our data provide further evidence for the high prevalence of anti-HBc in patients with HCV related chronic liver disease, particularly those with hepatocellular carcinoma, suggesting that HBV infection, probably including latent infection, may play an important role in carcinogenesis in these patients.

(Gut 1999;45:284–288)

Keywords: hepatitis B virus; anti-HBc; hepatitis C virus; hepatocellular carcinoma

Hepatitis C virus (HCV) has been known to be a causative agent of chronic liver disease, and this persistent viral infection is closely linked to the development of primary hepatocellular carcinoma (HCC).<sup>1–4</sup> Although the mechanism of carcinogenesis in patients with HCV infection has not been clarified, several investigators have recently shown that anti-HCV positive patients with HCC also often have hepatitis B virus (HBV) related antibodies in their

serum,<sup>5–9</sup> suggesting that HBV infection may be involved in the development of HCC in patients who are anti-HCV positive. On the other hand, we have recently shown that the HBV genome is invariably present in the liver tissue of healthy donors who are hepatitis B surface antigen (HBsAg) negative but positive for antibody to hepatitis B core antigen (anti-HBc).<sup>10</sup> Moreover, many previous studies have documented that HBV genomes are often detected in the liver tissue of patients even if they are negative for HBsAg.<sup>11–21</sup> These data suggest that HBV infection may often be observed in the liver of subjects who are positive for anti-HBV markers, and therefore it is possible that a considerable number of patients who have hitherto been regarded as having “HCV related chronic liver disease” may also have latent HBV infection. Thus, we studied 2014 patients in Japan with HCV related chronic liver disease, including those with HCC, in order to look for serological markers of HBV infection. We discuss the significance of the presence of anti-HBV markers in patients with HCV infection in the development of HCC.

## Patients and methods

### PATIENTS

Patients with HCV related chronic liver disease who visited Kyoto University or its affiliated hospitals in Japan between April 1996 and March 1997 were considered to be eligible for this study. Of these, all patients fulfilling the following criteria were randomly selected and included: (a) serum anti-HCV positive at entry; (b) serum HBsAg negative at entry; (c) information on other serological markers of HBV available. These cases were classified into the following three groups on the basis of clinical findings, blood chemistry, ultrasonography, angiography, and/or histological findings of specimens obtained by fine needle biopsy and/or at operation: chronic hepatitis (CH), liver cirrhosis (LC), and HCC. Information on cigarette smoking, alcohol consumption, and medical and family history was carefully obtained at the time of enrollment through interviews by the doctors in charge and the experienced public health nurses, and if the information from any of the patients was unre-

**Abbreviations used in this paper:** HCV, hepatitis C virus; HBV, hepatitis B virus; HBc, hepatitis B core; HCC, hepatocellular carcinoma; HBsAg, hepatitis B surface antigen; CH, chronic hepatitis; LC, liver cirrhosis.

Table 1 Characteristics of patients with hepatitis C virus related chronic liver disease

	CH	LC	HCC	Total
No of patients	973	430	611	2014
Age (years)	59.1 (0.6)	60.3 (0.4)	65.8 (0.3)*	61.4 (0.3)
Sex (M/F)	522/451	210/220	475/136*	1207/807
Daily drinkers (%)	30.7	29.4	45.5*	34.8
Smoking index 400 (%)	25.4	22.7	39.7*	29.2
Past history of blood transfusion (%)	38.0	36.1	30.6	35.4

Age is given as mean (SE). Values for drinking, smoking, and past history of blood transfusion represent percentages of the total number of patients in each group. CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma.

\*Significantly higher than LC or CH group ( $p < 0.01$ ).

liable or unclear, these patients were excluded from the evaluation. Drinking habits were classified into the following two grades: (a) non-drinkers and occasional drinkers; (b) daily drinkers. Daily drinkers were defined as those drinking alcohol 5 days/week for 15 years. A smoking index was obtained by multiplying the average number of cigarettes smoked a day by the total number of years of smoking. Accordingly, smoking habits were also classified into the following two grades based on the smoking index: (a) non-smokers and light smokers (smoking index <400); (b) heavy smokers (smoking index 400 or above). In all cases, the presence or absence of anti-HBV antibodies in the serum was examined. Using these procedures, 2014 patients were finally enrolled into the study. For the control group, a total of 352 subjects were randomly selected with the following restrictions: (a) a visit during the same period to the same hospital; (b) no liver disease confirmed by blood chemistry and ultrasonography; (c) anti-HCV negative at entry.

#### SEROLOGICAL EVALUATION

The tests for antibody to HBsAg (anti-HBs) and anti-HBc in patient sera were by enzyme immunoassay and/or radioimmunoassay using commercially available kits (Dainabott, Tokyo, Japan). Serum HBV DNA was determined by polymerase chain reaction assay as described previously.<sup>10</sup> The diagnosis of HCV infection was made by the presence in the serum of anti-HCV, as assessed by second or third generation assays.

#### STATISTICAL ANALYSES

The  $\chi^2$  test was used to compare frequencies entered in 2 by 2 contingency tables, and all  $p$  values were based on two sided tests. For the subgroup analyses, odds ratios and 95% confidence intervals were determined. All calculations were performed using SAS software (SAS Institute Inc).

Table 2 Anti-HBV serological markers in different clinical stages of hepatitis C virus related chronic liver disease

	CH	LC	HCC	Total
Anti-HBs positive	25.6	28.1	35.4*	29.1
Anti-HBc positive	43.8	49.8	59.4*	49.9
Age (years)				
<60	36.3 (160/441)	41.8 (46/110)	58.9* (56/95)	40.6 (262/646)
≥60	50.0 (266/532)	52.8 (169/320)	59.5* (307/516)	54.2 (742/1368)

Values represent percentages of the total number of patients in each group. Values in parentheses show the actual number of patients (patients with anti-HBc positive/total patients). HBV, hepatitis B virus; CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma; anti-HBs, antibody to hepatitis B surface antigen; anti-HBc, antibody to hepatitis B core antigen.

\*Significantly higher than LC or CH group ( $p < 0.01$ ).

## Results

Table 1 summarises the clinical features of the enrolled patients. A total of 2014 patients were considered to be eligible for the study. Of these, 973 had a diagnosis of CH, 430 had LC, and 611 had HCC. The mean age of the patients with HCC was significantly higher than that of those with CH or LC (65.8 *v* 59.1 and 60.3 respectively). The proportion of women patients was much smaller for HCC than CH or LC (22.3% *v* 46.4 and 51.2% respectively). Both the smoking and alcohol indices were significantly higher in patients with HCC than in those with either CH or LC in accordance with many previous reports.<sup>8 22-24</sup> At the time of enrollment, all patients were interviewed about their previous history; of the 1775 patients whose information was considered to be reliable, 629 (35.4%) had received a blood transfusion. The percentage of patients with a history of blood transfusion was not significantly different among patients with CH, LC, and HCC (38.0, 36.1, and 30.6% respectively). Information on the history of intravenous drug use and previous HBV vaccination was also obtained at enrollment, and a total of 12 and eight patients respectively had such histories. However, no association was found between these factors and the clinical stage of liver disease.

Table 2 shows serological status with regard to anti-HBV antibodies. A total of 52 anti-HCV positive patients with HBV marker (12 CH, 15 LC, and 25 HCC) were selected randomly and examined for the presence of circulating HBV DNA sequences, as detected by amplification of the S region.<sup>10</sup> Serum HBV DNA was not present in any of the patients. Of those with HCV related chronic liver disease, 29.1% were positive for anti-HBs, a value that is clearly higher than that observed in the general population.<sup>25</sup> More significantly, a total of 1004 patients (49.9%) were positive for the anti-HBc antibody; again, this rate is higher than that observed in the general population.<sup>25</sup> The proportion of patients who were positive for the anti-HBs and anti-HBc increased with progression of the liver disease. In particular, the percentage of patients testing positive for anti-HBs and the percentage positive for anti-HBc were both significantly higher in cases of HCC than in cases of either CH or LC. It is widely recognised that the prevalence of anti-HBV serological markers increases with age, and the mean age of patients with HCV related chronic liver disease is generally high.<sup>23 24</sup> Thus it may be that the high prevalence of anti-HBV serological markers in these patients is merely a reflection of their advanced age. In order to clarify this, subjects without HCV infection were also tested for anti-HBc antibody, and compared with anti-HCV positive patients (table 3). Anti-HBc was detected in 44 of 352 (12.2%) controls and was distributed around 10% in any age group. This result was consistent with a previous report on blood donors in Japan.<sup>25</sup> Even after matching for age, the rate of seropositivity for anti-HBc in HCV related chronic liver disease was significantly higher than that in the control of any age group. Fur-

Table 3 Anti-HBc positivity in patients with hepatitis C virus (HCV) related chronic liver disease and control patients matched by age

Age (years)	Control	Anti-HCV positive chronic liver disease
20–29	5.4	9.1*
30–39	10.8	27.2*
40–49	11.8	41.5†
50–59	12.6	43.6†
≥60	14.8	54.2†

Values represent percentages of the total number of patients in each group. Anti-HBc, antibody to hepatitis B core antigen.

\*Significantly higher than control ( $p < 0.05$ ).

†Significantly higher than control ( $p < 0.01$ ).

thermore, in order to assess the influence of the difference in mean age between HCC and non-HCC groups on the rate of anti-HBc positivity, the anti-HCV positive patients were split into two age groups (table 2). The positive rate for anti-HBc in the HCC group was found to be significantly higher than that in the CH and LC groups not only in patients under the age of 60 years, but also in those over the age of 60 years. The difference was more pronounced in the younger age group.

To exclude the influence of other factors on the increased positivity of anti-HBc in patients with HCC, the subgroup analyses by sex, alcohol intake, cigarette smoking, and history of blood transfusion were performed between the CH and HCC groups (table 4). We confirmed that the odds ratios for each factor were similar in each subgroup and these factors have no effect on seropositivity for anti-HBc in these patients. Furthermore, there were also no interactive effects of the history of intravenous drug abuse on the high prevalence of anti-HBc in the HCC group (data not shown).

According to a previous report, most blood donors with anti-HBc are usually also anti-HBs positive, while only 2.8% of patients are positive only for anti-HBc.<sup>25</sup> In contrast with the pattern seen in healthy blood donors, there were many patients with HCV infection who were positive only for anti-HBc but negative for anti-HBs. In fact, 24.1% of the total number of patients with HCV related chronic liver disease had anti-HBc in the absence of anti-HBs. In contrast, the positivity of anti-HBs alone was 4.6% among anti-HCV positive patients, and this group included all eight individuals who had received an HBV vaccination.

It may be speculated that the high prevalence of anti-HBV serological markers in patients with HCV infection is due to the fact that many

of them have had a blood transfusion in the past. Therefore, we evaluated the possible relation between a history of blood transfusion and the positive rate for anti-HBc in our study population. The positive rate for the anti-HBc was 46.9% and 50.9% in patients with and without a history of blood transfusion respectively. Thus, a history of blood transfusion cannot account for the higher rate of anti-HBV serological markers in patients with HCV infection. Similarly, other risk factors predisposing to viral transmission such as history of intravenous drug use or chance of sexual transmission were not different between anti-HBc positive and negative patients.

## Discussion

In this study, we found that the prevalence of anti-HBc is almost 50% in patients with HCV related chronic liver disease, which is significantly higher than that reported in healthy blood donors at a blood centre in Japan.<sup>25</sup> It is also higher than that observed in our control group without liver disease (12.2%). Several previous reports have already shown a high prevalence of anti-HBV serological markers in HCV infected patients.<sup>5–9</sup> However, most of the previous reports have mainly focused on patients with HCC, and moreover have not assessed the anti-HBV serological status in relation to age. It is known that the prevalence of anti-HBV serological markers increases with age. Moreover, the mean age of patients with HCV related chronic liver disease is generally high,<sup>23, 24</sup> raising the possibility that the high prevalence of anti-HBV markers in HCV related chronic liver disease found in this study merely reflects their advanced age. Indeed, our patients with anti-HCV are older than the blood donors in the previous report. Therefore we selected subjects without HCV infection as a control group in order to compare the rates of seropositivity in age matched groups. This showed that the prevalence of anti-HBV serological markers was higher in patients with HCV related chronic liver disease than in control subjects irrespective of age.

It is also known that many patients with anti-HCV have a history of blood transfusion.<sup>23, 24</sup> Thus it could be postulated that the high prevalence of anti-HBV markers in patients with HCV infection is merely attributable to having received a blood transfusion in the past. In this study, however, we found that the percentage of patients with a history of blood transfusion was not different in those with and without anti-HBc. Moreover, although the prevalence of anti-HBc increases as the liver disease progresses, the percentage of patients with a history of blood transfusion was not different among patients with CH, LC, and HCC. Taking the results together, we could not find any relation between a previous history of blood transfusion and being positive for anti-HBc. In addition, we confirmed that other factors, such as sex, smoking, and drinking, had no influence on the seropositivity for anti-HBc. No remarkable differences were observed between the anti-HBc positive and negative group in the prevalence of histories predispos-

Table 4 Odds ratios of anti-HBc positivity and hepatocellular carcinoma with hepatitis C virus infection

Subgroup	Odds ratio	95% Confidence interval
Sex		
Male	1.77	1.37 to 2.27
Female	1.84	1.25 to 2.71
Alcohol intake		
Nondrinkers and occasional drinkers	1.48	1.10 to 1.98
Daily drinkers	1.84	1.27 to 2.67
Cigarette smoking		
Nonsmokers and light smokers	1.58	1.18 to 2.11
Heavy smokers	2.10	1.39 to 3.17
Past history of blood transfusion		
Positive	1.79	1.22 to 2.62
Negative	1.64	1.25 to 2.14

Comparison was performed between patients with hepatocellular carcinoma and chronic hepatitis. Anti-HBc, antibody to hepatitis B core antigen.

ing to viral infection such as intravenous drug use. Thus, by analysing a large number of diverse patients including those with HCC, CH, and LC in Japan, we have clearly shown that the prevalence of anti-HBV serological markers is high in HCV related chronic liver disease even when other factors such as a past history of blood transfusion and differences in age are excluded.

It has been generally accepted that patients who are negative for HBsAg, but positive for anti-HBs and/or anti-HBc (low titre), have previously had transient HBV infection. However, it has been recently shown that traces of HBV are often detectable in the blood for many years after clinical recovery from acute hepatitis despite the presence of serum antibodies against the HBV and HBV specific cytotoxic T lymphocytes.<sup>26,27</sup> Furthermore, we have recently shown the transmission of HBV from healthy donors who were negative for HBsAg but positive for anti-HBc (low titre) to recipients in living related liver transplantation.<sup>10</sup> In addition, Koike *et al.*<sup>21</sup> have reported that clonally integrated HBV DNA sequences were identified at a high rate in patients with anti-HCV positive HCC who were seronegative for HBsAg. Taking these results together, it is strongly suggested that seronegativity for HBsAg does not exclude the existence of HBV DNA, and that HBV is often present in the liver tissue of patients who are positive for anti-HBc (low titre) even in the absence of HBsAg.<sup>11-21,28</sup> Thus it is conceivable that the patients with HCV related chronic liver disease who are positive for anti-HBc may have dual sustained infection with both HBV and HCV in the liver.

In this study, we found a strong correlation between the clinical stage of liver disease and the prevalence of anti-HBV antibodies. Indeed, the prevalence of the anti-HBc in patients with HCC was about 60%, which was significantly higher than that observed in CH and LC. These results are in accordance with several previous reports,<sup>5-9</sup> and suggest that HBV co-infection may be involved in hepatocarcinogenesis in patients with HCV related chronic liver disease. In contrast with our data, however, there is one study which showed that the rate of positivity for anti-HBV markers in patients with anti-HCV positive HCC was not high.<sup>29</sup> The reasons for these discrepant findings are unclear. They may be due to geographical difference, even though both studies were carried out in Japanese patients. However, it is more likely to be attributable to the different methods of evaluation for HBV infection. HBV serostatus was evaluated mainly by measuring anti-HBs status in the previous study. Our data on HBV transmission in liver transplantation, however, suggest that it is more important to test for anti-HBc rather than anti-HBs to predict latent HBV infection.<sup>10</sup>

In relation to the importance of anti-HBc in evaluating latent HBV infection, an interesting observation in this study is the characteristic pattern of anti-HBV serological status in patients with HCV infection. Generally, healthy blood donors who are positive for anti-

HBc also tend to be positive for anti-HBs. In this study, however, we found that as many as 24.1% of the patients with HCV related chronic liver disease were positive for anti-HBc but negative for anti-HBs. The reason for the difference in anti-HBV serological status between healthy blood donors and patients with HCV related chronic liver disease is unclear at present.<sup>30</sup> It may be that HBV infection occurs in anti-HCV positive patients at a younger age than the healthy population, because anti-HBc remains detectable long after anti-HBs is no longer detectable.<sup>31</sup> However, an alternative explanation may be that co-existing HCV protein has a negative effect on the synthesis of HBsAg, with a resulting decrease in anti-HBs production. Indeed, a previous study has shown an inverse relation between HBV and HCV replication.<sup>32,33</sup> In another report, HCV core protein has been shown to dramatically suppress HBsAg synthesis.<sup>34</sup>

In conclusion, this study has clearly shown a high prevalence of anti-HBc in HCV related chronic liver disease, including not only HCC but also CH and LC. Considering the accumulating evidence showing that the seropositivity of anti-HBc is often associated with sustained hepatic infection with HBV, our data suggest the possibility that HBV infection, including latent infection, may be important in the development of HCC in patients with HCV related chronic liver disease.

The following institutions and investigators also participated in the study: Dr N Kitajima, Miki City Hospital; Dr K Nishina, Kurashiki Central Hospital; Dr T Suyama, Shiga Medical Center for Adults; Dr M Nabeshima, Kyoto Katsura Hospital; Dr S Ono, Aiko Municipal Hospital; Dr O Nishida, Otsu Red-Cross Hospital; Dr H Komori, Nishi-Kobe Medical Center; Dr T Tamada, Takatsuki Red Cross Hospital. The work was supported by Grants-in-Aid for Scientific and Cancer Research from the Ministry of Education, Science and Culture, a Grant from Ministry of Health and Welfare, Japan, and a Grant from the Japan Society for the Promotion of Science (no JSPS-RFTF97100201).

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