appeared to be significantly associated with HCC development, which supported the previous reports [17–19]. These data might indicate that different HBV mutation patterns might be predictive for HCC in HBV/C1/Cs and C2/Ce-infected carriers in the context of HBeAg status.

In this cross-sectional study, however, HBV DNA level was retracted from predictive factors for HCC. One of the reasons is that HBV DNA data were available only at the time of diagnosis of HCC, when it has already decreased. A recent prospective study in Taiwan indicated that high HBV DNA levels at baseline and genotype C were independent predicting factors for HCC, but the mean viral load at the time of diagnosis of HCC was significantly lower than that at baseline [29]. Our recent cross-sectional case-control study [19] also showed that HBV DNA level was retracted from predicting factor for HCC.

In conclusion, the present multi-center cross-sectional control study indicated that subgenotype C2/Ce, T1653, V1753 and T1762/A1764 mutations in the enhancer II/core promoter are independent factors strongly associated with HCC development as well as the elder age, male sex and HBeAg positivity. The mutation patterns are associated with subgenotypes and HBeAg, suggesting clinical importance of the HBV/C subgenotyping and detection of the mutation pattern for the prediction of HCC. Further prospective studies in countries where HBV genotype C is endemic are required to confirm whether the accumulation of these mutations during the follow-up causes clinical and virological differences between HBV-infected carriers with HBV/C1/Cs and C2/Ce subgenotypes.

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Short Communication

Prevalence of hepatitis B virus infection in Japanese patients with HIV

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Patients with HIV infection are frequently infected with hepatitis viruses, which are presently the major cause of mortality in HIV-infected patients after the widespread use of highly active antiretrovirus therapy. We previously reported that approximately 20% of HIV-positive Japanese patients were also infected with hepatitis C virus (HCV). Hepatitis B virus (HBV) infection may also be an impediment to a good course of treatment for HIV-infected patients, because of recurrent liver injuries and a common effectiveness of some anti-HIV drugs on HBV replication. However, the status of co-infection with HIV and HBV in Japan is unclear. We conducted a nationwide survey to determine the prevalence of HIV–HBV co-infection by distributing a questionnaire to the hospitals belonging to the HIV/AIDS Network of Japan. Among the 5998 patients reported to be HIV positive, 377 (6.4%) were positive for the hepatitis B surface antigen. Homosexual men accounted for two-thirds (70.8%) of the HIV–HBV co-infected patients, distinct from HIV–HCV co-infection in Japan in which most of the HIV–HCV co-infected patients were recipients of blood products. One-third of HIV–HBV co-infected patients had elevated serum alanine aminotransferase levels at least once during the 1-year observation period. In conclusion, some HIV-infected Japanese patients also have HBV infection and liver disease. A detailed analysis of the progression and activity of liver disease in co-infected patients is needed.

Key words: co-infection, hepatitis B, HIV, liver disease.

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is a major public health problem worldwide, along with hepatitis C virus (HCV) and HIV infections. In the USA, the estimated prevalence of HBV is less than 1%, but approximately 1 million people are persistently infected.[1] The prevalence of HIV in the USA is also <1%, and the virus is estimated to have infected approximately 800 000 people.[2] Because of the common transmission routes, that is, parenteral transmission routes, many people with HIV infection are also infected with HBV. Among the HIV-positive people in the USA, the prevalence of HBV co-infection is 6–14%.[1,2] Before the introduction of highly active antiretroviral therapy (HAART) in 1996, most patients with HIV infection died of HIV-associated opportunistic infections, such as *Pneumocystis jiroveci* pneumonia and cytomegaloviral infection. Since the widespread use of HAART, the mortality associated with HIV infection has declined. However, the reduction in mortality due to opportunistic infection, has left patients co-infected with HIV and hepatitis viruses faced with the menace of progressive liver diseases due to HBV infection,[3,4] in addition to HCV infection.[5]

HBV co-infection or superinfection of HIV-infected patients leads to several problematic situations. First, HBV infection tends to develop into persistent infection in HIV-infected patients,[1,6,7] which is a rare event in healthy adults, although it substantially depends on the genotype of HBV.[8] It results in the acceleration of the development of cirrhosis and eventually hepatocellular carcinoma. Second, some nucleoside reverse transcriptase inhibitors (NRTI) used in HAART also have

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inhibitory effects on the replication of HBV.[9–12] A careless administration or discontinuation of NRTI on HIV–HBV co-infected patients may cause reactivation and/or aggravation of hepatitis B. In addition, the administration of anti-HBV drugs in HIV–HBV co-infection may lead to the development of drug resistance.[11,12] Third, liver injury occurs more frequently in patients on HAART who are co-infected with HIV and HBV than those infected with HIV only.[9,10]

Importantly, co-infection with HIV and HCV increases the morbidity and mortality of HIV-infected patients in Japan,[13] where the prevalence of HIV infection is increasing linearly, and is exceptionally high among developed countries.[14] There are more than 14 000 HIV-positive people in Japan as of 2006, according to the AIDS National Survey in Japan,[14] and approximately 0.8 million chronic HBV carriers.[15] However, the prevalence of co-infection with HIV and HBV in Japan has not been clarified to date. Therefore, we conducted a nationwide study by distributing a postal mail-based questionnaire to the hospitals belonging to the HIV/AIDS Network of Japan.

PATIENTS AND METHODS

IN THE QUESTIONNAIRE, the following information was obtained from the hospitals regarding the number of patients who visited the hospitals at least once between January and December in 2006: (i) the number of HIV-positive patients; (ii) the number of hepatitis B surface antigen (HBsAg)-positive patients among (i); (iii) the number of patients among (ii) who were determined at least once to have a serum alanine aminotransferase (ALT) level higher than 100 IU/L; (iv) the number of HIV-positive patients that contracted HIV from blood products; (v) the number of HBsAg-positive patients among (iv), (vi) the number of patients among (v) who were determined at least once to have a serum ALT level higher than 100 IU/L; (vii) the number of HIV-positive patients among homosexual men, (viii) the number of HBsAg-positive patients among (vii), (ix) the number of patients among (viii) who were determined at least once to have a serum ALT level higher than 100 IU/L; (x) the number of HIV-positive patients that contracted HIV through intravenous drug use (xi) the number of HBsAg-positive patients among (x), (xii) the number of patients among (xi) who had at least one determination of a serum ALT level more than 100 IU/L; (xiii) the number of HIV-positive patients whose transmission routes were classified as "others"; (xiv) the number of HBsAg-positive patients among (xiii); and (xv) the number of patients among (xiv) who were determined at least once to have a serum ALT level higher than 100 IU/L.

The questionnaire was sent to the 372 hospitals belonging to the HIV/AIDS Network of Japan by mail. Answers were mostly returned by mail and in some cases by fax. The list of the hospitals in the HIV/AIDS Network of Japan can be viewed at http://www.acc.go.jp/mLhw/mLhw_frame.htm.

RESULTS

THE QUESTIONNAIRE WAS sent to all 372 hospitals that were on the list of the hospitals in the HIV/AIDS Network of Japan in January 2006. Two hundred and seven hospitals (55.6%) responded within the indicated period. In total, 5998 patients were reported to be HIV positive. The collection rate of 55.6% was higher than that (47.8%) for a questionnaire HIV–HCV co-infection study carried out in 2003.[15] It may appear rather low, particularly considering the number of reported HIV-positive people in 2006, which was approximately 14 000, according to the AIDS National Survey in Japan.[14] However, not all of the HIV-positive people were going to hospitals, and the answers to the questionnaire were obtained from most of the major hospitals in the HIV/AIDS Network in big cities around Japan. This suggests that not all, but a majority of HIV-positive Japanese patients were enrolled in the study.

Among the 5998 patients reported to be HIV positive, 377 (6.3%) patients were positive for HBsAg (Table 1). Of these 377 patients, 122 (32.4%) had elevated serum ALT levels at least one time during the 1-year observation period.

The HBV prevalence rates, when fractionated by the routes of transmission, were as follows: among the 508 HIV-positive patients who contracted HIV from blood products, such as unheated concentrated coagulation factors, only 30 (5.9%) were HBsAg positive, which shows a marked contrast to the prevalence of HCV in this cohort (Fig. 1).[16] Among the 23 intravenous drug users, three (13.0%) were HBsAg positive. Among the 3213 HIV-positive patients who were homosexual men, 267 (8.3%) were HBsAg positive. In the remaining 2254 patients who were HIV-positive and whose route of HIV transmission was classified as "others", most contracted HIV heterosexually. This number (2254) showed a substantial increase from the 1316 obtained in the questionnaire for the HIV–HCV co-infection study in 2003, while the total number of HIV-positive patients increased from 4877 to 5998.[16] Among these, 77 (3.4%)

Table 1 Prevalence rates of hepatitis B virus infection among HIV-positive patients

Routes of transmission	No. patients	HBsAg positive (% in HIV positive according to route)	ALT >100 IU/L (% in HBsAg positive according to route)
Blood products	508 (5.9%)	30 (40.0%)	12
Homosexual men	3213 (8.3%)	267 (32.2%)	86
Drug addicts	23 (13.0%)	3 (66.7%)	2
Others (heterosexual etc.)	2254 (3.4%)	77 (28.6%)	22
Total	5998	377 (6.3%)	122 (32.4%)

ALT, serum alanine aminotransferase; HBsAg, hepatitis B surface antigen.

were HBsAg positive. In terms of the route of HIV infection, 267 (70.8%) of the 377 patients were homosexual men among the HIV–HBV co-infected patients. This shows a contrast to the status of HIV–HCV co-infection, in which the majority of HIV–HCV co-infected Japanese patients contracted both viruses from blood products.[16]

There were one or more HIV-positive patients in 154 (74.4%) of the 207 hospitals in the HIV/AIDS Network of Japan (Table 2). Twenty four (11.6%) of 207 hospitals had 20–49 HIV-positive patients, and 16 (7.7%) hospitals had 50 or more HIV-positive patients. There were one or more patients who were co-infected with HIV and HBV in 64 (30.9%) of the 207 hospitals. There were 10 or more HIV–HBV co-infected patients in nine (4.3%) hospitals, all of which had 50 or more HIV-positive patients (Table 2). HIV–HBV co-infected patients were concentrated in specific hospitals in big cities around Japan. In particular, in the Kanto area, HIV–HBV co-infected patients were concentrated in the HIV/AIDS Network hospitals in the Tokyo city area.

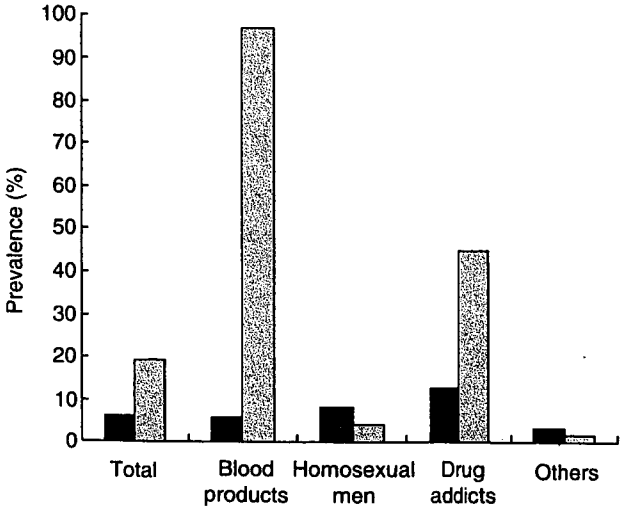

Figure 1 Prevalence rates of persistent hepatitis B virus and hepatitis C virus infections in the HIV-positive population sorted by the HIV risk group. (■), HBsAg, hepatitis B surface antigen; (▨), anti-HCV, antibody to hepatitis C virus. *Prevalence rates of anti-HCV are obtained from Koike K *et al.*[16]

DISCUSSION

ALONG WITH THE increase in the number of HIV-infected patients in Japan, co-infection with HIV and hepatitis viruses has become a major medical issue. HBV infection of HIV-positive patients raises several difficult problems: HBV infection tends to develop into persistent infection, even in adults; some NRTI used in HAART also have inhibitory effects on the replication of HBV, the improper administration, or discontinuation of which may lead to drug resistance; and HIV–HBV co-infected patients on HAART have liver injuries more frequently than HIV-monoinfected patients. It is important to determine the status of HBV infection in HIV-positive patients.

According to the statistics of the Ministry of Health, Labor, and Welfare of Japan, the number of reported HIV-positive people was slightly over 14 000 in 2006.[14] In the present study, 6.4% of HIV-positive patients were positive for HBsAg, the most reliable marker for ongoing HBV infection. It might have been advantageous if

Table 2 Number of hospitals categorized according to the number of patients infected with HIV and those co-infected with HIV and hepatitis B virus (HBV)

No. HIV (+)/ HBV (+)	No. HIV(+)				
	0	1–19	20–49	50+	Total
0	53	76	13	1	143
1–9	0	38	11	6	55
10+	0	0	0	9	9
Total	53	114	24	16	207

serum HBV-DNA levels were determined, but unfortunately, HBV-DNA level determination was not a routine laboratory test in most hospitals. In addition, considering that the antibody to the hepatitis B core antigen might be the only marker of ongoing HBV infection in some immuno-compromised patients, it would also be advantageous if this viral marker were available. These issues should be investigated in future studies. Comments from hospitals to the questionnaire included one indicating that not all HIV-positive patients underwent a test for serum HBsAg, suggesting the actual prevalence of HBsAg in HIV-infected patients might be higher than 6.4%.

In a previous questionnaire study of HIV–HCV co-infection, the prevalence of HCV infection among HIV-infected patients was 19.2%;[16] the prevalence of HBV infection (6.4%), is one-third of it. The lower positivity for HBsAg than for the anti-HCV antibody among those who contracted HIV through blood products accounts for this difference: almost all (96.9%) of the patients who contracted HIV through blood products were also anti-HCV antibody positive.[16] It should be noted that among the homosexual male patients who were HIV positive, 8.3% were HBsAg positive, which is twice as high as that of the anti-HCV antibody in these populations. A higher prevalence of HBV infection as a sexually transmitted infection than that of HCV[17] may explain the high prevalence of HBV infection in HIV-positive homosexual men. Similarly, a HBV prevalence of 3.4% in heterosexually transmitted HIV-positive patients is higher than that of the general Japanese population of the same age.[15]

Of the 377 patients who were HBsAg positive, 122 (32.4%) had elevated serum ALT levels at least once in the 1-year observation period. In this type of study using a questionnaire, it is difficult to obtain the details of patients' data, including age, body weight, and the degrees of liver injuries and fibrosis. If detailed items were included in the questionnaire, then the collection rate would be low. This time, to obtain a high collection rate, we asked whether the patients with HBsAg showed an elevated ALT level higher than 100 IU/L at least once during the 1-year observation period. We thereby do not have details on liver disease in HIV–HBV co-infected patients in the current study. Nonetheless, one-third of HIV–HBV co-infected patients have moderate liver injuries, either chronic hepatitis B or adverse effects of drugs, and are waiting for an aid for the amelioration of liver disease. A detailed analysis of the progression and activity of liver disease in HIV–HBV co-infected patients is expected.

The collection rate of the present questionnaire from the hospitals belonging to the HIV/AIDS Network was 55.6% (207 of 372). This was higher than that (47.8%) in the HIV–HCV co-infection questionnaire study carried out in 2003. The reason for this increase is not clear, but presumably the questionnaire conducted in 2003 has raised awareness among hospital staff regarding the relevance of hepatitis virus and HIV co-infection in clinical practice.

In the current study, both Japanese patients and those of other nationalities/ethnicities were included in the study. Although the ratio of newly diagnosed HIV-positive foreign people has been declining to approximately 10% in 2006, the one in total HIV positive still accounts for approximately 25% in Japan. Because the rates of the HBV carrier are different among countries, it is ideal to analyze the HBV prevalence separately according to the nationalities/ethnicities. However, in the current survey to the hospitals in HIV/AIDS Network of Japan, nationality/ethnicity was not itemized in order to make the questionnaire simple. If we would attempt to obtain such data under the approval of the ethical committee in each hospital, the response rate to questionnaire would be extremely lowered.

To establish measures that decrease the morbidity and mortality of HIV–HBV co-infected patients, it is essential to determine the current status of co-infection. In the present study, the number and transmission routes of HIV–HBV co-infected patients in Japan were determined for the first time, although detailed information on the severity and progression of liver disease in HIV–HBV co-infected patients has not been obtained yet. Undoubtedly, this will be the first step towards improving the prognosis and quality of life of Japanese patients co-infected with HIV and HBV.

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B型慢性肝炎に対するインターフェロン治療

——現況と今後の展望

Interferon therapy for chronic hepatitis B

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◎わが国でのB型慢性肝炎に対するIFN治療はHBe抗原陽性例のみが保険適用で，しかも投与期間が24週に限られている．その治療効果は若年，女性，ALT高値，HBV-DNA低値例などの好条件群では良好であるが，おしなべての成績は満足できるものではない．欧米からはPeg-IFN治療の良好な成績が多数報告されているが，わが国ではようやくPeg-IFN-α2aの治験が開始されたところである．若年例には核酸アナログ剤が使用困難で，Peg-IFN治療が大きな福音になると思われる．1日も早い保険適用が望まれる．

Key word：B型慢性肝炎，インターフェロン治療，HBステージ分類，ペグインターフェロン(Peg-IFN)

HBVキャリアは，その自然経過において80%以上がHBe抗原陰性，HBe抗体陽性，HBV-DNA低値の，いわゆる臨床的治癒の状態となる．しかし，少数ではあるが肝硬変に進展したり肝細胞癌を合併する例が存在し，B型肝細胞癌による死亡者数はこの20年間，約5,000名の状態が続いている．B型肝細胞癌発癌には，HBV増殖の多寡が密接に関係していることが明らかになり[1]，抗ウイルス薬によるHBV-DNA量の低下が発癌抑止にきわめて重要とされている．B型慢性肝炎に対する抗ウイルス薬としては，30～35歳以上の高年例には核酸アナログ剤が第一選択で，それより若年例ではインターフェロン(IFN)が用いられることが多い．

わが国でのB型慢性肝炎に対するIFN治療は1986年に1カ月投与が保険適用となり，2002年からは現行の6カ月投与が認可された．6カ月投与が可能となって，その有効性はある程度向上したが，HBe抗原陰性例は保険適用外である点など課題も多い．欧米で有用性が多く報告されているペグインターフェロン(Peg-IFN)は現在ようやく国内治験がはじまったばかりで市販までにはいまだ時間を要するが，近い将来，若年例に対する第一選択薬になると考えられる．

B型慢性肝炎治療ガイドラインとステージ分類

表1は平成18年度厚生労働科学研究“B型およびC型肝炎ウイルスの感染者に対する治療の標準化に関する臨床的研究”によるB型慢性肝炎の治療ガイドラインである．35歳未満でHBe抗原陽性例にはIFN長期間欠投与が選択されている．また35歳以上でも，HBe抗原陽性で7 log copies/ml以上の高ウイルス例にはIFN長期間欠投与も考慮することが示されている．著者らが提唱したHBキャリアのステージ分類[2](表2)においても，HBステージⅠ(HBe抗原陽性：HBV-DNA 7.6 log copies/ml以上)の若年(Ⅰa)で肝線維化F2以上の例およびHBステージⅡ(HBe抗原陽性：HBV-DNA 7.6 log copies/ml未満)の若年(Ⅱa)例にはIFNを第一選択薬にあげている．また，HBステー

表 1　B型慢性肝炎の治療ガイドライン(2007 年度版)

	HBV-DNA	≧7 log copies/ml	<7 log copies/ml
35 歳未満	HBe 抗原陽性	IFN 長期間歇	IFN 長期間歇
	HBe 抗原陰性	経過観察 進行例はエンテカビル	経過観察
35 歳以上	HBe 抗原陽性	①エンテカビル ②IFN 長期間歇	エンテカビル
	HBe 抗原陰性	エンテカビル	エンテカビル

表 2　HBVキャリアのステージ分類

HB ステージ	0	I	II	III	IV	V
HBs 抗原	+	+	+	+	+	−**
HBe 抗原	+	+	+	−	−	−
HBV-DNA (copies/ml)	不問	$10^{7.6}$≦	$10^{7.6}$>	10^{5}≦	10^{5}>	不問
ALT	持続正常	持続正常以外	持続正常以外	不問	不問	不問
年齢	不問	若年/高年(Ia/Ib)*	若年/高年(IIa/IIb)*	不問	不問	不問
発癌リスク	きわめて小	小/大	小/きわめて大	きわめて大	きわめて小	きわめて小
治療	不要	F2 以上 IFN/エンテカビル	IFN/エンテカビル	エンテカビル	不要	不要

*：若年は男性 30 歳未満，女性 35 歳未満，高年は男性 30 歳以上，女性 35 歳以上．
**：HBs 抗原(+)の時期が確認されていること．

ジIII，IVは，HBe 抗原陰性期でステージIIIは HBV-DNA 5.0 log copies/ml 以上，ステージIVは HBV-DNA 5.0 log copies/ml 未満例である．ステージIVはいわゆる臨床的治癒の状態でおおむね抗ウイルス治療の必要はないが，ステージIIIはもっとも発癌リスク(「サイドメモ」参照)の高い集団であり，速やかな治療介入が必要となる．ステージIIIで核酸アナログ剤が使用困難な場合，IFN の保険適用がないので，現在もっとも対応に苦慮することが多く，一刻も早い Peg-IFN の保険適用が望まれる．

これまでのIFN治療成績

わが国の IFN 1 カ月投与成績のまとめ[3]によると，投与終了 1 年後，2 年後の HBe 抗原陰性化率はそれぞれ 29%，55%，HBe 抗原抗体セロコンバージョン率は 12%，29%で，自然経過よりも高率であるとしている．また，1 カ月投与と 6 カ月投与の国内治療成績の集計[3]では，投与終了 6 カ月後の HBe 抗原陰性化率は 4 週投与，24 週投与でそれぞれ 11%，28%と長期投与の有効性が確認されている．1 カ月投与に対する 6 カ月投与の最大の利点は投与期間中に HBe 抗原抗体セロコンバージョンが生じる可能性が高く，投与終了後の急性増悪の出現を防止できることである．欧米でも 6 カ月投与が標準投与法であるが，Wong ら[4]の

サイドメモ

B型慢性肝炎の肝癌発癌リスク

B 型慢性肝炎例は 50 歳ぐらいまでに，約 90%が HBe(e)抗原陽性から e 抗体陽性にセロコンバージョンする．したがって，高齢者はほとんどの症例が e 抗体陽性の状態である．まだ，e 抗原陽性例が多い 50 歳前後までの肝癌発癌例は，e 抗原陽性例のほうが e 抗原陰性例に比べ有意に高率である．高齢者では e 抗原陽性例の絶対数が少なく，発癌例は e 抗体陽性が多数を占める．e 抗体陽性例の発癌は HBV-DNA 量と明らかな関連があり，4.0〜5.0 log copies/ml 以上群は高率に発癌し，それ未満ではきわめて低率であることが明らかになっている．HBV キャリア全体の肝癌発癌リスクは e 抗原持続陽性例，e 抗原陰性 HBV-DNA 高値持続群，e 抗原陰性 HBV-DNA 出没群の順に高く，e 抗原陰性 HBV-DNA 持続低値になってようやく，低リスクになると考えられる．HBV-DNA 量の定期的なモニタリングがきわめて重要である．

比較対照試験の集計でも投与終了後6カ月の時点でのHBe抗原陰性化率33%と，自然経過例12%に比べ有意に高率であったとしている．

欧米におけるPeg-IFNおよびIFN・多剤併用治療成績

Marcellinら[5]は，HBe抗原陰性例に対しPeg-IFN-α2a単独，ラミブジン(LAM)単独およびPeg-IFN-α2a・LAM併用群の無作為比較試験(RCT)を行い，Peg-IFN-α2a単独，Peg-IFN-α2a・LAM併用群はLAM単独群に比べ，有意にHBV-DNA抑制率が高率であったと報告している．また，Lauら[6]はHBe抗原陽性例に対して同様の検討を行い，Peg-IFN-α2a群はLAM単独群に比べ有意にHBe抗原陰性化率，HBV-DNA抑制率およびHBs抗原陰性化率が高率で，Peg-IFN-α2aの有用性を認めている．

Peg-IFN-α2bに関しても良好な報告がなされている．すなわち，Janssenら[7]はHBe抗原陽性例に対してPeg-IFN-α2b単独群とPeg-IFN-α2b・LAM併用群とのRCTを行い，Peg-IFN-α2b単独群において投与終了後26週後のHBe抗原陰性化率が36%と良好であり，これは併用群35%に劣っていないことを報告した．また，Chanら[8]はPeg-IFN-α2b・LAM併用群とLAM単独群とのRCTにて併用群が単独群に比べ，良好なvirological responseが得られ，変異株出現も低率であったとしている．さらに投与3年後においても，併用群は単独群に比べvirological responseを維持すると報告した[9]．Flinkら[10]は，標準的なIFNやLAMによる治療が無効であったHBe抗原陽性例に対してPeg-IFN-α2b単独治療を行い，約1/3の症例で投与終了後26週の時点でHBe抗原の消失を認めている．一方，YMDD変異株出現例に対するPeg-IFN-α2bの有効性については明らかな見解は示されていない[11]．アデフォビルとの併用についてはWursthornら[12]がPeg-IFN-α2bとアデフォビルとの併用によって，HBs抗原の減少を伴う著明なHBV-DNA量と肝内cccDNA量の低下を認めたと報告している．

IFN治療の適応と今後の展望

1986年より母児感染予防事業が施行されて，わが国のHBVキャリア率は激減した．しかし，20歳以上のキャリアはいまだ多数存在し，自然経過で臨床的治癒の状態に至らずに，肝硬変，肝細胞癌に進行する例も存在することはさきに述べた．これらの症例のうち，本人あるいはパートナーが妊娠，出産を望んでいる場合は男女の差なく核酸アナログ剤は使用できない．IFN治療の適応はまさにこれらの症例であり，30〜35歳までの若年の間の，HBe抗原からHBe抗体へのセロコンバージョンとHBV-DNA増殖の沈静化が目的となる．これまでの国内外での検討より，効果が期待できる治療前因子として女性，HBV-RNA量低値，投与前ALT高値および組織診断でactivityの高い症例があげられている．とくに，20歳代の女性の治療効果はきわめて良好であるが，女性は妊娠・出産を機に病態が安定化することが多く，IFNを含む抗ウイルス治療介入には慎重を期する必要がある．一方，男性では若年発癌も含めて，B型肝細胞癌発癌のリスクは有意に高く，若年でも肝線維化F2以上では積極的にIFN治療を導入する必要があると考えている．

現在，HBe抗原陽性例のみが保険適用であるが，6カ月の長期投与でも十分満足できる成績は得られていない．IFN製剤の投与期間制限がC型慢性肝炎と同様に撤廃されれば，IFN治療の有用性は大きく向上すると考えられるが，現在その動きはない．Peg-IFNの効果は前述のとおり良好であり，現在，Peg-IFN-α2aの治験が開始されたところであるが，HBe抗原陰性例や48週までの投与期間がデザインされており，より早期の認可が強く望まれる．

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Comparison of complete sequences of hepatitis B virus genotype C between inactive carriers and hepatocellular carcinoma patients before and after seroconversion

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Background. Most patients who acquire chronic hepatitis B virus (HBV) infection by perinatal transmission become inactive carriers (IC) after hepatitis B e (HBe) antigen seroconversion, whereas some patients have persistent abnormal serum transaminase levels and develop hepatocellular carcinoma (HCC) in the anti-HBe-positive phase. The aim of this study was to investigate the HCC-related mutations of HBV. ***Methods.*** Complete sequences of HBV were examined among eight IC and eight HCC patients infected with HBV genotype C before and after seroconversion. ***Results.*** The frequency of the T1653 mutation tended to be higher among HCC patients after seroconversion (16.7% vs. 62.5%; $P = 0.086$). The prevalence of a basal core promoter double mutation (T1762/A1764) was high among both IC and HCC patients after seroconversion (83.3% vs. 87.5%; $P = 0.825$). Among the HCC patients, a pre-S deletion mutant was detected in 62.5% patients before seroconversion, and in 37.5% patients after seroconversion. The core deletion mutant was also detected in 50% of HCC patients only before seroconversion. Deletion mutants of the pre-S or core region before seroconversion were significantly associated with HCC patients (0% vs. 62.5%; $P = 0.007$, 0% vs. 50%; $P = 0.021$, respectively). ***Conclusions.*** Our data showed a significant association of pre-S and core deletion mutants before seroconversion with HCC development. The T1653 mutation after seroconversion was frequently found in HCC patients infected with HBV genotype C. These results suggest that mutations may be predictive factor for development of HCC.

Key words: hepatocellular carcinoma, core deletion, pre-S deletion mutant, T1653 mutation

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most frequent cancer and the third leading cause of cancer-related death in the world, with an estimated annual prevalence of >500000 cases worldwide.[1] It is now accepted that HBV infection has hepatocarcinogenic potential in humans. Several mutations in the HBV genome have been reported to occur during the course of persistent viral infection, and there is increasing evidence of an association between these molecular alterations and the development of end-stage liver disease in patients with HBV infection.[2–6] Nevertheless, it is still unclear whether a specific mutation or a specific combination of mutations is associated with the development of severe disease, because previous studies focused on only a few mutations such as pre-S deletion, basal core promoter (BCP) double mutation, and precore (PC) mutation. Recently, several lines of evidence have indicated that complex HBV variants with deletions in the pre-S or core region and mutations in the enhancer II region are associated with end-stage liver disease.[7–9] Both the pre-S and core regions play an essential role in the interaction with immune responses because they contain B- and T-cell epitopes.[10–12] Pre-S and core deletion mutants with altered epitopes may survive despite the host immune system.

During persistent HBV infection, carriers frequently undergo seroconversion from hepatitis B e antigen (HBeAg) to the corresponding antibody (anti-HBe). Most patients who acquire chronic HBV infection with HBV genotype C (which is the common genotype in East Asian countries) by perinatal transmission become inactive carriers (IC) after seroconversion. A subgroup of patients have persistent abnormal serum transaminase levels and develop HCC in the anti-HBe-positive phase. Because most previous studies examined only a serum sample collected at one time point in each patient,

that is, they were cross-sectional studies, the association between different clinical events after seroconversion and specific HBV genomic mutations has not been clearly defined. To investigate this issue, complete HBV sequences were examined in eight IC and eight HCC patients before and after HBeAg seroconversion.

Materials and methods

Serum samples

Serum samples were obtained from 16 patients (eight patients were IC and the other eight were HCC patients) at the Nagoya City University Graduate School of Medical Sciences and National Hospital Organization Osaka National Hospital before and after seroconversion. Sixteen patients were infected with HBV genotype C. IC were defined as individuals who were hepatitis B surface antigen (HBsAg) positive with normal alanine aminotransferase (ALT) and α-fetoprotein levels over a 5-year period (with at least 12 evaluations at 3-month intervals) and without the presence of portal hypertension. HCC patients were diagnosed on the basis of results of abdominal ultrasonography, angiography, computed tomography, magnetic resonance imaging, or liver biopsy as well as by their having an elevated serum α-fetoprotein level (>400 ng/ml).

HBV Genotyping

HBV genotypes were determined by the restriction fragment length polymorphism method from the *S* gene sequence amplified by polymerase chain reaction (PCR)[13] or enzyme immunoassay (EIA) with monoclonal antibodies for distinct epitopes in the pre-S2 region products,[14] with commercial kits (HBV genotype EIA; Institute of Immunology, Tokyo, Japan). The genotypes were also confirmed by a phylogenetic tree analysis.

HBV DNA extraction

Serum samples were stored at −80°C until the assay. DNA was extracted from 100 μl of serum by using QIAamp DNA blood kits (Qiagen, Hilden, Germany).

Determination of the complete nucleotide sequences of HBV/C

The complete nucleotide sequences of 30 HBV/C isolates from 16 patients (HBV DNA in two serum samples from IC after seroconversion could not be amplified) were determined by a method reported previously[15] with a slight modification. In brief, two overlapping fragments of HBV genome were amplified by PCR, and eight overlapping HBV DNA fragments were amplified further by PCR with nested primers. Amplification was performed in a 96-well cycler (GeneAMP9600; Perkin-Elmer Cetus, Norwalk, CA, USA), and the PCR products were electrophoresed in 3.0% (wt/vol) agarose, stained with ethidium bromide, and observed under UV light.[3] Standard precautions were taken to avoid contamination during PCR. A negative control serum was also processed and included in each run to ensure specificity. Twelve overlapping HBV DNA fragments thus amplified were sequenced directly with a Prism BigDye kit (Applied Biosystems, Foster City, CA) in an ABI 3100 DNA automated sequencer.

Statistical analysis

Statistical analyses were performed with χ-squared and Fisher's exact tests for categorical variables. The Mann-Whitney *U* test was used for continuous variables, as appropriate. Differences were considered to be significant with P values <0.05. The statistical analysis software used was Stata software, version 8.0 (Statacorp LP, College Station, TX, USA).

Results

Table 1 compares age, ALT level, platelet count, HBV DNA, and rate of cirrhosis before and after HBeAg seroconversion, as well as age at seroconversion and the intervals between two sampling points for all patients. ALT level, platelet count, HBV DNA, and rate of cirrhosis after seroconversion were significantly higher among HCC patients than in IC.

The alignment of sequences covering the enhancer II and core promoter regions is shown in Fig. 1. We could not amplify HBV DNA in two serum samples from IC because of the small amount of HBV DNA in the samples. The box alpha and basal core promoter contained mutational hot spots, but box beta did not. The frequency of the T1653 mutation tended to be higher among HCC patients after seroconversion [IC vs. HCC: 1/6 (16.7%) vs. 5/8 (62.5%); $P = 0.086$] (Fig. 1 and Table 2), whereas the T1653 mutation did not differ between the two groups before seroconversion [IC vs. HCC: 1/8 (12.5%) vs. 2/8 (25%); $P = 0.522$]. The prevalence of the BCP double mutation was high among both IC and HCC patients after seroconversion [IC vs. HCC: 5/6 (83.3%) vs. 7/8 (87.5%); $P = 0.825$]. The prevalence of S1753 was low among IC and HCC patients before and after seroconversion (Fig. 1 and Table 2). The S1753 mutant was not recognized in the patients who were infected with the T1653 mutation clone. Deletion mutants of the core or pre-S region before seroconversion were significantly associated with HCC patients

Table 1. Comparison of clinical characteristics between IC and HCC patients before and after HBeAg seroconversion

Features	Before seroconversion			After seroconversion		
	Inactive carriers ($n = 8$)	HCC patients ($n = 8$)	Differences P value	Inactive carriers ($n = 8$)	HCC patients ($n = 8$)	Differences P value
Male, n (%)				4 (50)	7 (87.5)	0.106
Age (years)[a]	31.8 ± 8.4	40.0 ± 10.6	0.246	43.6 ± 10.0	51.6 ± 13.8	0.317
ALT (U/L)[a]	199.6 ± 220.5	234.6 ± 242.2	0.875	20.3 ± 7.2	40.4 ± 16.9	0.009*
Platelet count ($\times 10^4/mm^3$)[a]	17.5 ± 2.0	15.1 ± 4.3	0.268	17.5 ± 3.3	11.5 ± 5.4	0.027*
HBV DNA (LGE/ml)[a]	7.2 ± 0.5	7.2 ± 0.5	0.869	4.3 ± 0.7	5.7 ± 1.2	0.022*
Cirrhosis (%)	0 (0)	2 (25)	0.131	0 (0)	5 (62.5)	0.007*
Age at seroconversion (years)				37.8 ± 8.1	47.3 ± 14.2	0.226
Intervals between two sampling points (years)				11.8 ± 2.9	11.6 ± 4.4	1.0

IC, inactive carriers; HCC, hepatocellular carcinoma; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; HBV, hepatitis B virus; LGE, log genome equivalents

*Statistically significant

[a]Mean ± SD

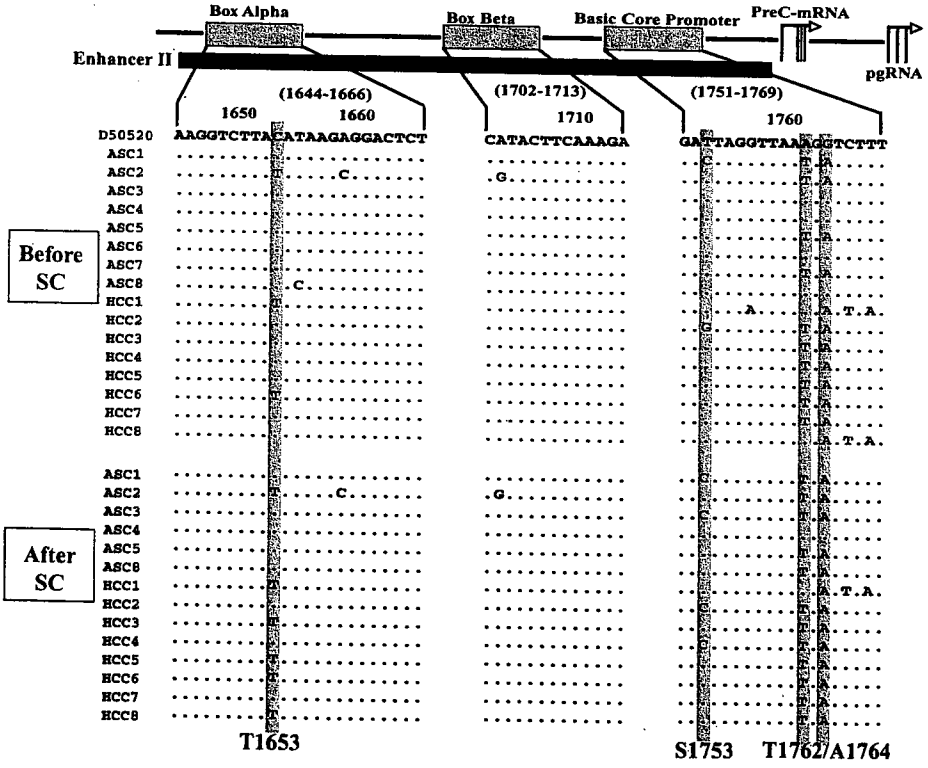

Fig. 1. Nucleotide sequences that cover enhancer II and core promoter regions in inactive carriers (IC) and hepatocellular carcinoma (HCC) patients. The wild-type sequence for genotype C is represented by D50520. *Dots* indicate nucleotides identical to the wild type. *SC*, seroconversion

(core deletion mutant: 0/8 [0%] vs. 4/8 [50%]; $P = 0.021$, pre-S deletion mutant 0/8 [0%] vs. 5/8 [62.5%]; $P = 0.007$, respectively) (Table 2).

The pre-S deletion mutant was detected in only one IC as a minor clone (Fig. 2), whereas among HCC patients, deletion mutants (major clones) were detected in five of eight (62.5%) patients before seroconversion, and in three of eight (37.5%) after seroconversion. Only two patients (patients 4 and 5) had pre-S deletion mutants before and after seroconversion. Two HCC patients (patients 1 and 2) had pre-S deletion mutants only before seroconversion (Fig. 3). Three HCC patients (patients 4–6) before seroconversion and one patient (No. 7) after seroconversion were coinfected with wild-type virus and pre-S deletion mutants (the deletion mutants were the major clones). Most deletions were identified in the 3′ terminus of the pre-S1 region and the 5′ terminus of the pre-S2 region.

Table 2. Comparison of HBV mutations between IC and HCC patients before and after HBeAg seroconversion

Features	Before seroconversion			After seroconversion		
	Inactive carriers ($n = 8$) n (%)	HCC patients ($n = 8$) n (%)	Differences P Value	Inactive carriers ($n = 8$) n (%)	HCC patients ($n = 8$) n (%)	Differences P Value
T1653 mutation	1 (12.5)	2 (25)	0.522	1/6 (16.7)	5/8 (62.5)	0.086
S1753 mutation	1 (12.5)	1 (12.5)	1.0	2/6 (33.3)	2/8 (25)	0.730
A1896 mutation	3 (37.5)	0 (0)	0.055	4/6 (66.7)	3/8 (37.5)	0.280
BCP double mutation	4 (50)	7 (87.5)	0.106	4/6 (66.7)	7/8 (87.5)	0.325
Core deletion Mutant	0 (0)	4 (50)	0.021*	0/6 (0)	0/8 (0)	1.0
Pre-S deletion Mutant	0 (0)	5 (62.5)	0.007*	1/8 (12.5)	3/8 (37.5)	0.248

BCP, basal core promoter
* Statistically significant

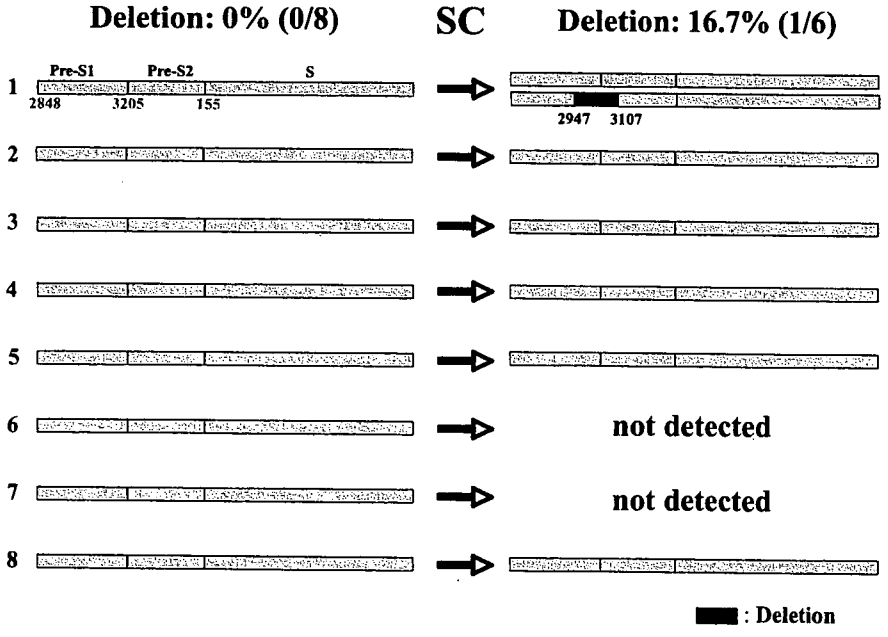

Fig. 2. Pre-S region deletion mutant in IC. The nucleotide sequences of the pre-S1, preS-2 and S are shown as bars. *Shading* of a bar indicates a deletion region. A pre-S deletion mutant was detected in only one IC after seroconversion as a minor clone (the *lower bar* shows the minor clone)

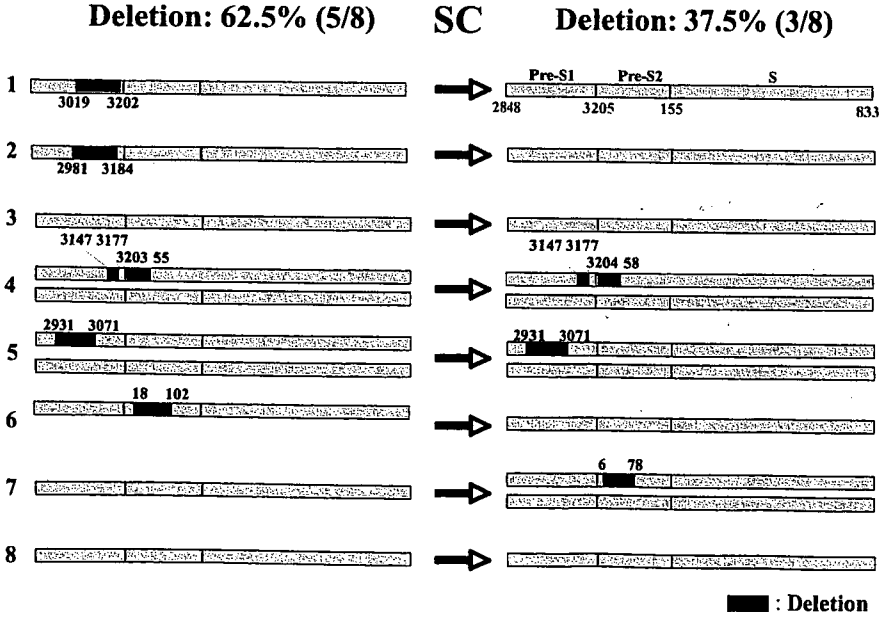

Fig. 3. The pre-S region deletion mutant in HCC patients. Pre-S deletion mutants were identified in five of eight (62.5%) HCC patients before seroconversion as a major clone (*upper bars* show major clones). Three of five deletion mutants before seroconversion were undetectable after seroconversion. Deletions were often in the C terminus of the pre-S1 domain and in the N terminus of the pre-S2 domain

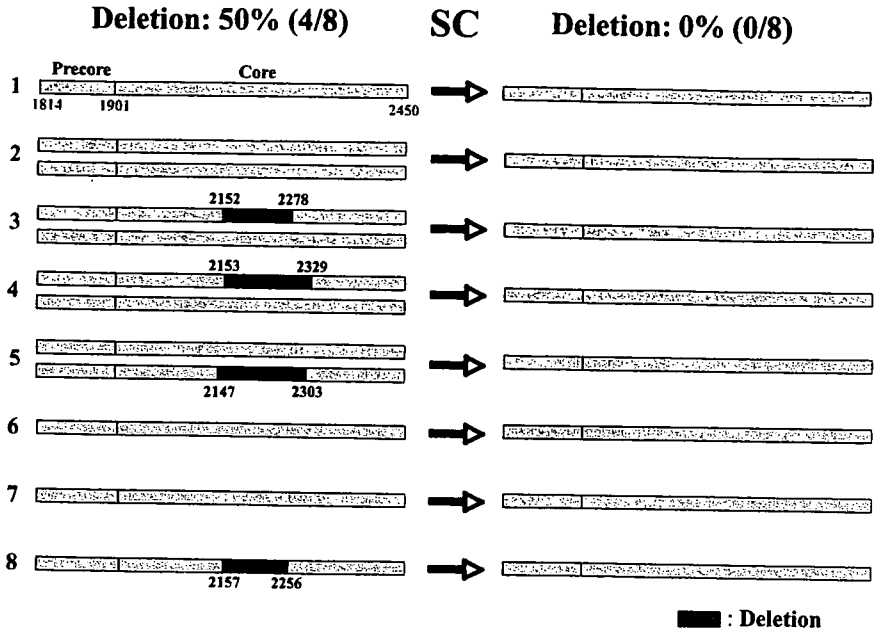

Fig. 4. Precore core region deletion mutant in HCC patients. The nucleotide sequences of precore and core are shown as bars. Core deletion mutants were identified in four of eight (50%) patients, only before seroconversion. All core deletion mutants around the center of the core domain were undetectable after seroconversion

Table 3. Clinical and virological characteristics among IC with HBV genotype C

Patient	Sex	Status of SC	Age (Years)	T1653 mutation	S1753 mutation	A1896 mutation	BCP double mutation	Core deletion mutant	Pre-S deletion mutant
1	M	Before	36	(−)	(+)	(−)	(+)	(−)	(−)
		After	45	(−)	(+)	(+)	(+)	(−)	(+)
2	M	Before	41	(+)	(−)	(+)	(+)	(−)	(−)
		After	55	(+)	(−)	(+)	(+)	(−)	(−)
3	M	Before	31	(−)	(−)	(+)	(−)	(−)	(−)
		After	46	(−)	(+)	(+)	(+)	(−)	(−)
4	M	Before	24	(−)	(−)	(−)	(−)	(−)	(−)
		After	34	(−)	(−)	(−)	(−)	(−)	(−)
5	M	Before	28	(−)	(−)	(+)	(+)	(−)	(−)
		After	35	(−)	(−)	(+)	(+)	(−)	(−)
6	M	Before	41	(−)	(−)	(−)	(−)	(−)	(−)
		After	56	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
7	M	Before	36	(−)	(−)	(−)	(+)	(−)	(−)
		After	49	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
8	F	Before	17		(−)	(−)	(−)	(−)	(−)
		After	29		(−)	(−)	(−)	(−)	(−)

SC, seroconversion

Figure 4 shows the deletion mutants in the precore/core region among HCC patients. Core deletion mutants were detected in four patients with HCC only before seroconversion. Core deletion mutants were identified around the center of the core region in these four patients. Three HCC patients (patients 3–5) before seroconversion were coinfected with wild-type virus and core deletion mutants. One HCC patient (patient 8) before seroconversion was infected with only the core deletion mutant.

Table 3 summarizes the virological characteristics of IC. We could not amplify HBV DNA in two serum samples of IC after seroconversion. No IC was infected with the core deletion mutant. The pre-S deletion mutant was identified in only one IC after seroconversion. BCP double mutations and A1896 mutations were identified in four of six (66.7%) IC after seroconversion. Table 4 shows the virological characteristics of HCC patients. The T1653 mutation was negatively correlated with the S1753 mutation in this population. The prevalence of T1762/A1764 was high in HCC patients. Of interest, a core or pre-S deletion mutant was detected in seven of eight (87.5%) HCC patients before seroconversion.

Table 4. Clinical and virological characteristics of HCC patients with HBV genotype C

Patient	Sex	Status of SC	Age (years)	T1653 mutation	S1753 mutation	A1896 mutation	BCP double mutation	Core deletion mutant	Pre-S deletion mutant
1	M	Before	40	(+)	(−)	(−)	(−)	(−)	(+)
		After	56	(+)	(−)	(+)	(−)	(−)	(−)
2	M	Before	31	(−)	(+)	(−)	(+)	(−)	(+)
		After	38	(−)	(+)	(+)	(+)	(−)	(−)
3	M	Before	38	(−)	(−)	(−)	(+)	(+)	(−)
		After	54	(+)	(−)	(+)	(+)	(−)	(−)
4	M	Before	55	(−)	(−)	(−)	(+)	(+)	(+)
		After	72	(−)	(+)	(−)	(+)	(−)	(+)
5	M	Before	28	(−)	(−)	(−)	(+)	(+)	(+)
		After	34	(+)	(−)	(−)	(+)	(−)	(+)
6	M	Before	36	(+)	(−)	(−)	(+)	(−)	(+)
		After	44	(+)	(−)	(−)	(+)	(−)	(−)
7	M	Before	35	(−)	(−)	(−)	(+)	(−)	(−)
		After	46	(−)	(−)	(−)	(+)	(−)	(+)
8	F	Before	57	(−)	(−)	(−)	(−)	(+)	(−)
		After	69	(+)	(−)	(−)	(+)	(−)	(−)

Discussion

Many previous studies have reported that the clinical course of chronic HBV infection may be modified by several specific viral mutations,[6,16–19] but most studies examined only serum samples collected from each patient at one time point. In this study, we compared viral mutations in IC and HCC patients before and after HBeAg seroconversion. ALT, HBV DNA levels, and rate of cirrhosis were significantly higher among the HCC patients than among IC only after seroconversion. Platelet count was lower among HCC patients than among IC only after seroconversion. Interestingly, even though clinical characteristics did not differ before seroconversion, deletion mutants of the core or pre-S region were significantly more associated with HCC patients than with IC. Core deletion mutants detected before seroconversion become undetectable in serum samples derived from the same patients after seroconversion. As well, pre-S deletion mutants were undetectable in three patients after seroconversion. However, the core and pre-S deletions being undetectable after seroconversion by direct sequencing does not exclude the possibility that they remained as minor clones. Preikschat et al.,[9] who sequenced cloned HBV genomes, reported the existence of deletion mutants as only minor clones; deletions and insertions in core promoter/enhancer II regions, deletions in the *C* gene, or deletions in the pre-S region were distributed on individual genomes in various combinations. Although it is unclear why major deletion mutants decreased after seroconversion, both core and pre-S deletion mutants may be predictive factors for HCC at an early stage in chronic HBV carriers.

Recently, Chen et al.[7] reported that combinations of HBV mutations (deletion in the pre-S region and/or mutations in the BCP and/or PC regions) were strongly associated with liver disease progression; a combination of mutations rather than a single mutation was associated with the development of progressive liver diseases, and pre-S deletion and BCP mutations in particular were significantly associated with the development of progressive liver diseases. In the present study, BCP mutation was identified frequently in HCC patients but also frequently in IC. The combination of a pre-S deletion with other mutants was not significantly associated with the development of HCC, owing to the small sample size in this study.

In our previous case–control study, a BCP double mutation was frequently found in each clinical group (40 IC, 40 chronic hepatitis, and 40 HCC patients), but the frequency of the T1653 mutation was significantly higher among HCC patients than among IC.[20] In this study, the T1653 mutation was identified in five of eight (62.5%) HCC patients after seroconversion and in only one of six (16.7%) IC after seroconversion, suggesting that the T1653 mutation is one of the factors promoting HCC development. However, the combination of pre-S or core deletion mutants with the T1653 mutation was not significantly associated with HCC development.

Both pre-S and core regions play an essential role in the immune response interaction because they contain B- and T-cell epitopes. Pre-S deletion and core deletion mutants thus allow escape from the host's immune function. In this study, most pre-S and core deletion regions in the HCC group encompassed B- and T-cell epitopes: most of the pre-S deletions in the 3′-terminus of the pre-S1 region and the 5′-terminus of the pre-S2 region

in HCC patients, and all of the core deletions around the center of the core region in HCC patients. These pre-S and core deletion sites including B- and T-cell epitopes[21–25] were consistent with those reported in previous papers describing patients infected with pre-S and core deletion mutants.[7,26–28]

Previous studies have shown that pre-S deletion mutants tend to accumulate at the later stage of HBV infection[3,4,29,30] and have demonstrated a marked decrease in the synthesis and secretion of small surface protein leading to retention of envelope protein and viral particles within hepatocytes, resulting in the ground-glass appearance of hepatocytes.[4] Recently, Hsieh et al.[31] identified two types of ground-glass hepatocytes containing two types of mutant L proteins with deletion within the pre-S1 and pre-S2 regions, respectively. They found that these pre-S deletion mutants accumulate in the endoplasmic reticulum (ER), resulting in strong ER stress. They concluded that the pre-S mutation causes ground-glass hepatocytes to induce oxidative DNA damage and mutations in hepatocytes in the late stages of HBV infection.

Yuan et al.[28] described the characteristics of a core deletion mutant: (1) Deletion often occurs within core amino acids 80 to 120. It does not usually extend into the partially overlapping polymerase. (2) The exact end points and sizes of deletions vary from variant to variant. (3) Deletions appear to be more often in frame than out of frame. (4) Internal deletions coincide with a potent T-cell epitope, suggesting an immune system escape function for this mutation. In the present study, the features of the core deletion mutant were mostly consistent with these characteristics.

In conclusion, our data showed a significant association of pre-S deletion and core deletion mutants before seroconversion with HCC development. The T1653 mutation after seroconversion was frequently found in HCC patients infected with HBV genotype C. These results suggest that these mutations may be a predictive factor for HCC development.

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HCV RNA定量キット コバスTaqMan HCV「オート」の検討

—TaqMan HCV「オート」に関する共同研究：中間報告—

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要　旨

リアルタイムPCR法の一種であるTaqMan PCR法を増幅・検出原理とするHCV RNA定量キット，コバスTaqMan HCV「オート」(以下，TaqMan HCV）に関する検討を行った。その結果，TaqMan HCVは，既存HCV RNA定量測定法と良好な相関を示し，治療ガイドラインで低/高ウイルス量の境界となる5.0 Log IU/mL［100 K IU/mL］付近でほぼ1対1の相関を示したことにより，TaqMan HCVも同一の基準で判断してよいと考えられた。また，治療中のウイルス量推移パターンも既存HCV RNA定量測定法と近似する挙動を示し，さらに長期間の観察が可能であったことが確認され，治療中の経過観察に応用できると考えられた。一方，既存HCV RNA定性法と比較したところ，TaqMan HCVのほうが高感度であることが示され，TaqMan HCVで測定を行うことにより，再燃例を低減することが期待できると考えられた。

また，リバビリン併用PEG-IFNα2b療法における治療効果予測についてTaqMan HCVを用いて検討した結果，HCV dynamicsの解析が有効であることが示唆された。

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Evaluation of HCV RNA measurement kit COBAS® TaqMan® HCV「AUTO」—Cooperative study : Interium report

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Key words：HCV RNA，アンプリコア，TaqMan，リアルタイムPCR