Prospective study of interferon therapy for compensated cirrhotic patients with chronic hepatitis C by monitoring serum hepatitis C RNA. Hepatology. 1999; 29:1573-80. [PMID: 10216145]

20. **Shiratori Y, Moriyama M, Imazeki F, Ohkubo H, Tanaka N, Arakawa Y, et al.** A prospective study of interferon therapy modified by pre-treatment viral load in cirrhotic patients. Tokyo-Chiba Hepatitis Research Group. Liver. 2000; 20:271-80. [PMID: 10959805]

21. **Kato N, Yokosuka O, Hosoda K, Ito Y, Ohto M, Omata M.** Quantification of hepatitis C virus by competitive reverse transcription-polymerase chain reaction: increase of the virus in advanced liver disease. Hepatology. 1993;18:16-20. [PMID: 8392026]

22. **Okamoto H, Kurai K, Okada S, Yamamoto K, Lizuka H, Tanaka T, et al.** Full-length sequence of a hepatitis C virus genome having poor homology to reported isolates: comparative study of four distinct genotypes. Virology. 1992; 188:331-41. [PMID: 1314459]

23. **Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ.** Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology. 1994;19:1513-20. [PMID: 8188183]

24. **Farmer DG, Rosove MH, Shaked A, Busuttil RW.** Current treatment modalities for hepatocellular carcinoma. Ann Surg. 1994;219:236-47. [PMID: 8147605]

25. **Shiina S, Imamura M, Omata M.** Percutaneous ethanol injection therapy (PEIT) for malignant liver neoplasms. Semi Intervent Radiol 1997; 14: 295-303.

26. **Livraghi T, Giorgio A, Marin G, Salmi A, de Sio I, Bolondi L, et al.** Hepatocellular carcinoma and cirrhosis in 746 patients: long-term results of percutaneous ethanol injection. Radiology. 1995;197:101-8. [PMID: 7568806]

27. **Livraghi T, Solbiati L, Meloni MF, Gazelle GS, Halpern EF, Goldberg SN.** Treatment of focal liver tumors with percutaneous radio-frequency ablation: complications encountered in a multicenter study. Radiology. 2003;226:441-51. [PMID: 12563138]

28. **Seki T, Wakabayashi M, Nakagawa T, Imamura M, Tamai T, Nishimura A, et al.** Percutaneous microwave coagulation therapy for patients with small hepatocellular carcinoma: comparison with percutaneous ethanol injection therapy. Cancer. 1999;85:1694-702. [PMID: 10223562]

29. **Takayasu K, Wakao F, Moriyama N, Muramatsu Y, Yamazaki S, Kosuge T, et al.** Postresection recurrence of hepatocellular carcinoma treated by arterial embolization: analysis of prognostic factors. Hepatology. 1992;16:906-11. [PMID: 1328010]

30. **Kleinbaum DG.** Survival Analysis: A Self-Learning Text. New York: Springer-Verlag; 1995.

31. **Yoshida H, Arakawa Y, Sata M, Nishiguchi S, Yano M, Fujiyama S, et al.** Interferon therapy prolonged life expectancy among chronic hepatitis C patients. Gastroenterology. 2002;123:483-91. [PMID: 12145802]

32. **Heathcote EJ, Shiffman ML, Cooksley WG, Dusheiko GM, Lee SS, Balart L, et al.** Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. N Engl J Med. 2000;343:1673-80. [PMID: 11106716]

33. **Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al.** Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet. 2001;358:958-65. [PMID: 11583749]

34. **Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL Jr, et al.** Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med. 2002;347:975-82. [PMID: 12324553]

35. **McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, et al.** Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology. 2002;123:1061-9. [PMID: 12360468]

Journal of Gastroenterology and Hepatology (2005) **20**, 850–856 DOI: 10.1111/j.1400-1746.2005.03823.x

HEPATOLOGY

Possible contribution of prior hepatitis B virus infection to the development of hepatocellular carcinoma

HIRONORI TANAKA,* YOSHIAKI IWASAKI,* KAZUHIRO NOUSO,† YOSHIYUKI KOBAYASHI,* SHIN-ICHIRO NAKAMURA,* EIJI MATSUMOTO,* NOBUYUKI TOSHIKUNI,* TOSHIHIKO KANEYOSHI,‡ TOSHIYA OHSAWA,§ KOUICHI TAKAGUCHI,¶ KOZO FUJIO,** TOMONORI SENOH,†† TOHRU OHNISHI,‡‡ KOHSAKU SAKAGUCHI* AND YASUSHI SHIRATORI*

**Department of Gastroenterology, Hepatology and Infectious Disease, Okayama University School of Medicine, ‡Department of Gastroenterology, Kurashiki Central Hospital, §Department of Medicine, Okayama Saiseikai General Hospital, Okayama, †Department of Medicine, Hiroshima City Hospital, **Department of Medicine, Fukuyama City Hospital, ††Fukuyama National Hospital, Hiroshima, ¶Department of Medicine, Kagawa Prefectural Central Hospital and ‡‡Department of Medicine, Mitoyo General Hospital, Kagawa, Japan*

Abstract

Background: The prevalence of prior hepatitis B virus (HBV) infection in hepatocellular carcinoma (HCC) patients and its role in hepatocarcinogenesis are not clear. The aim of the present study is to clarify the importance of prior HBV infection in development of HCC.

Methods: Of 1288 consecutive HCC patients between January 1999 and October 2002, 1008 patients were enrolled. To determine the influence of prior HBV infection in hepatitis B surface antigen (HBsAg)-negative HCC, the prevalence of antibody to hepatitis B core antigen (anti-HBc) was examined according to age, and the clinical features were compared between the anti-HBc positive and the negative groups.

Results: The proportion of HBsAg-negative HCC patients, HCC patients with antibody to hepatitis C virus (anti-HCV; C-HCC) and HCC patients negative for both HBsAg and anti-HCV (nBnC-HCC), increased with age. The anti-HBc-positive rates in C-HCC patients also increased with age. Those rates in nBnC-HCC patients were >50% in all age groups. Furthermore, it was found that the anti-HBc-positive rates of these patients were higher than those of corresponding control patients. Tumor size and a positive rate for vessel involvement both in C-HCC and nBnC-HCC patients were larger and higher, respectively, in anti-HBc-positive patients compared with anti-HBc-negative patients, although the difference in nBnC-HCC did not reach statistical significance because of the small numbers. These tumor characteristics were similar to those of B-HCC patients.

Conclusion: A possible contribution of prior HBV infection to the development of HCC is indicated.

Key words: antibody to hepatitis B core antigen, hepatitis B, hepatocellular carcinoma, occult HBV infection, prior HBV infection.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors, having a wide geographic distribution.[1,2] The incidence of HCC is increasing, not only in Asia, but also in Western countries.[3–6] Many environmental factors, including aflatoxins, alcohol, and genetic background have been implicated in the development of HCC.[7,8] However, hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are by

Correspondence: Dr Hironori Tanaka, Department of Gastroenterology, Hepatology and Infectious Disease, Okayama University School of Medicine, 2-5-1 Shikata-cho, Okayama 700-8558, Japan. Email: hironori@cc.okayama-u.ac.jp
Accepted for publication 28 July 2004.

far the best-documented and important factors associated with the progression from chronic hepatitis to cirrhosis and eventually to HCC.[9–11] In addition, HBV infection is particularly prevalent in the Asia–Pacific region including Japan, where patients usually acquire the infection at birth or in their early childhood.

Chronic HBV infection is characterized by persistence of hepatitis B surface antigen (HBsAg) and viremia. Early studies revealed that the clearance of HBsAg in patients with HBV infection is associated with the disappearance of viremia and remission of the disease. However, Brechot *et al.* demonstrated a high frequency of HBV-DNA-positive viral infection even in patients with HBsAg-negative liver disease,[12] and several investigations have also confirmed the persistence of HBV-DNA in HBsAg-negative patients,[13] and in particular, a high prevalence in patients with antibody to hepatitis B core antigen (anti-HBc).[14] In addition, HBV-DNA persisted even after seroconversion from HBsAg, to antibody to HBsAg.[15] Moreover, HBV was transmitted by liver transplantation from HBsAg-negative donors with anti-HBc to the recipient, and most of these donors were negative for HBV-DNA in the serum even by polymerase chain reaction (PCR).[16] Another report showed that hepatitis B developed in 18 of 23 recipients of livers from anti-HBc-positive donors (78%) compared with only three of 651 recipients of livers from anti-HBc-negative donors (0.5%) ($P < 0.0001$).[17] These data indicated that a low level of HBV-DNA persisted in the liver of some patients, especially in those with anti-HBc who cleared HBsAg from either acute self-limited or chronic HBV infection, in spite of the negativity for HBV-DNA in the serum. Demonstration of this clinical entity has resulted in introduction of the concept of 'occult HBV infection' which defines the presence of HBV infection with undetectable HBsAg.[18]

It has also been reported that HBV sequences, including the *X* gene of the HBV-DNA, are often found in HCC tissue in patients without HBsAg,[19–21] suggesting a role of these HBV genes in the development of HCC. An increased risk of developing HCC was also reported in individuals with HBV-related antibodies as the marker of past HBV infection.[22,23]

However, few reports have described the association of prior HBV infection with the clinicopathological findings for HCC patients without HBsAg in a large population. Thus, an investigation of prior HBV infection in a large number of HCC patients without HBsAg may be important to examine risk for the development of HCC.

Furthermore, the spontaneous HBsAg clearance rate has been reported to be approximately 0.5–1.3% annually in long-term follow-up studies,[24–26] but the prevalence of HBsAg and HBV-related antibodies by age in HCC patients has not been fully clarified. Thus, it may also be important to examine the anti-HBc-positive rate in HCC patients by age, because there is a difference in the prevalence of prior HBV infection among age groups.

In the present study we investigated the prevalence of anti-HBc according to age in HCC patients without HBsAg and difference in clinical characteristics between the anti-HBc-positive and -negative groups.

METHODS

Patients

Between January 1999 and October 2002, 1288 consecutive patients with HCC who were treated at Okayama University Hospital and affiliated hospitals (Okayama Hepatocellular Carcinoma Group) were enrolled and viral markers and various clinical characteristics were analyzed.

We collected the clinical parameters of patients such as sex, age, albumin, alanine aminotransferase (ALT), total bilirubin, platelet, prothrombin time, α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP) at the time of diagnosis.

A total of 342 patients with antihepatitis C antibody (anti-HCV)-positive chronic liver disease (C-CLD; 265 patients with chronic hepatitis and 77 with liver cirrhosis) who attended the hospital during this period were analyzed. They received abdominal ultrasonography (US) examination every 3–6 months for the early detection of HCC, but were found to be free of HCC.

For the control, a total of 212 subjects was randomly selected according to the following criteria: (i) a visit during the same period to the Okayama University Hospital; (ii) no liver disease confirmed by blood chemistry and US; and (iii) negativity for HBsAg and antihepatitis C antibody (anti-HCV) at entry.

Informed consent was obtained from all patients and the study was carried out in accordance with the Helsinki Declaration.

Viral markers

The HBsAg in sera was examined by enzyme immunoassay (EIA; International Reagents, Kobe, Japan or Dainabot, Tokyo, Japan), electrochemiluminescence immunoassay (ECLIA; Roche Diagnostics, Mannheim, Germany), chemiluminescent enzyme immunoassay (CLEIA; Fujirebio, Tokyo, Japan), or counting immunoassay (CIA; Sysmex, Kobe, Japan). Anti-HBc was examined by radioimmunoassay (Dainabot) or EIA (International Reagents), and the serum was considered anti-HBc positive when the percent inhibition in the assay was >50%. Hepatitis B e antigen (HBeAg), and antibody to hepatitis B e antigen (anti-HBe) were examined by radioimmunoassay, EIA, or CLEIA, using a commercially available kit.

Anti-HCV in sera was determined by a second- or third-generation enzyme-linked immunoassay (International Reagents or Dainabot, or Ortho Diagnostic Systems, Tokyo, Japan).

Diagnosis of hepatocellular carcinoma

Hepatocellular carcinoma was diagnosed by the typical features found on dynamic computed tomography scans (Asteion, Toshiba Medical System, Tokyo, Japan and HiSpeed, GE Yokogawa Medical Systems, Tokyo, Japan and Prosheed, Siemens-Asahi Medical Technol-

ogies, Tokyo, Japan). The typical features consist of the findings of a lesion that reveals both hyperattenuation in the arterial phase and hypoattenuation in the portal phase on a dynamic computed tomography scan. Ultrasonography, magnetic resonance imaging, and angiography were also performed to confirm the diagnosis of HCC. Because it was difficult to confirm the diagnosis of HCC in 392 patients, a US-guided tumor biopsy was performed. Vessel involvement was defined by several imaging techniques as an invasion of HCC tumor into the first and/or second branch or the main trunk of the portal vein.

Statistics

Statistical analysis was performed by the χ^2 test or the Fisher exact probability test in the case of categorical variables. The Mann–Whitney *U*-test was used for continuous variables. $P < 0.05$ was considered statistically significant. All calculations were performed using SAS software ver. 8.2 (SAS Institute, Cary, NC, USA).

RESULTS

Viral markers in patients with hepatocellular carcinoma

Of 1288 patients with HCC, 318 patients were negative for anti-HCV and 970 were positive. Of 318 patients without anti-HCV, 207 were positive for HBsAg (B-HCC), and 111 were negative (nBnC-HCC). Among the latter patients with anti-HCV, 44 were positive for HBsAg (BC-HCC), 926 were negative. Of the 926 patients, we enrolled 646 patients as C-HCC, because 280 patients were not tested for anti-HBc.

At the time of diagnosis, various clinical characteristics both of all patients ($n = 1288$) and enrolled patients ($n = 1008$), excluding the 280 anti-HBc undetermined HCC patients, were compared (Table 1), indicating that clinical characteristics were similar between the two groups.

Viral markers according to age

All HCC patients younger than 40 years were negative for anti-HCV, and most in the 40s were B-HCC. The incidence of B-HCC decreased with age. However, the number of B-HCC patients was comparatively higher in the 50s and 60s (<40 years old, $n = 12$; 40s, $n = 35$; 50s, $n = 80$; 60s, $n = 58$; 70s, $n = 22$; ≥80 years old, none; Fig. 1). Conversely, the proportion of patients with C-HCC increased with age and was >70% in the 60s and 70s. The proportion of patients with BC-HCC was around 3–6% in all age groups, and nBnC-HCC increased slightly with age, reaching a level of 38% in patients >80 (Fig. 1).

Of the 207 B-HCC patients, 60 patients were positive for HBeAg, 140 for anti-HBe, and seven were negative for both. The prevalence of HBeAg was constant among the patients younger than 70 (31–33%), but it was reduced to 9% in the patients in their 70s. Conversely, the positive rate of anti-HBe increased in the 70s, to 86% (Fig. 2).

Differences in the clinical features by viral markers

As indicated in Table 2, B-HCC patients were younger compared with C-HCC, BC-HCC, and nBnC-HCC patients. The levels of albumin and ALT and the platelet

Table 1 Baseline clinical characteristics (median [IQR])

	All patients	Entry patients	*P*
Patients (*n*)	1288	1008	NS
Age (years) (mean ± SD)	65 ± 9	64 ± 9	NS
Male/female	940/348	744/264	NS
Biochemical variables			
Albumin (g/dL)	3.6 (3.1–3.9)	3.6 (3.1–3.9)	NS
ALT level (IU/L)	52 (35–75)	52 (35–74)	NS
Total bilirubin level (mg/dL)	0.90 (0.64–1.40)	0.90 (0.66–1.40)	NS
Prothrombin time (%)	82 (69–93)	83 (69–94)	NS
Platelet count ($\times 10^3$/mL)	105 (70–147)	107 (71–151)	NS
Tumor markers			
AFP (ng/mL)	41 (12–272)	39 (11–290)	NS
DCP (mAU/L)	72 (22–941)	64 (21–859)	NS
Tumor-related factors			
Tumor size (mm)	28 (19–46)	29 (19–49)	NS
No. tumors, *n* 1/2/3/≥4	687/254/94/253	531/199/80/198	NS
Vessel involvement (*n*) (+/–)	247/1041	198/810	NS

AFP, α-fetoprotein; ALT, alanine aminotransferase; DCP, des-γ-carboxy prothrombin; IQR, interquartile range; NS, not significant.

Table 2 Differences in the clinical features of HCC patients according to viral markers (median [IQR])

	B-HCC	C-HCC	BC-HCC	nBnC-HCC
Patients (*n*)	207	646	44	111
Male/female	165/42**	466/180	29/15	84/27
Age (years) (mean ± SD)	54 ± 27***	65 ± 8	63 ± 9	68 ± 10
Biochemical variables				
Albumin (g/dL)	3.7 (3.2–4.0)*	3.5 (3.1–3.9)	3.5 (3.0–3.8)	3.8 (3.1–4.2)
ALT level (IU/L)	48 (33–69)*	58 (39–83)	49 (35–69)	33 (23–51)
Total bilirubin level (mg/dL)	0.9 (0.7–1.4)	0.9 (0.7–1.4)	1.0 (0.7–1.4)	0.9 (0.6–1.5)
Prothrombin time (%)	81 (68–93)	82 (69–93)	77 (69–95)	89 (73–102)
Platelet count ($\times 10^3$/mL)	117 (76–167)*	99 (65–138)	105 (70–170)	149 (107–194)
Tumor markers				
AFP (ng/mL)	120 (15–2665)*	35 (12–157)	43 (11–466)	13 (5–150)
DCP (mAU/L)	193 (28–5645)*	44 (19–265)	122 (22–1272)	270 (39–2802)
Tumor-related factors				
Tumor size (mm)	34 (20–60)*	25 (18–40)	29 (20–53)	43 (26–61)
Multiple tumor, *n* (%)†	109 (53)*	290 (45)	29 (66)	49 (44)
Vessel involvement (+), *n* (%)§	65 (31)*	94 (15)	11 (25)	28 (25)

AFP, α-fetoprotein; ALT, alanine aminotransferase; DCP, des-γ-carboxy prothrombin; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; IQR, interquartile range; NS, not significant.

B-HCC, HCC patients with positivity for HBsAg; C-HCC, HCC patients with positivity for anti-HCV; BC-HCC, HCC patients with positivity for both HBsAg and anti-HCV; nBnC-HCC, HCC patients with negativity for both HBsAg and anti-HCV.

†No. cases with more than two tumors.

*$P < 0.01$ vs C-HCC; **$P < 0.05$ vs C-HCC; ***$P < 0.01$ vs C-HCC, BC-HCC, and nBnC-HCC.

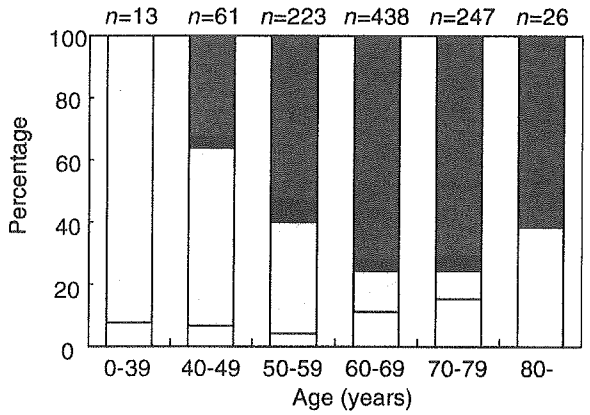

Figure 1 Distribution of viral markers by Age in hepatocellular carcinoma (HCC) patients.Although most HCC patients younger than 40 years were B-HCC, incidence of B-HCC decreased with age. Conversely, the proportion of C-HCC increased with age and was >70% in the 60s and 70s. nBnC-HCC increased in patients older than 70, and reaching a level of 38% in patients older than 80. B-HCC (■), positivity for hepatitis B surface antigen (HBsAg); C-HCC (□), positivity for anti-HCV; BC-HCC (■), positivity for both HBsAg and anti-HCV; nBnC-HCC (□), negativity for both HBsAg and anti-HCV.

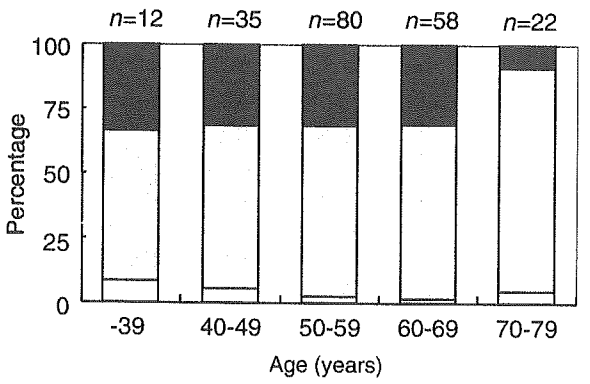

Figure 2 Status of HBeAg and anti-HBe by age in B-HCC. The prevalence of HBeAg was constant among the patients who were younger than the 60s (31–33%), but it was reduced to 9% in the 70s. Conversely, the positive rate of anti-HBe increased with age, and the rate was 86% in the 70s. B-HCC, hepatocellular carcinoma (HCC) patients with positivity for hepatitis B surface antigen (HBsAg); HBeAg (■), hepatitis B e antigen; anti-HBe (□), hepatitis B e antibody; both negative (□), negative for both HBeAg and anti-HBe.

count of B-HCC patients were significantly better than those of C-HCC patients. The B-HCC patients were characterized by a larger tumor (34 mm vs 25 mm), more abundant nodules (multiple, 53% vs 45%), and a higher frequency of vessel involvement (31% vs 15%) compared with C-HCC patients ($P < 0.01$ for each comparison).

The nBnC-HCC patients were characterized by the oldest age at detection and the best liver functions in all parameters. As for tumor characteristics, a solitary tumor was the most frequent, but tumor size was the largest, and vessel involvement was second in frequency to HCC patients.

Anti-HBc in C-HCC, nBnC-HCC, C-CLD, and control

To determine the influence of prior HBV infection, we analyzed the prevalence of anti-HBc in the groups of patients with C-HCC, nBnC-HCC, C-CLD and in control patients. Of the 646 C-HCC, 385 (60%) were positive for anti-HBc (Table 3). The prevalence of anti-HBc in C-HCC increased with age, and the rate was >50% for patients over the 50s. In contrast, a positive rate of anti-HBc in C-CLD was around 40%, even in patients in their 50s or 60s, although the positive rate was 50% among the patients older than the 70. The positive rate of anti-HBc in C-HCC was higher than that in C-CLD, and a significant difference was found in the 50s and 60s.

In all age groups of nBnC-HCC, the anti-HBc-positive rate was >50% and was higher than that of the control, although significant differences was observed only in those younger than 50 because of the small numbers of patients.

Anti-HBc and clinical features of HBsAg-negative HCC

We further classified C-HCC and nBnC-HCC into two groups by anti-HBc status and analyzed the differences in the clinical features (Table 4). There were no significant differences in the status of the liver function tests between those with and without anti-HBc. However, in C-HCC, tumor size was larger (27 mm vs 23 mm, $P = 0.014$) and the vessel involvement positive rate was higher (18% vs 10%, $P = 0.003$) in patients with anti-HBc. In addition, the tumor characteristics of anti-HBc positive nBnC-HCC were more advanced compared with those that were negative for anti-HBc (tumor size: 48 mm vs 37 mm; vessel involvement: 30% vs 19%), although the differences did not reach statistical significance because of the small number.

DISCUSSION

In the present study we demonstrated the prevalence of hepatitis viral markers in HCC patients by age. The proportion of HBsAg-negative HCC, C- and nBnC-HCC, increased with age, and the anti-HBc-positive rates in these patients demonstrated an increasing tendency with age (40% [10/25] < 50 years old; 57% [74/129] in the 50 s; 61% [222/364] in the 60s; 60% [143/239] ≥70 years old). Furthermore, we demonstrated that the anti-HBc-positive rates of these patients were higher than those of corresponding control patients. In particular, significant difference was found for the 50–69-year age group of C-HCC, and in patients <50 years old in the nBnC-HCC group. It also has been reported that the positive rate of anti-HBc in Japanese subjects without HCV infection was distributed around 5–15% in all age groups.[27] These results suggest that the positivity for anti-HBc may be associated with HCC development in HBsAg-negative HCC patients.

We also investigated the clinical features of HBsAg-negative HCC patients, C- and nBnC-HCC, in relation to presence or absence of anti-HBc, and demonstrated that the tumor characters of HCC, including tumor size and vessel involvement positive rate, were more advanced in HBsAg-negative HCC patients with anti-HBc than in those without it, although the difference in nBnC-HCC did not reach statistical significance because of the small numbers (Table 4). As indicated in Table 2, B-HCC was characterized with larger tumors in younger patients with less severe liver dysfunction compared with C-HCC,[28,29] although we should take into consideration the possibility that tumor size and vessel involvement depend on the rate of periodical check-up. The results may indicate that the growth is

Table 3 Anti-HBc positivity in patients with C-HCC, nBnC-HCC, C-CLD, and control patients matched by age

Age (years)	C-HCC % (*n*/*n*)	nBnC-HCC % (*n*/*n*)	C-CLD % (*n*/*n*)	Control % (*n*/*n*)	*P*1	*P*2
≤49	35 (7/20)	60 (3/5)	35 (36/122)	10 (7/68)	0.609	0.017
50–59	57 (68/120)	67 (6/9)	39 (43/111)	35 (13/37)	0.008	0.133
60–69	61 (193/315)	59 (29/49)	40 (35/87)	41 (24/58)	<0.001	0.082
≥70	61 (117/191)	54 (26/48)	50 (11/22)	49 (24/49)	0.360	0.686
Total	60 (385/646)	58 (64/111)	37 (125/342)	32 (68/212)	<0.001	<0.001

Anti-HBc, antibody to hepatitis B core antigen; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma.

C-HCC; HCC patients with positivity for anti-HCV; nBnC-HCC, HCC patients with negativity for both HBsAg and anti-HCV; C-CLD, antihepatitis C antibody positive chronic liver disease.

*P*1 value evaluated between C-HCC and C-CLD by Fisher's exact test.

*P*2 value evaluated between nBnC-HCC and control by Fisher's exact test.

Table 4 Clinical features of C-HCC and nBnC-HCC in relation to presence or absence of anti-HBc (median [IQR])

	C-HCC			nBnC-HCC		
	anti-HBc (+)	anti-HBc (−)	*P*	anti-HBc (+)	anti-HBc (−)	*P*
Patients (*n*)	385	261		64	47	
Male/female	289/96	177/84	0.049	52/12	21/15	NS
Age (years) (mean ± SD)	66 ± 7	65 ± 8	NS	68 ± 10	68 ± 10	NS
Biochemical variables						
Albumin (g/dL)	3.5 (3.1–3.9)	3.5 (3.1–3.9)	NS	3.8 (3.2–4.2)	3.7 (2.9–4.2)	NS
ALT level (IU/L)	59 (38–84)	55 (40–77)	NS	36 (25–57)	28 (20–44)	NS
Total bilirubin level (mg/dL)	0.9 (0.7–1.4)	0.9 (0.7–1.3)	NS	0.8 (0.6–1.4)	1.0 (0.6–1.5)	NS
Prothrombin time (%)	82 (69–93)	82 (70–94)	NS	89 (74–104)	86 (72–100)	NS
Platelet count ($\times 10^3$/mL)	97 (67–139)	102 (65–137)	NS	157 (120–197)	147 (80–190)	NS
Tumor markers						
AFP (ng/mL)	38 (13–169)	31 (11–149)	NS	14 (5–454)	13 (5–81)	NS
DCP (mAU/L)	45 (19–285)	43 (19–238)	NS	311(38–2835)	147 (30–3610)	NS
Tumor-related factors						
Tumor size (mm)	27 (18–44)	23 (18–35)	0.014	48 (30–66)	37 (23–60)	NS
Multiple tumors, *n* (%)†	178 (46)	112 (43)	NS	28 (44)	21 (45)	NS
Vessel involvement (+), *n* (%)†	69 (18)	25 (10)	0.031	19 (30)	9 (19)	NS

AFP, α-fetoprotein; ALT, alanine aminotransferase; DCP, des-γ-carboxy prothrombin; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; IQR, interquartile range; NS, not significant.

C-HCC, HCC patients with positivity for anti-HCV; nBnC-HCC, HCC patients with negativity for both HBsAg and anti-HCV.

†No. cases with more than two tumors.

P evaluated between absence and presence of anti-HBc.

faster from the beginning of its formation in the B-HCC group as compared with C-HCC. Thus, the tumor characters of HBsAg-negative HCC patients with anti-HBc were similar to those of B-HCC, suggesting an influence of prior HBV infection.

If we take into consideration that HBV is transmitted to recipients by liver grafts from almost all anti-HBc-positive donors, in spite of the negativity for HBV-DNA in the serum by PCR,[16] the prevalence of HBV persistence in the livers of anti-HBc-positive patients may be higher than that estimated from the positive result of the HBV-DNA by PCR in serum samples. Thus, the differences in tumor characteristics between patients with and without anti-HBc may reflect the persistence of HBV, the so-called occult HBV infection. Although occult HBV infection has been reported in several studies, the clinical relevance remains controversial.[13,14,18] In addition, there are no reports that showed the difference in tumor characteristics of HCC patients by the effect of occult HBV infection, although previous report indicated that prior HBV infection was a risk factor for poor prognosis after liver resection in patients infected with HCV.[30] Thus, the present data indicate an importance of considering the effect of occult HBV infection in HCC patients with HBsAg negativity.

In nBnC-HCC, tumor size was the largest in the four HCC groups. This may be explained by the fact that nBnC-HCC did not receive periodical medical check-ups of tumor markers and abdominal US to detect HCC at an early stage. Because the anti-HBc positive rate of nBnC-HCC patients younger than 50 was higher compared with that of control, the patients younger than 50 who have any liver problems should also receive periodic screening of HCC to detect HCC at an early stage, if they are positive for anti-HBc, even though they are negative for both HBsAg and anti-HCV.

Because Japan is an HBV endemic area, the incidence rate of HBV-related antibodies is higher than that in the USA or Europe. Thus, it is important to take geographic differences in the prevalence of HBV infection into consideration to interpret the present data. Further investigations are necessary to elucidate the influence of prior HBV infection on the development of HCC in other areas of the world.

In conclusion, the present study demonstrated that prior HBV infection may contribute to the development of HCC. Thus, testing for anti-HBc should be considered in order to establish the presence of prior HBV infection not only in patients positive for anti-HCV but also in patients who are negative for both HBsAg and anti-HCV who have any problem in liver function test.

ACKNOWLEDGMENTS

The authors thank Drs Yasuhiro Miyake, Hideaki Taniguchi, Ryo Terada, Akio Moriya, Tatsuya Fujikawa, and Mayumi Suzuki, Department of Medicine and Medical Science, Okayama University Graduate School of Medicine and Dentistry; Dr Yasuhiro Araki, Department of Medicine, Hiroshima City Hospital; Dr Hiroshi Ikeda, Department of Gastroenterology, Kurashiki Central Hospital; Drs Mitsuhiko Kawaguchi, and Kenji

Miyoshi, Department of Medicine, Okayama Saiseikai General Hospital; Dr Keiji Kita, Department of Medicine, Kagawa Prefectural Central Hospital, for their generous assistance.

REFERENCES

1 Parkin DM, Stjernsward J, Muir CS. Estimates of the worldwide frequency of twelve major cancers. *Bull. World Health Organ.* 1984; **62**: 163–82.
2 Befeler AS, Di Bisceglie AM. Hepatocellular carcinoma: diagnosis and treatment. *Gastroenterology* 2002; **122**: 1609–19.
3 Okuda K, Fujimoto I, Hanai A, Urano Y. Changing incidence of hepatocellular carcinoma in Japan. *Cancer Res.* 1987; **47**: 4967–72.
4 Deuffic S, Poynard T, Buffat L, Valleron AJ. Trends in primary liver cancer. *Lancet* 1998; **351**: 214–15.
5 Taylor-Robinson SD, Foster GR, Arora S, Hargreaves S, Thomas HC. Increase in primary liver cancer in the UK, 1979–94. *Lancet* 1997; **350**: 1142–3.
6 El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. *N. Engl. J. Med.* 1999; **340**: 745–50.
7 Chen CJ, Liang KY, Chang AS *et al.* Effects of hepatitis B virus, alcohol drinking, cigarette smoking and familial tendency on hepatocellular carcinoma. *Hepatology* 1991; **13**: 398–406.
8 Chen CJ, Zhang YJ, Lu SN, Santella RM. Aflatoxin B1 DNA adducts in smeared tumor tissue from patients with hepatocellular carcinoma. *Hepatology* 1992; **16**: 1150–5.
9 Chen PJ, Chen DS. Hepatitis B virus infection and hepatocellular carcinoma. molecular genetics and clinical perspectives. *Semin. Liver Dis.* 1999; **19**: 253–62.
10 Sherlock S. Viruses and hepatocellular carcinoma. *Gut* 1994; **35**: 828–32.
11 Okuda K. Hepatocellular carcinoma: recent progress. *Hepatology* 1992; **15**: 948–63.
12 Brechot C, Degos F, Lugassy C *et al.* Hepatitis B virus DNA in patients with chronic liver disease and negative tests for hepatitis B surface antigen. *N. Engl. J. Med.* 1985; **312**: 270–6.
13 Brechot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Brechot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely 'occult'? *Hepatology* 2001; **34**: 194–203.
14 Cacciola I, Pollicino T, Squadrito G, Cerenzia G, Orlando ME, Raimondo G. Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease. *N. Engl. J. Med.* 1999; **341**: 22–6.
15 Yotsuyanagi H, Yasuda K, Iino S *et al.* Persistent viremia after recovery from self-limited acute hepatitis B. *Hepatology* 1998; **27**: 1377–82.
16 Uemoto S, Sugiyama K, Marusawa H *et al.* Transmission of hepatitis B virus from hepatitis B core antibody-positive donors in living related liver transplants. *Transplantation* 1998; **65**: 494–9.
17 Dickson RC, Everhart JE, Lake JR *et al.* Transmission of hepatitis B by transplantation of livers from donors positive for antibody to hepatitis B core antigen. The National Institute of Diabetes and Digestive and Kidney Diseases Liver Transplantation Database. *Gastroenterology* 1997; **113**: 1668–74.
18 Hu KQ. Occult hepatitis B virus infection and its clinical implications. *J. Viral Hepat.* 2002; **9**: 243–57.
19 Paterlini P, Gerken G, Nakajima E *et al.* Polymerase chain reaction to detect hepatitis B virus DNA and RNA sequences in primary liver cancers from patients negative for hepatitis B surface antigen. *N. Engl. J. Med.* 1990; **323**: 80–5.
20 Nagaya T, Nakamura T, Tokino T *et al.* The mode of hepatitis B virus DNA integration in chromosomes of human hepatocellular carcinoma. *Genes Dev.* 1987; **1**: 773–82.
21 Paterlini P, Poussin K, Kew M, Franco D, Brechot C. Selective accumulation of the X transcript of hepatitis B virus in patients negative for hepatitis B surface antigen with hepatocellular carcinoma. *Hepatology* 1995; **21**: 313–21.
22 Chiba T, Matsuzaki Y, Abei M *et al.* The role of previous hepatitis B virus infection and heavy smoking in hepatitis C virus-related hepatocellular carcinoma. *Am. J. Gastroenterol.* 1996; **91**: 1195–203.
23 Yu MC, Yuan JM, Ross RK, Govindarajan S. Presence of antibodies to the hepatitis B surface antigen is associated with an excess risk for hepatocellular carcinoma among non-Asians in Los Angeles County, California. *Hepatology* 1997; **25**: 226–8.
24 Liaw YF, Sheen IS, Chen TJ, Chu CM, Pao CC. Incidence, determinants and significance of delayed clearance of serum HBsAg in chronic hepatitis B virus infection: a prospective study. *Hepatology* 1991; **13**: 627–31.
25 de Franchis R, Meucci G, Vecchi M *et al.* The natural history of asymptomatic hepatitis B surface antigen carriers. *Ann. Intern. Med.* 1993; **118**: 191–4.
26 Villeneuve JP, Desrochers M, Infante-Rivard C *et al.* A long-term follow-up study of asymptomatic hepatitis B surface antigen-positive carriers in Montreal. *Gastroenterology* 1994; **106**: 1000–5.
27 Marusawa H, Osaki Y, Kimura T *et al.* High prevalence of anti-hepatitis B virus serological markers in patients with hepatitis C virus related chronic liver disease in Japan. *Gut* 1999; **45**: 284–8.
28 Miyagawa S, Kawasaki S, Makuuchi M. Comparison of the characteristics of hepatocellular carcinoma between hepatitis B and C viral infection: tumor multicentricity in cirrhotic liver with hepatitis C. *Hepatology* 1996; **24**: 307–10.
29 Chen MF, Jeng LB, Lee WC, Chen TC. Surgical results in patients with dual hepatitis B- and C-related hepatocellular carcinoma compared with hepatitis B- or C-related hepatocellular carcinoma. *Surgery* 1998; **123**: 554–9.
30 Kubo S, Nishiguchi S, Hirohashi K *et al.* Clinical significance of prior hepatitis B virus infection in patients with hepatitis C virus-related hepatocellular carcinoma. *Cancer* 1999; **86**: 793–8.