90% of patients with AIDS have markers of past or ongoing HBV infection (18). Thus, HBV carriers are more frequent in the HIV-1-positive than in the HIV-1-negative population (4, 9). Among patients with HIV infection in Japan, 6.3% are HBsAg positive, in particular, 8.3% of HIV-infected MSM (16). In this study, coinfection with HIV was found in 6 of the 44 (13.6%) patients infected with HBV/A. All of them were men. Their median age was 27.7 ± 4.1 years, and five patients were positive for HBeAg. Thus, there is a possibility that HIV-1 and HBV/A coinfections are increasing among young people in Japan, and the high rate of HBeAg positivity may be influenced by immune suppression due to HIV infection.

In the phylogenetic analysis, the HBV/A2 isolates recovered in this study were homologous to those from Europe and the United States, and some of them clustered with the Japanese isolates. On the other hand, there were HBV/A1 isolates that formed a cluster with those from the Philippines and India. Furthermore, some isolates from patients with acute hepatitis who were infected with HBV/A in Japan were highly homologous to HBV/A isolates from patients with chronic hepatitis. This invites speculation that some HBV/A isolates were introduced into Japan from foreign countries, while others have already settled down there and spread from patients with chronic infection to their contacts. HBV/A would have been infiltrating throughout Japan by these two different routes.

Clinical differences among patients infected with HBV/A, -B, and -C were observed. The mean age was lower in the patients infected with HBV/A than in those infected with HBV/B or -C. As mentioned above, AHB patients infected with HBV/A have been increasing in the younger generation in Japan, and around 10% of them would have progressed to chronic infection. This is one of the reasons why the patients infected with HBV/A are younger than those infected with HBV/B or -C. Most patients infected with HBV/B were negative for HBeAg, while a high proportion of the patients infected with HBV/A and -C had it. In particular, this difference was remarkable in the patients who were older than 40 years of age. Thus, the seroconversion rate for the loss of HBeAg among younger people may be higher in infection with HBV/B than in that with HBV/A or -C. Inactive carriers were commoner in HBV/A than in HBV/C infection, as well.

These lines of evidence indicate that the activity of hepatitis is lower in HBV/B than HBV/C infection, and patients with HBV/B seroconvert from HBeAg to anti-HBe at young ages. In addition, cirrhosis and HCC were less frequent in the patients infected with HBV/B than in those infected with HBV/C. Therefore, the prognosis would be better in the patients infected with HBV/B than in those infected with HBV/C. These results are in accord with previous reports (5, 13, 28, 42). There have been few reports on the clinical features of patients with chronic hepatitis infected with HBV/A in Japan. Chu et al. have reported the distribution of HBV genotypes with reference to clinical characteristics in the United States (6). They have shown that HBV/A and HBV/C infections are accompanied by a higher frequency of HBeAg than HBV/B infection, while HBV/B is associated with a lower rate of hepatic decompensation than HBV/A and -C. In our study, inactive carriers were commoner, while cirrhosis and HCC were found less often in HBV/A than in HBV/C infection. HBeAg was more prevalent in the patients infected with HBV/A than in those infected with HBV/B who were older than 40 years of age. Therefore, it can be said that the prognosis is better for patients infected with HBV/A than for those infected with HBV/C; it may be poorer than for those infected with HBV/B.

In conclusion, HBV/A has been increasing among CHB patients in Japan. On the basis of phylogenetic analyses, some HBV/A isolates appear to have been imported from foreign countries. They clustered with HBV/A from AHB patients and have infiltrated throughout Japan. It is very likely that acute and chronic infections with HBV/A have been increasing in Japan. Obviously, immunoprophylaxis of perinatal HBV infection, implemented since 1986 on a national basis, has been insufficient to prevent horizontal HBV/A infection diffusing among high-risk groups by transmission routes shared by HIV infection. The foreseeable spread of HBV/A infection in Japan should be prevented by universal vaccination programs extended to high-risk groups or the general population.

ACKNOWLEDGMENTS

The study was supported in part by a grant-in-aid from the Ministry of Health, Labor and Welfare of Japan and a grant-in-aid from the Ministry of Education, Culture, Sports, Science and Technology.

We thank T. Kimura and K. Sato, Institutes of Immunology Co., Ltd. (Tokyo, Japan), for determining HBV genotypes in this study and Takashi Saito, Yamagata University Hospital; Akihiro Matsumoto, Shinshu University Hospital; Yasuhiro Asahina, Musashino Red Cross Hospital; Yoshito Ito, University Hospital, Kyoto Prefectural University of Medicine; Keiko Hosho, Tottori University Hospital; Morikazu Onji, Ehime University Hospital; Tatsuya Ide, Kurume University Hospital; and Hiroshi Sakugawa, Hospital, University of the Ryukyus, for their help throughout this work.

Kentaro Matsuura wrote the study protocol and the first draft of the manuscript and performed the experiments and statistical analysis. Yasuhito Tanaka contributed to the experimental work and the final version of the manuscript. Shuhei Hige, Gotaro Yamada, Yoshikazu Murawaki, Masafumi Komatsu, Tomoyuki Kuramitsu, Sumio Kawata, Eiji Tanaka, Namiki Izumi, Chiaki Okuse, Shinichi Kakumu, Takeshi Okanoue, Keisuke Hino, Yoichi Hiasa, Michio Sata, and Tatsuji Maeshiro contributed to the collection of the samples and clinical data from patients and to the final version of the manuscript. Fuminaka Sugauchi, Shunsuke Nojiri, Takashi Joh, and Yuzo Miyakawa contributed to the final version of the manuscript. Masashi Mizokami had the original idea and did the planning of the study and contributed to the final version of the manuscript. All of the authors have seen and approved the final draft of the manuscript.

REFERENCES

1. **Akuta, N., F. Suzuki, M. Kobayashi, A. Tsubota, Y. Suzuki, T. Hosaka, T. Someya, S. Saitoh, Y. Arase, K. Ikeda, and H. Kumada.** 2003. The influence of hepatitis B virus genotype on the development of lamivudine resistance during long-term treatment. J. Hepatol. **38**:315–321.
2. **Alter, M. J.** 2006. Epidemiology of viral hepatitis and HIV co-infection. J. Hepatol. **44**:S6–S9.
3. **Arauz-Ruiz, P., H. Norder, B. H. Robertson, and L. O. Magnius.** 2002. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. J. Gen. Virol. **83**:2059–2073.
4. **Bodsworth, N. J., D. A. Cooper, and B. Donovan.** 1991. The influence of human immunodeficiency virus type 1 infection on the development of the hepatitis B virus carrier state. J. Infect. Dis. **163**:1138–1140.
5. **Chu, C. J., M. Hussain, and A. S. Lok.** 2002. Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. Gastroenterology **122**:1756–1762.
6. **Chu, C. J., E. B. Keeffe, S. H. Han, R. P. Perrillo, A. D. Min, C. Soldevila-Pico, W. Carey, R. S. Brown, Jr., V. A. Luketic, N. Terrault, and A. S. Lok.** 2003. Hepatitis B virus genotypes in the United States: results of a nationwide study. Gastroenterology **125**:444–451.
7. **Chu, C. J., and A. S. Lok.** 2002. Clinical significance of hepatitis B virus genotypes. Hepatology **35**:1274–1276.
8. **Ding, X., M. Mizokami, G. Yao, B. Xu, E. Orito, R. Ueda, and M. Nakanishi.** 2001. Hepatitis B virus genotype distribution among chronic hepatitis B virus carriers in Shanghai, China. Intervirology **44**:43–47.

9. **Fujii, T., H. Taguchi, H. Katano, S. Mori, T. Nakamura, N. Nojiri, K. Nakajima, K. Tadokoro, T. Juji, and A. Iwamoto.** 1999. Seroprevalence of human herpesvirus 8 in human immunodeficiency virus 1-positive and human immunodeficiency virus 1-negative populations in Japan. J. Med. Virol. **57:**159–162.
10. **Gojobori, T., K. Ishii, and M. Nei.** 1982. Estimation of average number of nucleotide substitutions when the rate of substitution varies with nucleotide. J. Mol. Evol. **18:**414–423.
11. **Hadler, S. C., F. N. Judson, P. M. O'Malley, N. L. Altman, K. Penley, S. Buchbinder, C. A. Schable, P. J. Coleman, D. N. Ostrow, and D. P. Francis.** 1991. Outcome of hepatitis B virus infection in homosexual men and its relation to prior human immunodeficiency virus infection. J. Infect. Dis. **163:**454–459.
12. **Kao, J. H.** 2002. Clinical relevance of hepatitis B viral genotypes: a case of deja vu? J. Gastroenterol. Hepatol. **17:**113–115.
13. **Kao, J. H., P. J. Chen, M. Y. Lai, and D. S. Chen.** 2000. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology **118:**554–559.
14. **Kobayashi, M., Y. Arase, K. Ikeda, A. Tsubota, Y. Suzuki, S. Saitoh, F. Suzuki, N. Akuta, T. Someya, M. Matsuda, J. Sato, K. Takagi, Y. Miyakawa, and H. Kumada.** 2002. Viral genotypes and response to interferon in patients with acute prolonged hepatitis B virus infection of adulthood in Japan. J. Med. Virol. **68:**522–528.
15. **Koibuchi, T., A. Hitani, T. Nakamura, N. Nojiri, K. Nakajima, T. Jyuji, and A. Iwamoto.** 2001. Predominance of genotype A HBV in an HBV-HIV-1 dually positive population compared with an HIV-1-negative counterpart in Japan. J. Med. Virol. **64:**435–440.
16. **Koike, K., Y. Kikuchi, M. Kato, J. Takamatsu, Y. Shintani, T. Tsutsumi, H. Fujie, H. Miyoshi, K. Moriya, and H. Yotsuyanagi.** 2008. Prevalence of hepatitis B virus infection in Japanese patients with HIV. Hepatol. Res. **38:**310–314.
17. **Kurbanov, F., Y. Tanaka, K. Fujiwara, F. Sugauchi, D. Mbanya, L. Zekeng, N. Ndembi, C. Ngansop, L. Kaptue, T. Miura, E. Ido, M. Hayami, H. Ichimura, and M. Mizokami.** 2005. A new subtype (subgenotype) Ac (A3) of hepatitis B virus and recombination between genotypes A and E in Cameroon. J. Gen. Virol. **86:**2047–2056.
18. **Lebovics, E., B. M. Dworkin, S. K. Heier, and W. S. Rosenthal.** 1988. The hepatobiliary manifestations of human immunodeficiency virus infection. Am. J. Gastroenterol. **83:**1–7.
19. **Lok, A. S.** 1992. Natural history and control of perinatally acquired hepatitis B virus infection. Dig. Dis. **10:**46–52.
20. **Lusida, M. I., V. E. Nugrahaputra, Soetjipto, R. Handajani, M. Nagano-Fujii, M. Sasayama, T. Utsumi, and H. Hotta.** 2008. Novel subgenotypes of hepatitis B virus genotypes C and D in Papua, Indonesia. J. Clin. Microbiol. **46:**2160–2166.
21. **Mayerat, C., A. Mantegani, and P. C. Frei.** 1999. Does hepatitis B virus (HBV) genotype influence the clinical outcome of HBV infection? J. Viral Hepat. **6:**299–304.
22. **Miyakawa, Y., and M. Mizokami.** 2003. Classifying hepatitis B virus genotypes. Intervirology **46:**329–338.
23. **Norder, H., B. Hammas, S. Lofdahl, A. M. Courouce, and L. O. Magnius.** 1992. Comparison of the amino acid sequences of nine different serotypes of hepatitis B surface antigen and genomic classification of the corresponding hepatitis B virus strains. J. Gen. Virol. **73:**1201–1208.
24. **Noto, H., T. Terao, S. Ryou, Y. Hirose, T. Yoshida, H. Ookubo, H. Mito, and H. Yoshizawa.** 2003. Combined passive and active immunoprophylaxis for preventing perinatal transmission of the hepatitis B virus carrier state in Shizuoka, Japan during 1980–1994. J. Gastroenterol. Hepatol. **18:**943–949.
25. **Ogawa, M., K. Hasegawa, T. Naritomi, N. Torii, and N. Hayashi.** 2002. Clinical features and viral sequences of various genotypes of hepatitis B virus compared among patients with acute hepatitis B. Hepatol. Res. **23:**167–177.
26. **Okamoto, H., F. Tsuda, H. Sakugawa, R. I. Sastrosoewignjo, M. Imai, Y. Miyakawa, and M. Mayumi.** 1988. Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. J. Gen. Virol. **69:**2575–2583.
27. **Orito, E., T. Ichida, H. Sakugawa, M. Sata, N. Horiike, K. Hino, K. Okita, T. Okanoue, S. Iino, E. Tanaka, K. Suzuki, H. Watanabe, S. Hige, and M. Mizokami.** 2001. Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. Hepatology **34:**590–594.
28. **Orito, E., M. Mizokami, H. Sakugawa, K. Michitaka, K. Ishikawa, T. Ichida, T. Okanoue, H. Yotsuyanagi, S. Iino, et al.** 2001. A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotypes B and C. Hepatology **33:**218–223.
29. **Ozasa, A., Y. Tanaka, E. Orito, M. Sugiyama, J. H. Kang, S. Hige, T. Kuramitsu, K. Suzuki, E. Tanaka, S. Okada, H. Tokita, Y. Asahina, K. Inoue, S. Kakumu, T. Okanoue, Y. Murawaki, K. Hino, M. Onji, H. Yatsuhashi, H. Sakugawa, Y. Miyakawa, R. Ueda, and M. Mizokami.** 2006. Influence of genotypes and precore mutations on fulminant or chronic outcome of acute hepatitis B virus infection. Hepatology **44:**326–334.
30. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.
31. **Sakamoto, T., Y. Tanaka, E. Orito, J. Co, J. Clavio, F. Sugauchi, K. Ito, A. Ozasa, A. Quino, R. Ueda, J. Sollano, and M. Mizokami.** 2006. Novel subtypes (subgenotypes) of hepatitis B virus genotypes B and C among chronic liver disease patients in the Philippines. J. Gen. Virol. **87:**1873–1882.
32. **Sakamoto, T., Y. Tanaka, J. Simonetti, C. Osiowy, M. L. Borresen, A. Koch, F. Kurbanov, M. Sugiyama, G. Y. Minuk, B. J. McMahon, T. Joh, and M. Mizokami.** 2007. Classification of hepatitis B virus genotype B into 2 major types based on characterization of a novel subgenotype in Arctic indigenous populations. J. Infect. Dis. **196:**1487–1492.
33. **Salmon-Ceron, D., C. Lewden, P. Morlat, S. Bevilacqua, E. Jougla, F. Bonnet, L. Heripret, D. Costagliola, T. May, and G. Chene.** 2005. Liver disease as a major cause of death among HIV infected patients: role of hepatitis C and B viruses and alcohol. J. Hepatol. **42:**799–805.
34. **Sherlock, S. D. J.** 1997. Virus hepatitis, p. 265–392. *In* S. D. J. Sherlock (ed.), Diseases of the liver and biliary system, 10th ed. Blackwell Scientific Publications, London, United Kingdom.
35. **Shibayama, T., G. Masuda, A. Ajisawa, K. Hiruma, F. Tsuda, T. Nishizawa, M. Takahashi, and H. Okamoto.** 2005. Characterization of seven genotypes (A to E, G and H) of hepatitis B virus recovered from Japanese patients infected with human immunodeficiency virus type 1. J. Med. Virol. **76:**24–32.
36. **Shin, I. T., Y. Tanaka, Y. Tateno, and M. Mizokami.** 2008. Development and public release of a comprehensive hepatitis virus database. Hepatol Res. **38:**234–243.
37. **Stuyver, L., S. De Gendt, C. Van Geyt, F. Zoulim, M. Fried, R. F. Schinazi, and R. Rossau.** 2000. A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. J. Gen. Virol. **81:**67–74.
38. **Sugauchi, F., H. Kumada, S. A. Acharya, S. M. Shrestha, M. T. Gamutan, M. Khan, R. G. Gish, Y. Tanaka, T. Kato, E. Orito, R. Ueda, Y. Miyakawa, and M. Mizokami.** 2004. Epidemiological and sequence differences between two subtypes (Ae and Aa) of hepatitis B virus genotype A. J. Gen. Virol. **85:**811–820.
39. **Sugauchi, F., E. Orito, T. Ichida, H. Kato, H. Sakugawa, S. Kakumu, T. Ishida, A. Chutaputti, C. L. Lai, R. G. Gish, R. Ueda, Y. Miyakawa, and M. Mizokami.** 2003. Epidemiologic and virologic characteristics of hepatitis B virus genotype B having the recombination with genotype C. Gastroenterology **124:**925–932.
40. **Sugauchi, F., E. Orito, T. Ichida, H. Kato, H. Sakugawa, S. Kakumu, T. Ishida, A. Chutaputti, C. L. Lai, R. Ueda, Y. Miyakawa, and M. Mizokami.** 2002. Hepatitis B virus of genotype B with or without recombination with genotype C over the precore region plus the core gene. J. Virol. **76:**5985–5992.
41. **Sugauchi, F., E. Orito, T. Ohno, Y. Tanaka, A. Ozasa, J. H. Kang, J. Toyoda, T. Kuramitsu, K. Suzuki, E. Tanaka, Y. Akahane, T. Ichida, N. Izumi, K. Inoue, H. Hoshino, S. Iino, H. Yotsuyanagi, S. Kakumu, E. Tomita, T. Okanoue, S. Nishiguchi, Y. Murawaki, K. Hino, M. Onji, H. Yatsuhashi, M. Sata, Y. Miyakawa, R. Ueda, and M. Mizokami.** 2006. Spatial and chronological differences in hepatitis B virus genotypes from patients with acute hepatitis B in Japan. Hepatol. Res. **36:**107–114.
42. **Sumi, H., O. Yokosuka, N. Seki, M. Arai, F. Imazeki, T. Kurihara, T. Kanda, K. Fukai, M. Kato, and H. Saisho.** 2003. Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. Hepatology **37:**19–26.
43. **Suzuki, Y., M. Kobayashi, K. Ikeda, F. Suzuki, Y. Arfase, N. Akuta, T. Hosaka, S. Saitoh, T. Someya, M. Matsuda, J. Sato, S. Watabiki, Y. Miyakawa, and H. Kumada.** 2005. Persistence of acute infection with hepatitis B virus genotype A and treatment in Japan. J. Med. Virol. **76:**33–39.
44. **Tanaka, Y., I. Hasegawa, T. Kato, E. Orito, N. Hirashima, S. K. Acharya, R. G. Gish, A. Kramvis, M. C. Kew, N. Yoshihara, S. M. Shrestha, M. Khan, Y. Miyakawa, and M. Mizokami.** 2004. A case-control study for differences among hepatitis B virus infections of genotypes A (subtypes Aa and Ae) and D. Hepatology **40:**747–755.
45. **Tanaka, Y., E. Orito, M. F. Yuen, M. Mukaide, F. Sugauchi, K. Ito, A. Ozasa, T. Sakamoto, F. Kurbanov, C. L. Lai, and M. Mizokami.** 2005. Two subtypes (subgenotypes) of hepatitis B virus genotype C: a novel subtyping assay based on restriction fragment length polymorphism. Hepatol. Res. **33:**216–224.
46. **Usuda, S., H. Okamoto, H. Iwanari, K. Baba, F. Tsuda, Y. Miyakawa, and M. Mayumi.** 1999. Serological detection of hepatitis B virus genotypes by ELISA with monoclonal antibodies to type-specific epitopes in the preS2-region product. J. Virol. Methods **80:**97–112.
47. **Usuda, S., H. Okamoto, T. Tanaka, K. Kidd-Ljunggren, P. V. Holland, Y. Miyakawa, and M. Mayumi.** 2000. Differentiation of hepatitis B virus genotypes D and E by ELISA using monoclonal antibodies to epitopes on the preS2-region product. J. Virol. Methods **87:**81–89.
48. **Weinbaum, C. M., K. M. Sabin, and S. S. Santibanez.** 2005. Hepatitis B, hepatitis C, and HIV in correctional populations: a review of epidemiology and prevention. AIDS **19**(Suppl. 3):S41–S46.
49. **Yotsuyanagi, H., C. Okuse, K. Yasuda, E. Orito, S. Nishiguchi, J. Toyoda, E. Tomita, K. Hino, K. Okita, S. Murashima, M. Sata, H. Hoshino, Y. Miyakawa, and S. Iino.** 2005. Distinct geographic distributions of hepatitis B virus genotypes in patients with acute infection in Japan. J. Med. Virol. **77:**39–46.

J Gastroenterol
DOI 10.1007/s00535-009-0093-z

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

The influence of hepatitis B DNA level and antiviral therapy on recurrence after initial curative treatment in patients with hepatocellular carcinoma

Makoto Chuma · Shuhei Hige · Toshiya Kamiyama · Takashi Meguro · Atsushi Nagasaka · Kazuaki Nakanishi · Yoshiya Yamamoto · Mitsuru Nakanishi · Toshihisa Kohara · Takuya Sho · Keiko Yamamoto · Hiromasa Horimoto · Tomoe Kobayashi · Hideki Yokoo · Michiaki Matsushita · Satoru Todo · Masahiro Asaka

Received: 10 January 2009 / Accepted: 23 May 2009

Abstract
Background Prediction and prevention of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) recurrence is an important clinical issue. We investigated whether HBV DNA level and antiviral therapy are associated with HCC recurrence.
Methods This retrospective study involved 103 patients who underwent hepatic resection or radiofrequency ablation for initial HCC. Patients were divided into four groups. Thirty had high serum HBV DNA levels (>4 $\log_{10}$ copies/mL) and had not received antiviral therapy (high virus group; HVG). Thirty-four had low HBV DNA levels ($\leq$4 $\log_{10}$ copies/mL) and had not received antiviral therapy (low virus group; LVG). Twenty received antiviral therapy after HCC developed (therapeutic group A, TG-A). Nineteen received antiviral therapy before HCC developed (therapeutic group B, TG-B).
Results Cumulative HCC recurrence rates at 3 years in the HVG, LVG, TG-B, and TG-A were 71.1%, 42.2%, 42.3%, and 52.0%, respectively. Recurrence rates differed significantly between the HVG and LVG ($P = 0.016$) and between the HVG and TG-B ($P = 0.008$). Recurrence rate in the TG-A was marginally lower than in the HVG ($P = 0.10$). On multivariate analysis, high serum hepatitis B virus DNA levels (hazard ratio: HR 2.67; 95% CI 1.31–5.47; $P = 0.007$) and absence of antiviral therapy (HR 2.57; 95% CI 1.34–4.94; $P = 0.005$) were independent risk factors for hepatocellular carcinoma recurrence.
Conclusion HBV DNA level and antiviral therapy are associated with HCC recurrence. For patients with high HBV DNA levels, antiviral therapy before the development of HCC is important for prevention of recurrence.

Keywords Hepatocellular carcinoma · Hepatitis B virus · Recurrence · Antiviral therapy

M. Chuma (✉) · S. Hige · M. Nakanishi · T. Kohara · T. Sho · K. Yamamoto · H. Horimoto · T. Kobayashi · M. Asaka
Department of Gastroenterology and Hematology,
Hokkaido University, Kita 15, Nishi 7, Kita-ku,
Sapporo 060-8638, Japan
e-mail: mchuuma@med.hokudai.ac.jp

T. Kamiyama · K. Nakanishi · H. Yokoo · M. Matsushita · S. Todo
Department of General Surgery,
Hokkaido University, Sapporo, Japan

T. Meguro
Department of Internal Medicine,
Hokkaido Gastroenterology Hospital, Sapporo, Japan

A. Nagasaka
Department of Gastroenterology,
Sapporo Municipal Hospital, Sapporo, Japan

Y. Yamamoto
Department of Gastroenterology,
Hakodate Municipal Hospital, Hakodate, Japan

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignancy and the third leading cause of death from cancer worldwide [1, 2]. Hepatic resection or transplantation can provide a complete cure [3, 4], and radiofrequency ablation (RFA) is also recognized as a curative treatment option [5, 6]. Recent remarkable advances in curative treatment have improved the prognosis of patients with HCC, but these techniques remain unsatisfactory because of a high posttreatment recurrence rate [7, 8]. Previous studies have noted that factors contributing to recurrence include: tumor size, number, and differentiation; vascular

Published online: 25 June 2009

invasion; levels of alpha-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist II (PIVKA-II); gender; alcohol consumption; hepatitis C virus (HCV) infection; hepatic reserve; and degree of liver fibrosis [8–12].

Only a few recent studies have evaluated hepatitis B virus (HBV) replication status as a predictor of HCC recurrence [13–15], and interpretation of their results was complicated by use of antiviral therapy. Since HBV DNA level is reduced by antiviral agents, HBV DNA level at the time of HCC treatment differs significantly between patients who have received antiviral therapy and those who have not. To determine whether HBV DNA level at the time of HCC treatment is associated with HCC recurrence, it is therefore necessary to exclude patients who have received antiviral therapy after the development of HCC.

Furthermore, the efficacy of antiviral therapy in reducing the risk of HBV-related HCC recurrence is far from clear. Three anti-HBV agents are predominantly used in Japan. Lamivudine is a nucleotide analog that inhibits reverse transcriptase, ameliorates hepatitis, and improves histologic findings in the liver during long-term treatment by inhibiting the replication of HBV [16, 17]. Furthermore, lamivudine is considered to slow the progression of severe liver disease to cirrhosis as well as to HCC [17–19]. Adefovir dipivoxil and entecavir are potent inhibitors of HBV DNA polymerase which have been shown to be safe and effective for the treatment of patients with chronic hepatitis B infection (CHB) that does not respond to lamivudine [20, 21]. With regard to lowering the risk of HCC recurrence, the literature contains only one report for lamivudine [22] and none for adefovir dipivoxil or entecavir.

In this study, by strict classification of patients into groups, comparison of cumulative HCC recurrence rates between the groups, and multivariate analysis, we aimed (1) to clarify the influence of HBV DNA level in the absence of antiviral therapy on recurrence of HCC, and (2) to clarify the influence of antiviral therapy on the risk of HCC recurrence.

Patients and methods

Patients

Between January 2001 and December 2007, a total of 196 patients who were diagnosed with HBV-related HCC at our liver unit underwent hepatic resection or RFA as a primary treatment. HCC was diagnosed based on the American Association for the Study of Liver Disease (AASLD) guidelines [23]. All patients were positive for serum hepatitis B surface antigen (HBsAg) for at least 6 months before their diagnosis of HCC and were negative for antibodies to hepatitis C and human immunodeficiency virus. In this retrospective study, of the 196 patients who were assessed initially, 103 fulfilled the following criteria and were enrolled (hepatic resection, 52 patients; RFA, 51 patients). Inclusion criteria were: (1) hepatic resection or RFA for initial HCC treatment; (2) three or fewer lesions, each 3 cm or less in diameter; (3) no extrahepatic metastasis or vascular invasion; (4) curative treatment and no local recurrence after treatment; (5) no recurrence 3 months after treatment; (6) liver function of Child-Pugh class A or B; (7) no excessive alcohol intake (>65 g/day); and (8) no evidence of any other active neoplastic site.

Since HBV DNA level reduced to ≤ 4 $\log_{10}$ in all cases after administration of antiviral agents (lamivudine, adefovir dipivoxil or entecavir), the patients were divided into four groups (Fig. 1).

1. Thirty patients had consistently high serum HBV DNA levels (>4 $\log_{10}$ copies/mL) during serial examinations from the time of HCC diagnosis to recurrence (high virus group; HVG). These patients did not receive antivirals.
2. Thirty-four patients had consistently low serum HBV DNA levels (≤ 4 $\log_{10}$ copies/mL) during the serial follow-up (low virus group; LVG). These patients did not receive antivirals.
3. Twenty patients had high serum HBV DNA levels (>4 $\log_{10}$ copies/mL) when HCC was diagnosed and received antiviral therapy after the development of HCC (therapeutic group A, TG-A). Seventeen patients received antiviral therapy within 1 month after HCC treatment. The remaining three patients received antiviral therapy from diagnosis of active viral hepatitis B; the intervals between HCC treatment and the commencement of nucleotide analogue in these three patients were 12, 15, and 22 months.
4. Nineteen patients received antiviral therapy before the development of HCC (therapeutic group B, TG-B). In these patients, HBV DNA level was high (>4 $\log_{10}$ copies/mL) at commencement of the antiviral agents but low (≤ 4 $\log_{10}$ copies/mL) at HCC diagnosis.

As shown in Fig. 1, first, to determine whether HBV DNA level was associated with HCC recurrence, we selected the patients who had not received antiviral therapy (HVG plus LVG) and then compared cumulative HCC recurrence rates between the HVG and LVG. We then performed univariate and multivariate analysis of the hazard ratios for the recurrence of HCC in these patients. Second, to determine whether antiviral therapy was associated with lower risk of HCC recurrence, we selected the patients who had high serum HBV DNA levels when they had not received antiviral therapy (HVG plus TG-A plus

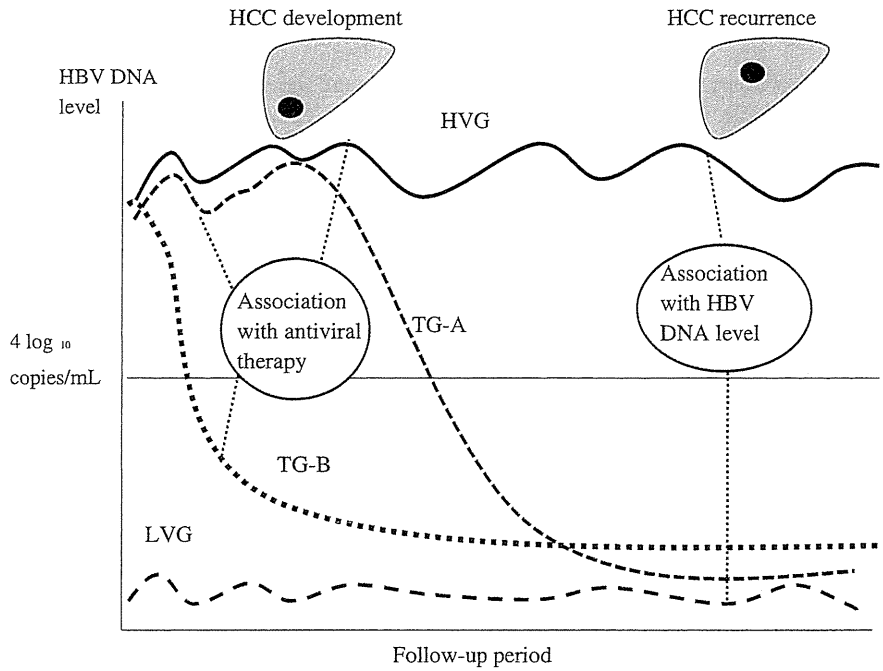

Fig. 1 Classification of patients according to HBV DNA level and use of antiviral therapy. Enrolled patients were divided into four groups according to HBV DNA level and use of antiviral therapy: (1) patients who did not have antiviral agents and who had consistently high serum HBV DNA levels during the time of HCC development to recurrence (high virus group; HVG), (2) patients who did not have antiviral agents and had consistently low serum HBV DNA levels (low virus group; LVG), (3) patients who had high serum HBV DNA levels at development of HCC and who received antiviral therapy after this (therapeutic group A; TG-A), and (4) patients who received antiviral therapy before HCC developed (therapeutic group B; TG-B)

TG-B) and then compared cumulative HCC recurrence rates between the HVG and TG-A and between the HVG and TG-B. We then performed univariate and multivariate analysis of the risk factors for recurrence of HCC in these patients.

Initial work-up and follow-up

The initial evaluation included a complete medical history and physical examination, focusing on the symptoms and signs often associated with HCC or chronic liver disease. All patients were tested at baseline for HBsAg, antibody to HBsAg, hepatitis B e antigen (HBeAg), antibody to HBeAg (anti-HBe), serum levels of alanine aminotransferase (ALT), albumin, bilirubin, AFP, and PIVKA-II, prothrombin time (PT), and complete blood cell counts. HBV DNA was quantified by polymerase chain reaction (PCR) assay (Amplicor HBV monitor assay, Roche Diagnostics, Manheim, Germany). The lower limit of detection of the assay was 2.6 log copies/mL.

During follow-up, clinical evaluations and biochemical tests were performed every 1–3 months. Patients underwent triphasic computed tomography of the liver every 3 months. The endpoint used in this study was the recurrence of HCC.

Antiviral therapy

In the TG-A, seven patients received lamivudine only (100 mg/day). Entecavir alone (0.5 mg/day) was used in five patients. Adefovir dipivoxil (10 mg/day) was used together with lamivudine to suppress lamivudine-resistant hepatitis B virus (HBV) in eight patients. In the TG-B, five patients received lamivudine only (100 mg/day). Entecavir (0.5 mg/day) was used in six patients; four of these patients were switched from lamivudine to entecavir. Adefovir dipivoxil (10 mg/day) was used together with lamivudine in eight patients.

Statistical analysis

Cumulative HCC recurrence rate was calculated by the Kaplan-Meier method and differences were compared by the log-rank test.

Univariate and multivariate analysis of the risk ratios for the recurrence of HCC were performed using Cox's proportional hazards regression analysis. The risk factors examined included gender, age, HBeAg status, ALT (>35 IU/L versus ≤35 IU/L), platelet count (>120 × 10^3/μL versus ≤120 × 10^3/μL), PT (>70 versus ≤70%), albumin (>3.5 mg/dL versus ≤3.5 mg/dL), bilirubin (>1.2 mg/dL versus ≤1.2 mg/dL), liver fibrosis (cirrhosis versus no cirrhosis), tumor differentiation (well and moderately differentiated versus poorly differentiated), AFP (>20 ng/mL versus ≤20 ng/mL), PIVKA-II (>40 mAU/mL versus ≤40 mAU/mL), tumor size (>2 cm versus ≤2 cm), tumor number (single versus multiple), and initial treatment (hepatic resection versus RFA). HBV DNA level (>4 $\log_{10}$ copies/mL versus ≤4 $\log_{10}$ copies/mL) was added to these factors when analyzing the influence of

HBV DNA level, and antiviral therapy (received versus not received) was added when analyzing the influence of antiviral therapy. Differences between the two groups were analyzed using the log-rank test. All P values were two-tailed, and those <0.05 were considered significant. Statistical analysis was performed using Stat View software (version 5.0; SAS Institute Inc., Cary, NC, USA).

Results

Baseline clinical characteristics

Baseline characteristics at the time of initial HCC treatment for the four groups are summarized in Table 1. The mean follow-up period for all patients was 40 (12–92) months. There were no significant differences among the four groups with regard to gender; age; HBeAg; levels of ALT, PT, albumin, PIVKA-II, AFP, or bilirubin; platelet count; Child-Pugh score; tumor size; tumor number; stage of HCC; initial HCC treatment; or follow-up period. However, there was a significant difference with respect to HBV DNA level among the four groups. Median HBV DNA levels in the HVG (5.9 log copies/mL, range 4.1–7.6) and TG-A (6.0 log copies/mL, range 4.1–8.1) were significantly higher than those in the LVG (<2.6 log copies/mL, range <2.6–3.6) and TG-B (<2.6 log copies/mL, range <2.6–4.0) ($P = 0.005$).

Overall recurrence rate

Overall, 56 of 103 patients (54.4%) had a recurrence of HCC, with the mean period until recurrence from initial treatment being 34.7 ± 22.7 months (range 7.0–67.0 months). The estimated recurrence rates at 1 and 3 years after curative treatment were 16.5% and 53.0%, respectively (Fig. 2).

Comparison of cumulative HCC recurrence rates between the HVG and LVG

To clarify the influence of HBV DNA level on HCC recurrence, we selected the patients who had not received

Table 1 Baseline characteristics of the four groups

Variables	HVG ($n = 30$)	LVG ($n = 34$)	TG-A ($n = 20$)	TG-B ($n = 19$)
Gender (men/women)	22/8	28/6	14/6	15/4
Age (years)[a]	55.6 ± 8.3	55.9 ± 8.3	55.7 ± 7.9	54.3 ± 9.2
HBeAg (+/−)	13/17	8/26	10/10	6/13
HBV DNA ($\log_{10}$ copies/ml)[b]	5.9 (4.1–7.6)	<2.6 (<2.6–3.6)	6.0 (4.1–8.1)	< 2.6 (<2.6–4.0)
Genotype (B/C)	1/17	0/11	0/10	0/7
ALT (IU/L)[a]	37.7 ± 16.8	23.5 ± 9.1	43.1 ± 19.6	27.9 ± 13.8
Platelet count ($\times 10^3/\mu L$)[a]	13.5 ± 6.2	14.3 ± 8.3	11.4 ± 4.9	12.0 ± 4.3
PT (%)[a]	83.7 ± 15.9	85.5 ± 15.7	84.6 ± 14.5	81.7 ± 12.4
Albumin (mg/dL)[a]	3.9 ± 0.4	3.9 ± 0.4	4.0 ± 0.6	4.1 ± 0.6
Bilirubin (mg/dL)[a]	0.9 ± 0.5	0.9 ± 0.4	0.9 ± 0.3	0.8 ± 0.3
Child-Pugh score (A/B)	27/3	31/3	17/3	15/4
Liver fibrosis (F1/F2/F3/F4)	3/2/4/16	1/2/2/15	1/0/2/11	1/0/3/7
Differentiation (well/mod./poor)	8/12/2	4/17/2	4/6/2	4/4/0
AFP (ng/mL)[b]	20.7 (1.0–3387.6)	15.1 (1.0–1124.0)	26.7 (2.8–2009.7)	26.0 (1.0–2870.3)
PIVKA-II (mAU/mL)[b]	40.0 (8.0–795.0)	33.0 (11.0–403.9)	36.5 (9.0–1651.0)	34.0 (12.0–1162.0)
Tumor size (cm)[a]	2.1 ± 0.7	1.9 ± 0.7	1.7 ± 0.5	1.8 ± 0.5
Multiple tumors (number, %)	7 (23.3%)	9 (26.5%)	5 (25.0%)	4 (21.0%)
TNM stage (I/II/III)	10/17/3	12/17/5	9/10/1	6/10/3
Treatment for HCC (OPE/RFA)	16/14	16/18	10/10	9/10
Follow-up period (months)	49.2 (12–89)	55.5 (15–92)	35.5 (12–67)	34.0 (12–58)

HVG high virus group (HBV DNA ≥ 4 log copies/mL), *LVG* low virus group (HBV DNA < 4 log copies/mL), *TG-A* antiviral therapy group after the development of HCC, *TG-B* antiviral therapy group before the development of HCC, *HBeAg* hepatitis B e antigen, *HBV* hepatitis B virus, *ALT* alanine aminotranferase, *PT* prothrombin time, *Differentiation* Tumor differentiation, *AFP* alpha-fetoprotein, *PIVKA-II* protein induced by vitamin K absence or antagonist II, *HCC* hepatocellular carcinoma, *OPE* hepatic resection, *RFA* radiofrequency ablation

[a] Mean ± SD

[b] Median (range)

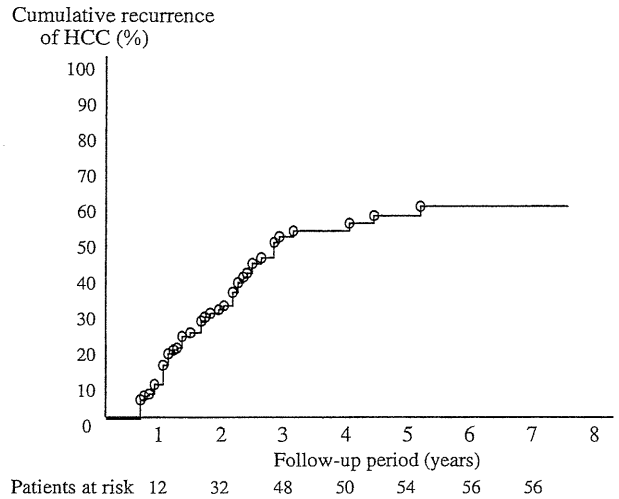

Fig. 2 Overall recurrence rate. The estimated hepatocellular carcinoma (*HCC*) recurrence rates at 1 and 3 years after curative treatment were 16.1 and 53.2%, respectively

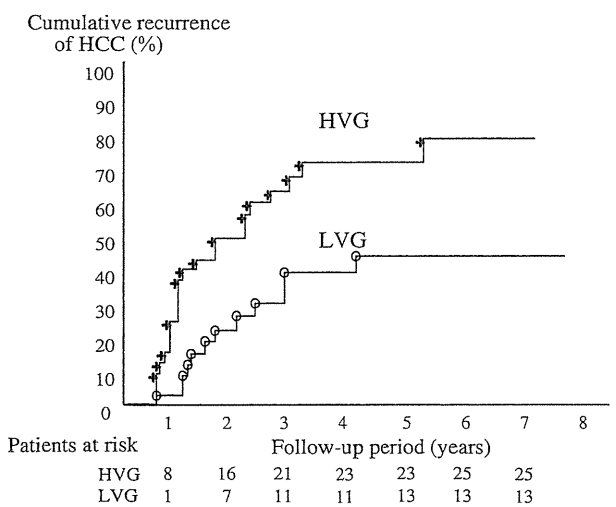

Fig. 3 Comparison of cumulative HCC recurrence rates between the high virus group (HVG) and low virus group (LVG). Cumulative HCC recurrence rates at 3 years were significantly higher in the HVG than in the LVG (71.1 versus 42.2%, $P = 0.016$)

antiviral therapy (the HVG and LVG), and compared cumulative HCC recurrence rates in these two groups. The cumulative HCC recurrence rates in the HVG and LVG were 26.7% and 9.4% at 1 year and 71.1% and 42.2% at 3 years, respectively. There were significant differences regarding the recurrence rates of HCC between the HVG and LVG ($P = 0.016$) (Fig. 3). The follow-up period for cases in which no recurrence was detected was 12–89 months in the HVG and 15–92 months in the LVG.

Multivariate analysis of risk factors for HCC recurrence in the absence of antiviral therapy

To evaluate the factors that affected recurrence after curative treatment, the 16 variables of interest shown in Table 2 were included in the analysis. In the multivariate analysis, only high serum HBV DNA level (hazard ratio 2.67; 95% CI 1.31–5.47; $P = 0.007$) was an independent risk factor for recurrence (Table 2).

Comparison of cumulative HCC recurrence rates between the TG-B and HVG, and between the TG-A and HVG

Next, to clarify the influence of antiviral therapy on the risk of HCC recurrence, we selected the patients who had a high HBV DNA level when they had not received antiviral therapy (the HVG plus TG-A plus TG-B), and compared cumulative HCC recurrence rates between the TG-A and HVG, and between the TG-B and HVG. The cumulative HCC recurrence rates in the TG-B and TG-A were 5.3% and 15.0% at 1 year, and 42.3% and 52.0% at 3 years, respectively. There were significant differences regarding the recurrence rates of HCC between the HVG and TG-B ($P = 0.008$). On the other hand, while recurrence rate was lower in the TG-A than in the HVG, this was not statistically significant ($P = 0.10$) (Fig. 4). The follow-up period for cases in which no recurrence was detected was 12–67 months in the TG-A and 12–58 months in the TG-B.

Analysis of risk factors including antiviral therapy for HCC recurrence

To evaluate the factors that affected recurrence after curative treatment, the 16 variables of interest shown in Table 3 were included in the analysis. In the multivariate analysis, multiple tumors (hazard ratio 2.81; 95% CI, 1.45–5.42; $P = 0.002$) and absence of antiviral therapy (hazard ratio 2.57; 95% CI 1.34–4.94; $P = 0.005$) were independent risk factors for recurrence (Table 3).

Cumulative HCC recurrence for each antiviral agent

Table 4 shows HCC recurrence rates for the antiviral agents used in the TG-A and TG-B. In the TG-A, cumulative HCC recurrence rate at 3 years was 47.9% in patients who were administered a single agent (lamivudine or entecavir) and 75.0% in patients who were administered two agents (lamivudine plus adefovir). In the TG-B, cumulative HCC recurrence rate at 3 years was 21.7% in patients administered a single agent and 63.5% in those given two agents. In both the TG-B and TG-A, HCC recurrence

Table 2 Factors affecting HCC recurrence in patients not given antiviral therapy (HVG plus LVG)

Characteristic	Univariate analysis	Multivariate analysis	Hazard ratio (95% CI)
Gender (male)	0.171	–	
Age (≥55 years)	0.204	–	
HBeAg status (positive)	0.433	–	
HBV DNA (≥4 log copies/mL)	0.015	0.007[a]	2.67 (1.31–5.47)
ALT (≥35 IU/L)	0.309	–	
Platelet count (<120 × 10^3/μL)	0.04	0.077	2.05 (0.93–4.51)
PT (<70%)	0.037	0.191	1.83 (0.74–4.54)
Albumin (<3.5 mg/dL)	0.382	–	
Bilirubin (≥1.2 mg/dL)	0.122	–	
Liver fibrosis (cirrhosis)	0.366	–	
Tumor differentiation (mod., poor)	0.703	–	
AFP (≥20 ng/mL)	0.336	–	
PIVKA-II (≥40 mAU/mL)	0.185	–	
Tumor size (≥2 cm)	0.072	–	
Tumor number (multiple)	0.155	–	
Initial treatment (resection versus RFA)	0.291	–	

HCC hepatocellular carcinoma, *HVG* high virus group (HBV DNA ≥ 4 log copies/mL), *LVG* low virus group (HBV DNA < 4 log copies/mL), *95% CI* 95% confidence interval, *HBeAg* hepatitis B e antigen, *HBV* hepatitis B virus, *ALT* alanine aminotranferase, *PT* prothrombin time, *mod.* moderately differentiated, *poor* poorly differentiated, *AFP* alpha-fetoprotein, *PIVKA-II* protein induced by vitamin K absence or antagonist II, *RFA* radiofrequency ablation

[a] Statistically significant

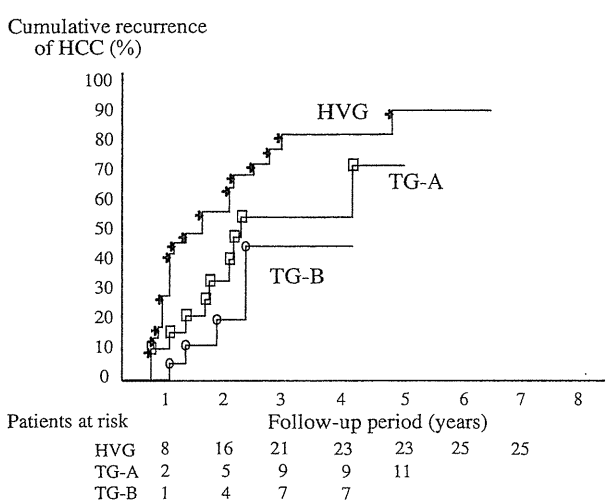

Fig. 4 Comparison of cumulative HCC recurrence rates between the antiviral therapy before HCC diagnosis group (TG-B) and the high virus group (HVG), and between the antiviral therapy after HCC diagnosis group (TG-A) and the HVG. The cumulative HCC recurrence rates at 3 years in the TG-B and TG-A were 42.3% and 52.0%, respectively. There were significant differences regarding HCC recurrence rates between HVG and TG-B ($P = 0.008$). On the other hand, although HCC recurrence rate was lower in the TG-A than in the HVG, this difference was not statistically significant ($P = 0.10$)

tended to be lower with single agents than with two agents, but this trend was not significant ($P = 0.07$ for the TG-B, $P = 0.14$ for the TG-A).

Discussion

Recurrence of hepatitis B-related HCC is extremely high even after curative treatment [7, 8], and prediction and prevention of HCC recurrence is therefore an important clinical issue. Several factors are reported to be associated with an increased risk of HCC recurrence after surgical resection or local ablation therapies, including tumor characteristics such as multiplicity, size, and portal invasion; AFP level; PIVKA-II level; and hepatic functional parameters such as albumin level, PT, and Child-Pugh class [8–12]. In addition, recent studies have suggested that a high viral load is another risk factor for recurrence [13–15]. However, these studies included patients who had received antiviral therapy and did not fully account for this. Furthermore, the efficacy of antiviral therapy in reducing the risk of HCC recurrence is far from clear. In our study, by clearly categorizing the patients and comparing cumulative HCC recurrence among the groups, we clarified (1)

Table 3 Factors affecting HCC recurrence in patients with high HBV DNA levels (HVG plus TG-A plus TG-B)

Characteristic	Univariate analysis	Multivariate analysis	Hazard ratio (95% CI)
Gender (male)	0.54	–	
Age (≥55 years)	0.661	–	
HBe Ag status (positive)	0.075	–	
Antiviral therapy (not received)	0.018	0.005[a]	2.57 (1.34–4.94)
ALT (≥35 IU/L)	0.902	–	
Platelet count (<120 × 10^3/μL)	0.36	–	
PT (<70%)	0.341	–	
Albumin (<3.5 mg/dL)	0.158	–	
Bilirubin (≥1.2 mg/dL)	0.392	–	
Liver fibrosis (cirrhosis)	0.49	–	
Tumor differentiation (mod., poor)	0.852	–	
AFP (≥20 ng/mL)	0.424	–	
PIVKA-II (≥40 mAU/mL)	0.229	–	
Tumor size (≥2 cm)	0.284	–	
Tumor number (multiple)	0.009	0.002[a]	2.81 (1.45–5.42)
Initial treatment (resection versus RFA)	0.851	–	

HCC hepatocellular carcinoma, *HBV* hepatitis B virus, *HVG* high virus group (HBV DNA ≥4 log copies/mL), *TG-A* antiviral therapy group after the development of HCC, *TG-B* antiviral therapy group before the development of HCC, *HBeAg* hepatitis B e antigen, *95% CI* 95% confidence interval, *ALT* alanine aminotranferase, *PT* prothrombin time, *mod.* moderately differentiated, *poor* poorly differentiated, *AFP* alpha-fetoprotein, *AFP* alpha-fetoprotein, *PIVKA-II* protein induced by vitamin K absence or antagonist II, *RFA* radiofrequency ablation

[a] Statistically significant

Table 4 Cumulative HCC recurrence according to antiviral agents

Antiviral agents	No. of patients	Recurrence rate (3 years, %)
TG-A ($n = 20$)		
Lamivudine	7	54.3
Entecavir	5	20.0
Single agent (lamivudine or entecavir)	12	47.9
Lamivudine plus adefovir dipivoxil	8	75.0
TG-B ($n = 19$)		
Lamivudine	5	0
Entecavir	2	0
Lamivudine then entecavir	4	50.0
Single agent (lamivudine or entecavir)	11	21.7
Lamivudine plus adefovir dipivoxil	8	63.5

TG-A antiviral therapy group after the development of HCC, *TG-B* antiviral therapy group before the development of HCC

the influence of HBV DNA level in the absence of antiviral therapy on the risk of HCC recurrence, and (2) the influence of antiviral therapy on recurrence of HCC. In patients who had not undergone antiviral treatment, multivariate analysis demonstrated that HBV DNA level >4 $\log_{10}$ copies/mL was an independent factor associated with higher cumulative risk of HCC recurrence after curative treatment.

The mechanism for recurrent carcinogenesis associated with HBV in the remaining liver in patients who have undergone curative treatment remains unclear. Both direct and indirect carcinogenic mechanisms are thought to be involved [24]. Active replication of HBV may initiate malignant transformation through a direct carcinogenic mechanism by increasing the probability of viral DNA insertion in or near proto-oncogenes, tumor-suppressor genes or regulatory elements of cellular DNA [25, 26]. The integration of viral DNA may increase the production of transactivator protein hepatitis B X antigen, which may promote neoplasia of hepatocytes, as well as bind to the p53 tumor-suppressor gene and disrupt its functions [27, 28]. Indirectly, continuing HBV replication can also induce chronic liver fibrosis and inflammation and mediate alteration in transforming growth factor-beta1 (TGF-β1) and alpha-M production, thereby leading to carcinogenesis [29, 30]. High HBV viral load can induce hepatocarcinogenesis via direct and indirect ways; hence, the risk of multicentric recurrent tumors in the liver remnant is thought to be increased.

Given the strong association between HBV DNA level and cancer recurrence, we next investigated and demonstrated that antiviral therapy is associated with lower risk of HCC recurrence. Multivariate analysis showed that absence of antiviral therapy and number of tumors were the

two independent factors associated with higher cumulative risk of HCC recurrence in patients with high serum HBV DNA level after curative treatment. The number of tumors has previously been associated with HCC recurrence. Recently, the efficacy of lamivudine in preventing hepatocellular carcinoma in chronic hepatitis B has been described [19], and one study demonstrated that lamivudine therapy reduced the recurrence of HCC in patients with chronic hepatitis B [22]. The authors stated that remission of active hepatitis in response to lamivudine therapy may decrease HCC development and metastatic potential. Taken together, these findings suggest that, although antiviral therapy itself does not have anticancer effects, it may suppress HCC recurrence directly and indirectly by decreasing HBV viral load. We further showed that, while recurrence rate of HCC was significantly lower in the TG-B than in the HVG, it was only marginally lower in the TG-A than in the HVG. This suggests that, for patients with high serum HBV DNA levels, it is important to give antiviral therapy before HCC develops to prevent HCC recurrence. In addition, we showed that the rate of HCC recurrence was marginally lower for single antiviral agent therapy than for therapy using two agents. The patients who received two agents were unresponsive to lamivudine and had high serum HBV DNA level in the lamivudine-refractory period, despite receiving antiviral therapy. The difference in HBV DNA level between these two modes of therapy may be associated with the difference in rate of HCC recurrence (data not shown). However, we were unable to further evaluate such relationships, as there were few patients in each therapeutic group. Further analysis needs to be performed in a larger population of patients with HBV-related HCC and with a longer follow-up period in order to clarify our findings.

Conclusion

Both HBV DNA level and absence of antiviral therapy appear to be associated with HCC recurrence. To prevent HCC recurrence for patients with high serum HBV DNA levels, it seems important to commence antiviral therapy before HCC develops. Large-scale prospective trials are necessary to elucidate the effects of HBV DNA viral load on recurrence after curative treatment and the protective roles of antiviral therapy.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005;55:74–108.
2. Befeler AS, Di Bisceglie AM. Hepatocellular carcinoma: diagnosis and treatment. Gastroenterology. 2002;122:1609–19.
3. Song TJ, Ip EW, Fong Y. Hepatocellular carcinoma: current surgical management. Gastroenterology. 2004;127:S248–60.
4. Ioannou GN, Perkins JD, Carithers RL Jr. Liver transplantation for hepatocellular carcinoma: impact of the MELD allocation system and predictors of survival. Gastroenterology. 2008; 134:1342–51.
5. Shiina S, Teratani T, Obi S, Sato S, Tateishi R, Fujishima T, et al. A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. Gastroenterology. 2005;129:122–30.
6. Livraghi T, Meloni F, Di Stasi M, Rolle E, Solbiati L, Tinelli C, et al. Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: is resection still the treatment of choice? Hepatology. 2008;47:82–9.
7. Poon RT, Fan ST, Lo CM, Liu CL, Ng IO, Wong J. Long-term prognosis after resection of hepatocellular carcinoma associated with hepatitis B-related cirrhosis. J Clin Oncol. 2000;18:1094–101.
8. Toyama T, Hiramatsu N, Yakushijin T, Oze T, Nakanishi F, Yasumaru M, et al. A new prognostic system for hepatocellular carcinoma including recurrent cases: a study of 861 patients in a single institution. J Clin Gastroenterol. 2008;42:317–22.
9. Adachi E, Maeda T, Matsumata T, Shirabe K, Kinukawa N, Sugimachi K, et al. Risk factors for intrahepatic recurrence in human small hepatocellular carcinoma. Gastroenterology. 1995;108:768–75.
10. Koike Y, Shiratori Y, Sato S, Obi S, Teratani T, Imamura M, et al. Risk factors for recurring hepatocellular carcinoma differ according to infected hepatitis virus—an analysis of 236 consecutive patients with a single lesion. Hepatology. 2000; 32:1216–23.
11. Sasaki Y, Yamada T, Tanaka H, Ohigashi H, Eguchi H, Yano M, et al. Risk of recurrence in a long-term follow-up after surgery in 417 patients with hepatitis B- or hepatitis C-related hepatocellular carcinoma. Ann Surg. 2006;244:771–80.
12. Poon RT, Fan ST, Ng IO, Lo CM, Liu CL, Wong J. Different risk factors and prognosis for early and late intrahepatic recurrence after resection of hepatocellular carcinoma. Cancer. 2000; 89:500–7.
13. Hung IF, Poon RT, Lai CL, Fung J, Fan ST, Yuen MF, et al. Recurrence of hepatitis B-related hepatocellular carcinoma is associated with high viral load at the time of resection. Am J Gastroenterol. 2008;103:1663–73.
14. Jang JW, Choi JY, Bae SH, Yoon SK, Woo HY, Chang UI, et al. The impact of hepatitis B viral load on recurrence after complete necrosis in patients with hepatocellular carcinoma who receive transarterial chemolipiodolization: implications for viral suppression to reduce the risk of cancer recurrence. Cancer. 2007;110:1760–7.
15. Huang Y, Wang Z, An S, Zhou B, Zhou Y, Chan HL, et al. Role of hepatitis B virus genotypes and quantitative HBV DNA in metastasis and recurrence of hepatocellular carcinoma. J Med Virol. 2008;80:591–7.
16. Dienstag JL, Perrillo RP, Schiff ER, Bartholomew M, Vicary C, Rubin M, et al. A preliminary trial of lamivudine for chronic hepatitis B infection. N Engl J Med. 1995;333:1657–61.
17. Dienstag JL, Goldin RD, Heathcote EJ, Hann HW, Woessner M, Stephenson SL, et al. Histological outcome during long-term lamivudine therapy. Gastroenterology. 2003;124:105–17.
18. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced disease. N Engl J Med. 2004;351:1521–31.

19. Matsumoto A, Tanaka E, Rokuhara A, Kiyosawa K, Kumada H, Omata M, et al. Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: a multicenter retrospective study of 2795 patients. Hepatol Res. 2005;32:173–84.
20. Peters MG, Hann HW, Martin P, Heathcote EJ, Buggisch P, Rubin R, et al. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. Gastroenterology. 2004;126:91–101.
21. Chang TT, Gish RG, Hadziyannis SJ, Cianciara J, Rizzetto M, Schiff ER, et al. A dose-ranging study of the efficacy and tolerability of entecavir in lamivudine-refractory chronic hepatitis B patients. Gastroenterology. 2005;129:1198–209.
22. Kubo S, Tanaka H, Takemura S, Yamamoto S, Hai S, Ichikawa T, et al. Effects of lamivudine on outcome after liver resection for hepatocellular carcinoma in patients with active replication of hepatitis B virus. Hepatol Res. 2007;37:94–100.
23. Bruix J, Sherman M. Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. Hepatology. 2005;42:1208–36.
24. Kew MC. Hepatitis viruses and hepatocellular carcinoma. Res Virol. 1998;149:257–62.
25. Kim CM, Koike K, Saito I, Miyamura T, Jay G. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. Nature. 1991;351:317–20.
26. Paterlini P, Poussin K, Kew MC, 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.
27. Wang XW, Gibson MK, Vermeulen W, Yeh H, Forrester K, Stürzbecher HW, et al. Abrogation of p53-induced apoptosis by the hepatitis B virus X gene. Cancer Res. 1995;55:6012–6.
28. Yoon YJ, Chang HY, Ahn SH, Kim JK, Park YK, Kang DR, et al. MDM2 and p53 polymorphisms are associated with the development of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. Carcinogenesis. 2008;29:1192–6.
29. Colombo M, Sangiovanni A. Etiology, natural history and treatment of hepatocellular carcinoma. Antiviral Res. 2003;60:145–50.
30. Pan J, Clayton M, Feitelson MA. Hepatitis B virus X antigen promotes transforming growth factor-beta1 (TGF-beta1) activity by up-regulation of TGF-beta1 and down-regulation of alpha2-macroglobulin. J Gen Virol. 2004;85:275–82.

臨牀消化器内科 2009 Vol.24 No.5

検査値の読み方　連載

新しいB型肝炎ウイルスのマーカー：HBコア関連抗原

髭　修平* 堀本　啓大* 小原　俊央*
中西　満* 中馬　誠* 浅香　正博*

はじめに

B型肝炎ウイルス(HBV)に関連した測定系には，おもに抗原/抗体の測定系とHBV-DNAを測定する核酸検査が使われているが，最近，核酸検査にreal-time PCRが導入され，感度，測定可能域の点で改良がみられている．その後，さらに新たなウイルスマーカーとしてB型肝炎ウイルスコア関連抗原(以下，HBcr抗原)が臨床応用された．

本稿では，HBV複製・増殖におけるHBcr抗原の位置づけと，われわれの測定結果をもとにして，臨床上の測定の意義について検討する．

Ⅰ．HBVの構造

HBVのゲノムは全長約3.2 kbの環状二本鎖DNAであるが，通常は，一部が一本鎖で存在する不完全二重鎖構造である．ウイルス粒子内では，HBVゲノムはコア粒子内に存在し，さらに，そのコア粒子は，3種類の異なるサイズのS蛋白からなる外皮で被われている．この感染性をもったウイルス粒子はDane粒子と呼ばれその他，HBV-DNAを内包しない中空粒子が多数存在することも知られている(図1)．

Ⅱ．HBVの複製・増殖(図1)

HBVが肝細胞内に侵入する際には，外皮から脱殻し，コア粒子が核内に移行する．このとき，不完全二重鎖のDNAは，自身のDNAポリメラーゼにより完全二本鎖となり，さらに閉環して閉環二本鎖DNA(covalently closed circular DNA；cccDNA)となり，超らせん状構造(supercoiled DNA)をとる．このcccDNAをもとにして，ウイルスの増殖では大別して以下の二つの過程をとる．

一つは，ウイルス遺伝子自体の複製である．cccDNAを鋳型にしてmRNAが転写されるが，そのなかで最長のものをpregenomic RNAと呼び遺伝子複製の基となる．逆転写反応によりHBVゲノムの(−)鎖が合成され，さらに(＋)鎖が合成されて不完全二本鎖DNAができ

Key words：HBコア関連抗原，肝組織中cccDNA，precore変異，核酸アナログ

Shuhei Hige／Hiromasa Horimoto／Toshihisa Kohara／Mitsuru Nakanishi／Makoto Chuma／Masahiro Asaka
*北海道大学大学院医学研究科消化器内科学(〒060-8638 北海道札幌市北区北15条西7丁目)

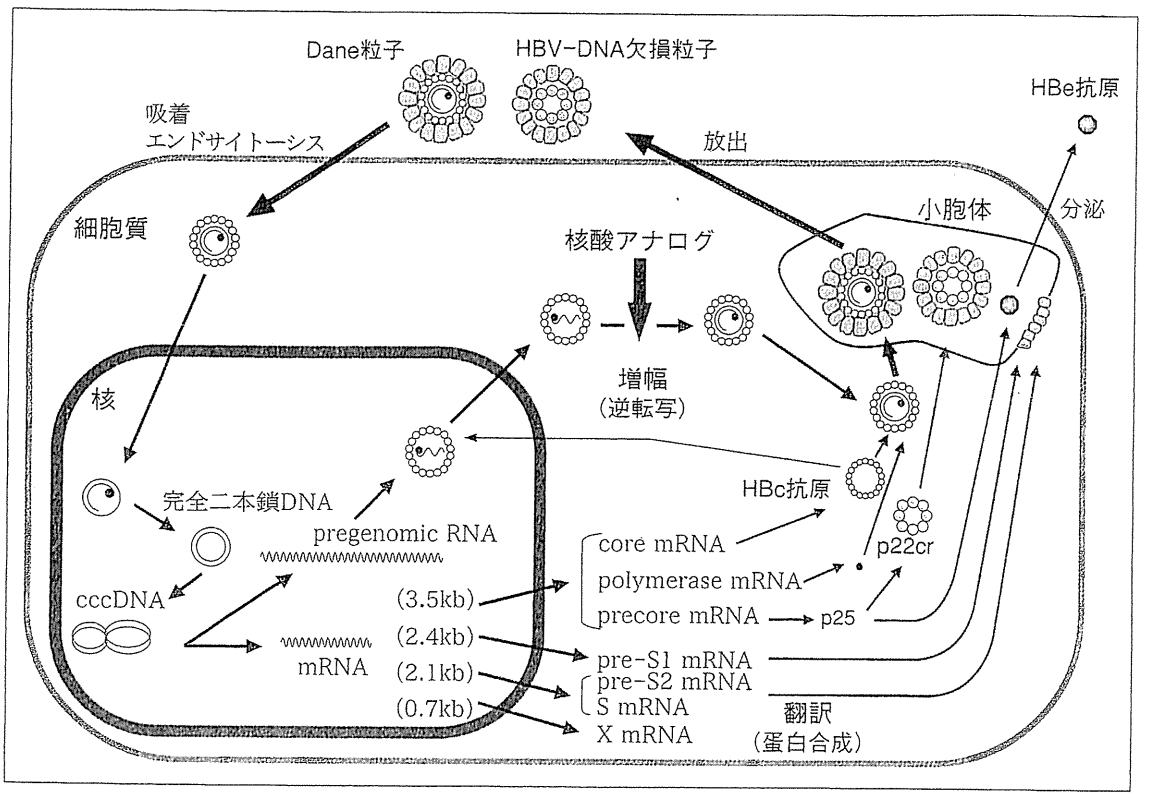

図1　B型肝炎ウイルスの複製と増殖

あがる.

もう一つは，ウイルス粒子に必要な蛋白や酵素の合成である．これも，cccDNA を鋳型として複数の長さの mRNA が作られる．これらから，HBV の四つの open reading frame(S，P，C，X)に対応する蛋白などが作られる．3.5 kb の mRNA には，上記の pregenomic RNA のほかに，core mRNA，precore mRNA，polymerase mRNA などが作られることが知られている.

Ⅲ．HBcr 抗原(図 2)

core mRNA からは HBc 抗原が作られる．HBc 抗原は二つの領域から成り立っているが，assembly domain は粒子形成に，arginine-rich domain は RNA の内部取り込みに作用する．precore mRNA から作られた蛋白(p 25)は，その両端が分解酵素で切断されて HBe 抗原となるが，HBc 抗原の有する arginine-rich domain をもたず，細胞外に分泌される．さらに，Kimura らは，precore mRNA から前半部分が peptidase で切断されず，後半部分の arginine-rich domain が不十分な蛋白(p22cr)が合成され，これが，HBV-DNA を内包しない Dane 粒子類似のウイルス粒子となることを報告している[1].

HBcr 抗原測定系は，HBV の core，precore mRNA から転写・翻訳される上記の蛋白(HBc 抗原，HBe 抗原，p22cr)全体を同時に測定する新しい HBV 関連マーカーで，2008 年から保険適応下で測定可能となった．これは，血中の蛋白を変性させ，立体構造を壊して上記の抗原蛋白を直線状にして，共通のエピトープを認識するように設定したモノクローナル抗体を用い，化学発光酵素免疫測定法(chemiluminescent

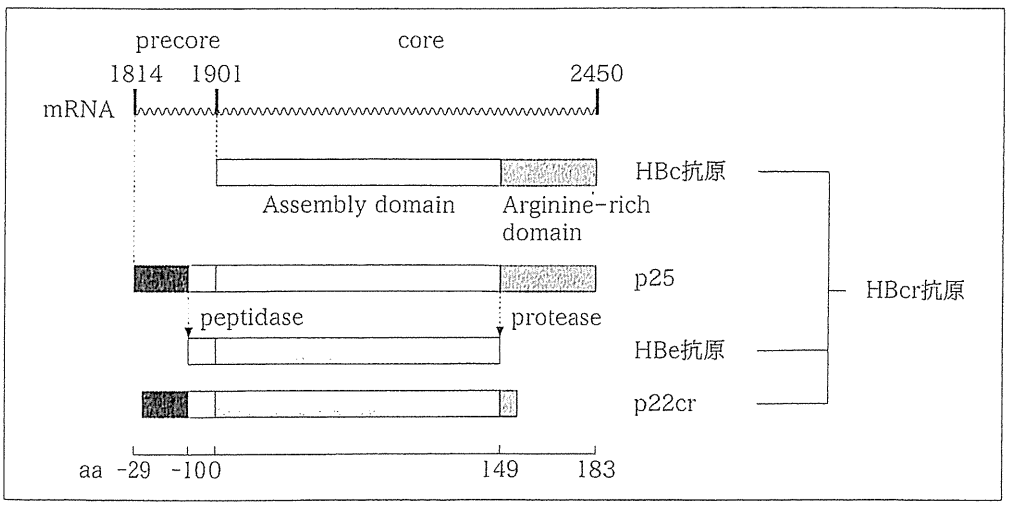

図2　precore および core mRNA からの翻訳蛋白と HBcr 抗原

enzyme immunoassay；CLEIA）で測定する．測定結果は対数で表示され，測定範囲は 3.0～7.0(logU/m*l*)である．

Ⅳ．HBcr 抗原測定の意義

図1および図2から HBcr 抗原の位置づけを推測すると，以下のような意義・特徴があるものと考えられる．

1．cccDNA を反映する指標としての有用性

肝組織内 cccDNA と血清 HBV-DNA 量の関連性については，Suzuki らの報告[2]があるが，核酸アナログによる抗ウイルス治療時には，逆転写の段階で薬物が作用するために HBV-DNA 量では評価は不能となる．

一方，これまでは HBe 抗原しか測定できなかった core，precore mRNA からの翻訳蛋白が，HBcr 抗原測定系により全体を測定できるようになった．こちらは，核酸アナログによる抑制を受けない経路であり，治療中でも cccDNA との関連を示していると考えられる．

2．precore 遺伝子変異の影響

図2に示されているとおり，HBe 抗原および p22cr は precore/core mRNA からの合成蛋白である．したがって，precore 領域後半の 28 番目あるいは 29 番目のアミノ酸が遺伝子変異によりストップコドンに変化した場合には，HBe 抗原や p22cr の基となる p25 が合成されない．したがって，precore 領域末端が完全に変異株に置換された場合には，HBcr 抗原は HBc 抗原のみを測定していることになる．

Ⅴ．HBcr 抗原の測定結果

1．肝組織中 cccDNA 量と HBcr 抗原量

肝生検組織中の cccDNA を測定した B 型肝疾患患者 24 例における，対応する血清中の HBcr 抗原量との関連を図3に示す．対象症例中 15 例では HBcr 抗原量が測定範囲を超えており，希釈測定にて定量判定した．その結果，両者には良好な相関関係を認めた($r=0.85$，$p<0.001$)．

2. HBe 抗原陽性例(無治療)の血清中 HBV-DNA 量と HBcr 抗原量

HBe 抗原陽性例 30 例の HBV-DNA 量と HBcr 抗原量を対応させて比較したところ，HBV-DNA 量が測定感度を超えた例が 6 例，HBcr 抗原量が測定感度を超えた例が 22 例あり，両者とも測定範囲内であった例は 8 例のみで，多くの例で定量的相関は示されなかった(**図 4**)．そこで，HBcr 抗原高値検体を希釈(10 倍希釈し，さらに感度以上の例は，さらに 10 倍希釈)測定し比較すると，良好な相関を示した($r=0.88$, $p<0.001$)(**図 5**)．

3. HBe 抗原陰性例(無治療)の血清中 HBV-DNA 量と HBcr 抗原量

HBe 抗原陰性例 39 例で両者の検討を行うと，HBcr 抗原が測定可能な例が 19 例，HBV-DNA 量が測定可能な例が 32 例で，HBV-DNA のみ測定可能な例のほうが多かったが，両者が測定可能な例では良好な相関を示した($r=0.75$, $p<0.01$)(**図 6**)．しかし，HBcr 抗原が測定感度以下で，かつ HBV-DNA 量が測定可能

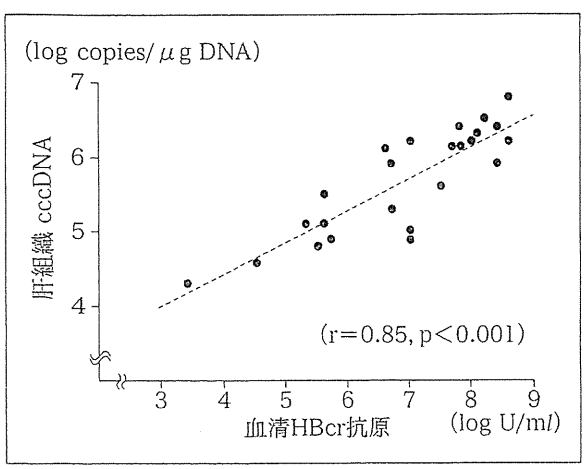

図 3　肝組織中 cccDNA 量と血清 HBcr 抗原量

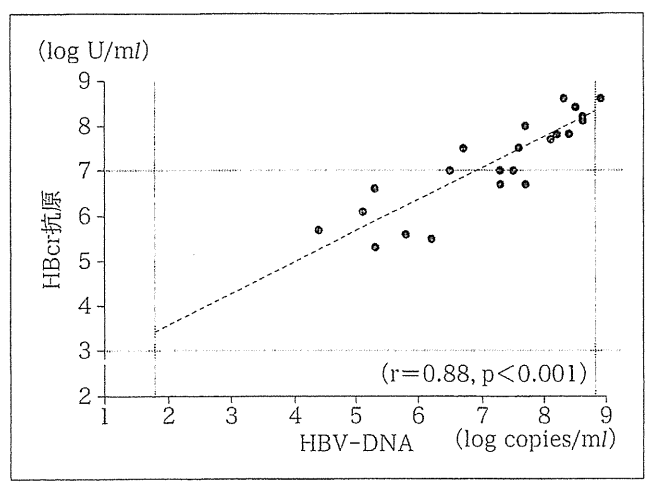

図 5　血清 HBV-DNA 量と HBcr 抗原量(希釈測定後)

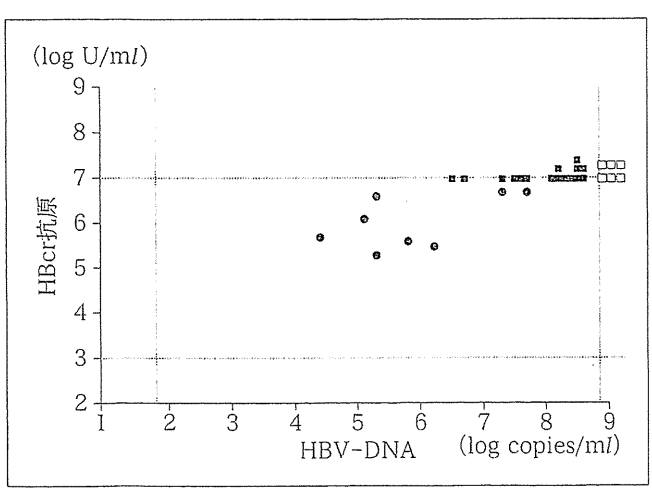

図 4　HBe 抗原陽性例の血清 HBV-DNA 量と HBcr 抗原量
●：両者測定可能，■：HBV-DNA のみ測定可能，□：両者測定不可能

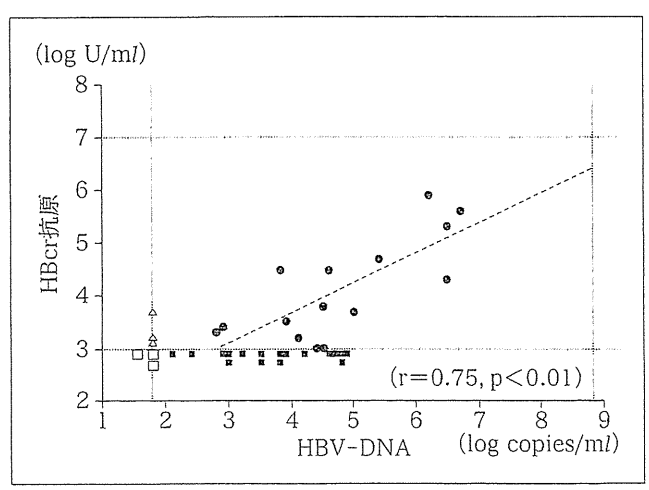

図 6　HBe 抗原陰性例の血清 HBV-DNA 量と HBcr 抗原量
●：両者測定可能，■：HBV-DNA のみ測定可能，△：HBcr 抗原のみ測定可能，□：両者測定不可能

な17例においては，HBV-DNA量が2.1(log copies/ml，以下，LC/ml)から4.9(LC/ml)まで分布しており，500倍以上の差を認めた．後述する，核酸アナログ治療後のパターンと逆であることに注目する必要があると思われる．

4. HBV precore変異株比率の違いとHBV-DNA量とHBcr抗原量

図2で示したように，HBV precore領域の変異の程度により，同じcccDNAレベルでも，そこから増幅されるHBV-DNA量と翻訳されるHBcr抗原量が異なる可能性が考えられる．無治療で，かつ，HBV-DNA量，HBcr抗原量，precore/core promoter遺伝子変異を測定したB型肝疾患患者41例で関連を検討した．このうち，HBV-DNA量およびHBcr抗原量が測定範囲内にあるのは14例であったが，precore領域の野生株の比率が高いほど，HBV-DNA量に対するHBcr抗原量の比率が高めの傾向を示した(r=0.63，p<0.01)(**図7**)．

5. 核酸アナログ投与例

ラミブジン(アデホビル併用を含む)あるいはエンテカビルを投与中の58例について，HBV-DNA量とHBcr抗原量の関連を検討した．

そのなかで，検査時点にHBV-DNA量が測定感度(1.8 LC/ml)未満に低下している46例における，HBe抗原/HBe抗体とHBcr抗原量の分布を**図8**に示す．HBe抗原陽性例やHBe抗体価が70%未満の症例のHBcr抗原は，ほとんどが4(log U/ml)以上であった．また，HBe抗体が90%以上の高力価であってもHBcr抗原量は必ずしも低値例ばかりではなく，症例により数百倍以上の差があることが示されている．これらは，核酸アナログ製剤治療によりHBV-DNAの複製は抑制されているものの，薬剤作用が及ばないHBVのcccDNAレベルは

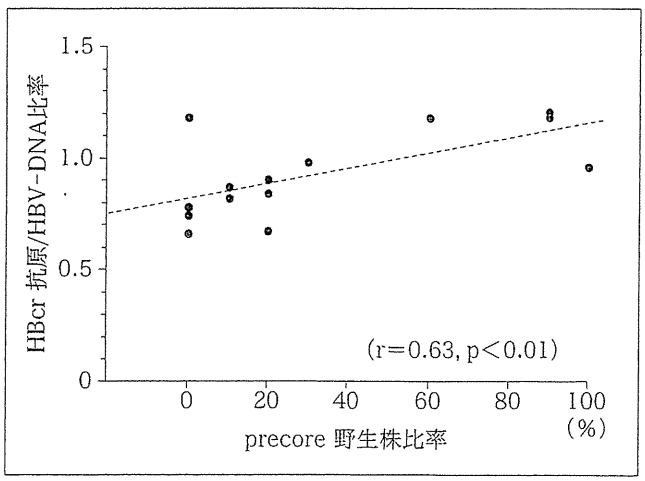

図7 precore野生株の比率とHBcr抗原/HBV-DNA比率

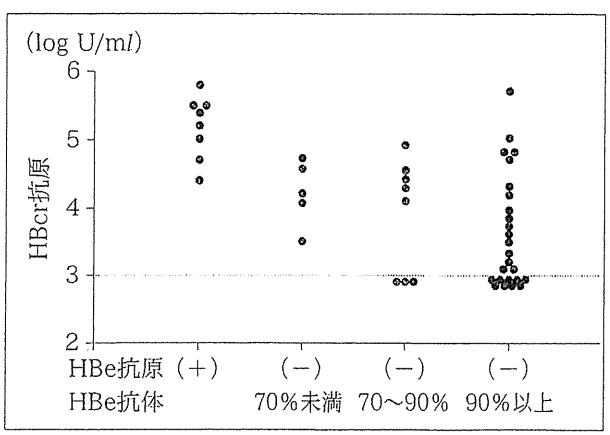

図8 核酸アナログ治療中かつHBV-DNA量測定感度以下の症例におけるHBe抗原/HBe抗体とHBcr抗原量の分布

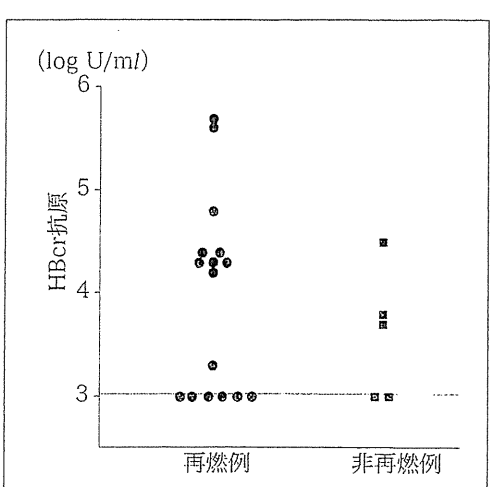

図9 核酸アナログ中止時のHBcr抗原量と中止後再燃の有無

必ずしも同時に抑制されず，ウイルスの活動性は維持されていることを示していると考えられる．

6. 核酸アナログ投与中止時のHBcr抗原量と再燃

核酸アナログ投与を中止したHBe抗体陽性症例21例について，中止後の再燃(ここでは，中止後1年以内のHBV-DNA量5 LC/m*l*以上への上昇とする)の有無と中止時点のHBcr抗原量を示す(**図9**)．21例中5例は再燃を認めなかったが，中止時のHBcr抗原量が測定感度未満であっても再燃例はみられる(図9)．

まとめ

HBcr抗原測定により，従来はHBe抗原しか測定できなかったprecore/core領域の合成蛋白を広く測定することが可能となり，新しいHBV関連マーカーとして評価されてきている．とくに，HBcr抗原が肝細胞内のcccDNA量を反映していることから，HBV-DNAの逆転写抑制を主作用とする核酸アナログを投与中で，HBV-DNA量が測定困難な症例におけるHBVレベルの評価が可能となることが期待されている[3]．本稿に示したとおり，われわれの検討では，HBcr抗原低値は，核酸アナログ治療中止の必要条件にはなるものと考えている．

また，HBcr抗原測定系の対象となる蛋白を考慮した場合，HBe抗原やprecore領域遺伝子変異の状態によってもHBcr抗原レベルが異なることも理解しておく必要がある．HBe抗原陽性例ではHBcr抗原量は測定範囲以上のことも多く，どのような症例やタイミングで測定するかの判断も必要である．

これらの臨床的意義を評価，確立するために，今後の検討が望まれる．

文　献

1) Kimura, T., Ohno, N., Terada, N., et al.：Hepatitis B virus DNA-negative Dane particles lack core protein but contain a 22-kDa precore protein without C-terminal arginine-rich domain. J. Biol. Chem. 280；21713-21719, 2005
2) Suzuki, F., Miyakoshi, H., Kobayashi, M., et al.：Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients. J. Med. Virol. 81；27-33, 2009
3) Matsumoto, A., Tanaka, E., Minami, M., et al.：Low serum level of hepatitis B core-related antigen indicates unlikely reactivation of hepatitis after cessation of lamivudine therapy. Hepatol. Res. 37；661-666, 2007

Gut Online First, published on November 7, 2011 as 10.1136/gutjnl-2011-300832

ORIGINAL ARTICLE

Hepatitis B virus strains of subgenotype A2 with an identical sequence spreading rapidly from the capital region to all over Japan in patients with acute hepatitis B

Yoko Tamada,[1,2] Hiroshi Yatsuhashi,[1,2] Naohiko Masaki,[3] Makoto Nakamuta,[4] Eiji Mita,[5] Tatsuji Komatsu,[6] Yukio Watanabe,[7] Toyokichi Muro,[8] Masaaki Shimada,[9] Taizo Hijioka,[10] Takeaki Satoh,[11] Yutaka Mano,[12] Toshiki Komeda,[13] Masahiko Takahashi,[14] Hiroshi Kohno,[15] Hajime Ota,[16] Shigeki Hayashi,[17] Yuzo Miyakawa,[18] Seigo Abiru,[1,2] Hiromi Ishibashi[1,2]

For numbered affiliations see end of article.

Correspondence to
Professor Hiroshi Yatsuhashi, Clinical Research Center, NHO National Nagasaki Medical Center and Department of Hepatology, Nagasaki University Graduate School of Biomedical Sciences, Address: 2-1001-1 Kubara, Omura, Nagasaki 856-8562, Japan; yatsuhashi@nmc.hosp.go.jp

Revised 3 October 2011
Accepted 5 October 2011

ABSTRACT

Objective To examine recent trends of acute infection with hepatitis B virus (HBV) in Japan by nationwide surveillance and phylogenetic analyses.

Methods During 1991 through 2009, a sentinel surveillance was conducted in 28 national hospitals in a prospective cohort study. Genotypes of HBV were determined in 547 patients with acute hepatitis B. Nucleotide sequences in the preS1/S2/S gene of genotype A and B isolates were determined for phylogenetic analyses.

Results HBV genotype A was detected in 137 (25% (accompanied by genotype G in one)) patients, B in 48 (9%), C in 359 (66%), and other genotypes in the remaining three (0.5%). HBV persisted in five with genotype A including the one accompanied by genotype G; another was co-infected with HIV type 1. The genotype was A in 4.8% of patients during 1991–1996, 29.3% during 1997–2002, and 50.0% during 2003–2008 in the capital region, as against 6.5%, 8.5% and 33.1%, respectively, in other regions. Of the 114 genotype A isolates, 13 (11.4%) were subgenotype A1, and 101 (88.6%) were A2, whereas of the 43 genotype B isolates, 10 (23.3%) were subgenotype B1, 28 (65.1%) were B2, two (4.7%) were B3, and three (7.0%) were B4. Sequences of 65 (64%) isolates of A2 were identical, as were three (23%) of A1, and five (18%) of B2, but none of the B1, B3 and B4 isolates shared a sequence.

Conclusions Acute infection with HBV of genotype A, subgenotype A2 in particular, appear to be increasing, mainly through sexual contact, and spreading from the capital region to other regions in Japan nationwide. Infection persisted in 4% of the patients with genotype A, and HBV strains with an identical sequence prevailed in subgenotype A2 infections. This study indicates the need for universal vaccination of young people to prevent increases in HBV infection in Japan.

Significance of this study

What is already known about this subject?

- In Japan, a national prevention programme was started in 1986 with selective vaccination of babies born to mothers who carry hepatitis B virus (HBV). Since then, the prevalence of hepatitis B surface antigen among younger generations has decreased sharply.
- However, retrospective studies indicate that the frequency of HBV genotype A is increasing among patients with acute hepatitis B (AHB) within the capital region of Japan.
- Infection with genotype A more often persists than infection with other genotypes.
- Because there is no reliable and comprehensive surveillance system for AHB in Japan, the incidence of AHB and factors responsible for changes over many years are not known.

What are the new findings?

- This is a prospective cohort study for surveillance of AHB throughout Japan in a national research programme.
- The incidence of AHB in Japan has not decreased, because genotype A infections have increased over time.
- Genotype A infections started to increase in the capital region of Japan, and then spread to other regions 5–6 years later.
- About 90% of genotype A found in AHB patients in Japan is subgenotype A2.
- Subgenotype A2 isolates from patients with AHB tend to preserve sequence identity over time, indicating that particular subgenotype A2 strains have been transmitted without undergoing mutations.

Hepatitis B virus (HBV) has been classified into 10 genotypes, designated A–J, based on a >8% divergence in the full-genome sequence.[1–7] Different genotypes are associated with distinct clinical manifestations, such as severity and progression of liver disease, as well as response to antiviral treatments.[8–10] Some genotypes are subclassified: genotype A into at least two subgenotypes, A1 (Asian/African type) and A2 (European type)[11–13];

Significance of this study

How might it impact on clinical practice in the foreseeable future?

- It needs to be noted that subgenotype A2 infections are spreading among sexually active generations in Japan.
- Although selective vaccination has prevented mother-to-baby transmission of HBV since 1986, it does not contain sporadic infections in Japan.
- Herd vaccination of younger generations needs to be considered in Japan.

B into B1 (Japanese type) and B2 (Asian type)[14 15]; and C into C1 (Southeast-Asian type) and C2 (East-Asian type).[16] Subgenotypes also influence the replication of HBV and clinical manifestation.[15 17 18]

According to a report from Japan in 2001,[19] genotype C was the most prevalent (84.7%), followed by genotype B (12.2%) and A (1.7%), among patients with chronic hepatitis B. In 2002, genotype A became the most prevalent in patients with acute hepatitis B (AHB) around Tokyo, the capital region of Japan.[20 21] Several reports have shown that infection with HBV genotype A is associated with particular sexual behaviours, such as homosexual activity and promiscuous sexual contacts, and tends to persist longer than that with HBV genotype C.[22 23] These reports have raised concerns about the horizontal HBV infection in adults, which, in general, is considered to resolve spontaneously. However, adult-acquired HBV infection may result in chronic HBV infection in some instances.

Information on changes in genotype distribution over time, as well as genotype-specific clinical manifestations, may help in planning preventive measures and antiviral therapy strategies. Therefore it is important to examine how genotype A infection has spread in Japan, and what clinical and virological characteristics it possesses.

We have been conducting a nationwide, sentinel surveillance on acute viral hepatitis for more than 30 years. As part of this surveillance, a prospective cohort study has been conducted on 547 patients with AHB in 28 medical centres over the 19 years from 1991 to 2009. Geographical and longitudinal distributions of HBV genotypes/subgenotypes were surveyed, and their influence on clinical outcome was evaluated.

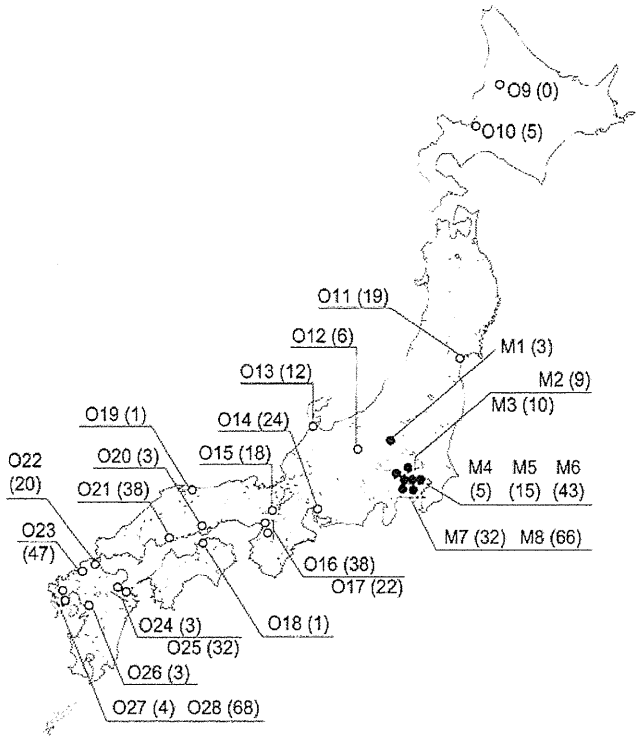

Figure 1 Locations of participating hospitals in Japan. Hospitals in the capital region (M1–M8) are indicated by eight closed circles, and those in other regions (O9–O28) by 20 open circles. Numbers in parentheses indicate the total number of enrolled subjects for each site. The hospitals are: M1, Nishigunma Hospital, Gunma; M2, Nishisaitama-Chuo Hospital, Saitama; M3, National Disaster Medical Center, Tokyo; M4, Tokyo Hospital, Tokyo; M5, Tokyo Medical Center, Tokyo; M6, National Center for Global Health and Medicine, Tokyo; M7, Sagamihara Hospital, Kanagawa; M8, Yokohama Medical Center, Kanagawa; O9, Asahikawa Medical Center, Hokkaido; O10, Hokkaido Medical Center, Hokkaido; O11, Sendai Medical Center, Miyagi; O12, Matsumoto Medical Center, Nagano; O13, Kanazawa Medical Center, Ishikawa; O14, Nagoya Medical Center, Aichi; O15, Kyoto Medical Center, Kyoto; O16, Osaka National Hospital, Osaka; O17, Osaka-Minami Medical Center, Osaka; O18, Zentsuji Hospital, Kagawa; O19, Yonago Medical Center, Tottori; O20, Okayama Medical Center, Okayama; O21, Kure Medical Center and Chugoku Cancer Center, Hiroshima; O22, Kokura Medical Center, Fukuoka; O23, Kyushu Medical Center, Fukuoka; O24, Beppu Medical Center, Oita; O25, Oita Medical Center, Oita; O26, Kumamoto Medical Center, Kumamoto; O27, Ureshino Medical Center, Saga; and O28, Nagasaki Medical Center, Nagasaki.

PATIENTS AND METHODS

Patients

A total of 681 patients with sporadic AHB were enrolled consecutively in a survey carried out by the Japan National Hospital Acute Hepatitis Study Group (JNHAHSG). They were admitted to 28 national hospitals from January 1991 to the end of December 2009. They were grouped geographically into two areas: the capital region (Gunma, Saitama, Tokyo and Kanagawa) and other regions (figure 1). Patients were also longitudinally categorised into three periods: 1st (1991–1996), 2nd (1997–2002) and 3rd (2003–2008). In addition, the year 2009 provided the most recent data. Of the 681 patients, 547 (80.3%) entered the study, for whom serum samples were available on admission and had been stored at −20°C.

The diagnosis of AHB was based on the following criteria: (1) acute onset of liver injury without a history of liver dysfunction; (2) detection of hepatitis B surface antigen (HBsAg) in the serum; (3) positivity for IgM antibody to HBV-core antigen (IgM anti-HBc) in high titres (detectable in sera diluted 10-fold); and (4) absence of past or family history of chronic HBV infection. Severe acute hepatitis (SAH) was defined as prothrombin time (PT) ≤40% and hepatic encephalopathy of grade ≤I. Fulminant hepatitis (FH) was diagnosed from PT ≤40% and hepatic encephalopathy of grade ≥II. Patients in whom HBsAg remained in the serum for >6 months after onset were considered to have acquired chronic HBV infection. The following information was collected from each patient: year and age at onset, gender, residential area, HBsAg, IgM anti-HBc, alanine aminotransferase, total bilirubin, PT, severity of liver disease, mortality, routes of transmission, sexual behaviours, travelling abroad in recent past, HBV genotype, mutations in precore (PreC) and core promoter (CP) regions, and RNA of hepatitis D virus. Antibody to HIV type 1 (anti-HIV) was

Tamada Y, Yatsuhashi H, Masaki N, *et al*. *Gut* (2011). doi:10.1136/gutjnl-2011-300832

determined in patients who were at high risk and gave consent to testing.

Informed consent was obtained from each patient. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and the Ministry of Education, Culture, Sports Science and Technology of Japan, and was approved by the ethics committee of each institution.

Extraction of HBV DNA

HBV DNA was extracted from serum (100 μl) by the SMITEST EX-R&D Nucleic Acid Extraction Kit (MBL Co, Nagoya, Japan) and used for genotyping/subgenotyping and detecting mutations in PreC and CP regions.

HBV genotypes

Genotypes were determined in Nagasaki Medical Center with the SMITEST HBV Genotyping Kit (MBL) by hybridisation with type-specific probes immobilised on a solid-phase support.[24]

Determination of HBV subgenotypes

For subgenotyping, HBV DNA was amplified by PCR with TaKaRa Ex Taq (Takara Bio, Shiga, Japan). PCR was performed with appropriate nested primers to amplify a ~1.2 kb sequence in the preS1/S2/S gene (nucleotides 2854–835 in the reference isolate (AB116077)). PCR products were purified, subjected to cycle sequencing reaction with the BigDye Terminator v1.1 (Applied Biosystems, Tokyo, Japan), and applied to the DNA sequencer (3100-Avant; Applied Biosystems).

Mutations in the PreC and CP regions

The A1896 mutation in the PreC region was detected by the enzyme-linked minisequence assay (SMITEST HBV PreC ELMA; Roche Diagnostics, Tokyo, Japan), and mutations in the CP region for T1762/A1764 by the enzyme-linked specific probe assay (SMITEST HBV Core Promoter Mutation Detection Kit; Roche Diagnostics). The results were recorded as 'wild-type' and 'mutant types' dominantly expressed by HBV isolates.[25]

Phylogenetic analyses

Nucleotide sequences were aligned, and phylogenetic trees were constructed by the CLUSTAL W program v1.83 (DDBJ homepage: http://clustalw.ddbj.nig.ac.jp/top-j.html). The statistical validity was assessed by bootstrap resampling with 1000 replicates. Reference HBV strains were retrieved from the GenBank database.

Statistical analysis

Results were expressed as percentage or mean±SD. Statistical differences were evaluated by χ^2 and Fisher exact tests for categorical variables, and analysis of variance and Scheffe's test for quantitative variables, using the SPSS software. The 95% CI, for the difference in means, was calculated in analyses for quantitative variables. $p<0.05$ was considered significant.

RESULTS

Distribution of HV genotypes

HBV genotypes were determined in the 547 patients with AHB. The genotype was A in 137 (25.0%) patients (accompanied by G in one (0.2%)), B in 48 (8.8%), C in 359 (65.6%), D in one (0.2%), E in one (0.2%), and H in one (0.2%). Because HBV genotype G is a defective virus and cannot replicate by itself,[26 27] the single patient with mixed genotypes A and G was included in the 137 patients with genotype A in further analyses. RNA of hepatitis D virus was detected in three of the 453 (0.7%) patients. Anti-HIV was examined in patients at high risk of infection and detected in 14 of the 53 (26.4%) who gave consent to testing.

Demographic and clinical differences among patients infected with HBV of distinct genotypes

Demographic and clinical characteristics of patients with different genotypes are compared in table 1. There was no difference in mean age among patients with genotypes A, B and C. The proportion of men was higher in patients with genotype A than B or C (94.2% vs 79.2%, $p<0.05$; or 56.0%, $p<0.0001$), and in those with genotype B than C (79.2% vs 56.0%, $p<0.05$).

Maximum levels of total bilirubin were higher in patients with genotype A than C (9.6±7.6 vs 7.1±6.2 mg/dl, $p<0.05$), with a difference of 2.5 mg/dl (95% CI 0.93 to 4.08), whereas the highest alanine aminotransferase activity and lowest PT values did not differ among patients with distinct genotypes.

SAH developed in four (2.9%) patients with genotype A, four (8.3%) with genotype B, and 26 (7.2%) with genotype C. FH developed in one (2.1%) patient with genotype B and eight (2.2%) with genotype C; no patients with genotype A developed FH. Eight (1.5%) patients died, including one with genotype B and seven with genotype C. There were no significant differences among patients with different genotypes in the frequency of SAH or FH or mortality.

The outcome of AHB was traceable in 514 of the 547 (94.0%) patients. Chronic infection with persistence of HBsAg for >6 months developed in five of the 123 (4.1%) patients with genotype A (including the one accompanied by genotype G), none of the 46 (0%) with genotype B, and none of the 342 (0%) with genotype C; it was more common in patients with genotype A than C ($p<0.05$). HBV infection persisted exclusively in the patients with genotype A, either alone (four patients) or together with genotype G (one).

Among the five patients who acquired chronic HBV infection, four (three with genotype A and one with mixed genotypes A and G) were examined for anti-HIV, and one with genotype A was found to be positive. HBV infection persisted in three (including the one with anti-HIV) of the five patients for >1 year after the onset, and the remaining two (both without anti-HIV) cleared HBsAg from the serum after retaining it for >6 months.

Mutations in the PreC and/or CP region were detected in 3.7% (4/109) of patients with genotype A, 15.4% (6/39) of those with genotype B, and 25.5% (79/310) of those with genotype C. They were significantly less common in patients with genotype A than B or C (A vs B, $p<0.05$; A vs C, $p<0.0001$). The only patient with genotype A who had the PreC mutation was simultaneously infected with genotype G.

Routes of transmission were identifiable in 275 of the 547 (50%) patients, and the main route was heterosexual contacts; those in the remaining patients could not be disclosed. The frequency of heterosexual activity did not differ among patients with distinct genotypes. However, homosexual activity was more common in patients with genotype A than B or C (21.2%, 0% and 0.8%, respectively (A vs B, $p<0.001$; A vs C, $p<0.0001$)). Among the 32 homosexual men, HBV genotype A was detected in 29 (91%). Consent to anti-HIV testing was given by 10 of the 29 patients, and four of these (40%) were positive.

Longitudinal changes in the distribution of genotypes

Figure 2 illustrates changes in the distribution of HBV genotypes through three 6-year periods over 18 years (1991–2008). In addition, data from 2009 are shown. HBV genotype A accounted