**Table 2** Clinical characteristics of the patients with age- and sex-matched controls

| Parameters | Seroconversion (+) | Seroconversion (−) | *P* |
|---|---|---|---|
| Patient numbers | 21 | 18 | |
| Gender (male/female) | 8/13 | 11/7 | n.s. |
| Median Age (years) | 30.0 (24–55) | 31.0 (18–46) | n.s. |
| HBV Genotype (A/B/C) | 1/3/17 | 0/0/26 | n.s. |
| HBV DNA level (average ± SD) (log copies/mL) | 7.4 ± 0.6 | 7.4 ± 0.5 | n.s. |
| HBsAg level (average ± SD) (log IU/mL) | 4.70 ± 4.79 | 4.70 ± 4.54 | n.s. |

HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

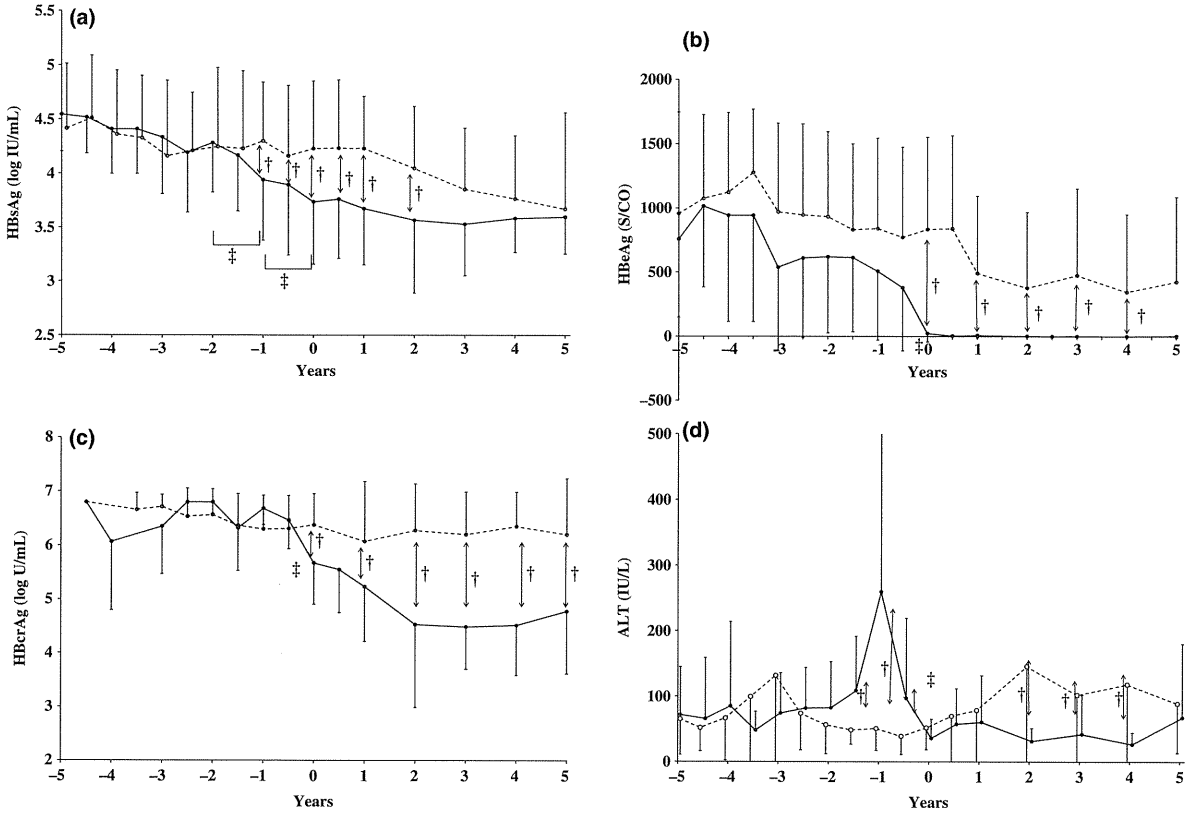


**Fig. 4** Comparison of serial changes in (a) HBsAg, (b) HBeAg, (c) HBcrAg level and (d) ALT during 10 years around HBeAg seroconversion with those of age-and sex-matched controls without seroconversion. The group with HBeAg seroconversion ($n$ = 21) is shown with closed circles and that without seroconversion ($n$ = 18) with open circles. The time point of seroconversion was designated as 0 year. (a) There was no significant difference between the two groups until 2 years before seroconversion, but 1 year before, on and after HBeAg seroconversion, there were significant differences between the two groups (†$P < 0.05$, unpaired $t$-test). In the patients with seroconversion, HBsAg showed a significant decrease 2 years before seroconversion (‡$P < 0.05$, paired $t$-test). (b) The HBeAg level differed significantly between the two groups after HBeAg seroconversion (†$P < 0.05$, unpaired $t$-test, ‡$P < 0.05$, paired $t$-test). (c) The core-related antigen of HBV (HBcrAg) level showed a significant difference between the two groups after seroconversion (†$P < 0.05$, unpaired $t$-test, ‡$P < 0.05$, paired $t$-test). (d) The level of ALT in the patients with HBeAg seroconversion showed a significant increase half a year before HBeAg seroconversion. The ALT level differed significantly between the two groups before and after HBeAg seroconversion (†$P < 0.05$, unpaired $t$-test, ‡$P < 0.05$, paired $t$-test). HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen.

DNA level at baseline showed a strong relation with tumour development, as we have reported previously [22], although HBsAg seroclearance at age <50 years was reported to be associated with a lower risk for the development of HCC [23]; that is, there was a difference in clinical outcome between the low level of HBsAg and its seroclearance. Therefore, the final goal of therapy for HBV carriers might be set as HBsAg seroclearance.

It is well known that seroconversion of HBeAg to its antibody is associated with a decrease in serum HBV DNA to low or undetectable levels, clinical remission and an improvement in hepatic inflammatory activity. Some studies reported that high pretreatment ALT levels and low serum HBV DNA levels were independently associated with an increased rate of seroconversion after treatment with either IFN [24–26] or nucleoside/nucleotide analogues (NA) [27]. Further analysis also showed that factors such as viral genotype [28], quantitative HBeAg [29] and active histological disease [30] also may be important predictors of seroconversion after treatment with IFN or NA [31]. The quantitative monitoring of HBsAg titre also has been suggested as a predictor of treatment response, especially for IFN-based therapies in chronic HBV infection [32]. In our study, HBsAg decreased significantly, compared with a group without seroconversion, for 2 years prior to seroconversion. In the age-and sex-controlled study, we analyzed the relationship between the level of HBsAg at 140 time points and the occurrence within 2 years of HBeAg seroconversion. In 10 of 19 patients (52.6%), when the level of HBsAg showed more than 50% decrease compared with the previous year, HBeAg seroconversion occurred within 2 years, which differed significantly (chi-square test, $P = 0.003$). Thus, this study suggested that quantitative measurement of the HBsAg titre might clinically be useful and that it becomes possible to build a treatment strategy by predicting whether seroconversion will occur. The reason why the level of HBsAg decreased before HBeAg seroconversion with preceding the decrease of HBeAg titre or HBcrAg level remains unclear. The current findings speculated us that it was most likely due to the integration of HBV into the host genome that potentially provides a separate template for the production of HBsAg or the cytokine-dependent modification of viral replication pathways [21].

In a recent report, there was no obvious decline in HBsAg at the time of HBeAg seroconversion, compared with the decline of HBV DNA, from the evaluation of HBsAg only at two points before seroconversion [12]. By the evaluation of serial changes in HBsAg levels before seroconversion, in addition to the difference of HBV genotype or race, our study might show differences from that finding.

HBV covalently closed circular DNA (cccDNA) is important for virus replication and impacts on clinical outcome [33], and HBsAg has been evaluated recently as a surrogate marker of cccDNA [34,35]. Because liver biopsy was not a routine procedure, we did not measure cccDNA directly in liver. Further studies are required to clarify the precise significance of HBsAg levels because a direct association between HBV cccDNA levels in liver and HBsAg levels in serum remains to be shown.

In conclusion, the titre of HBsAg is a new marker related to HBV replication and its serial measurement possibly may be a predictive factor for HBeAg seroconversion.

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*Scandinavian Journal of Gastroenterology*, 2011; 46: 1111–1117

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ORIGINAL ARTICLE

# Emergence of entecavir-resistant mutations in nucleos(t)ide-naive Japanese patients infected with hepatitis B virus: Virological breakthrough is also dependent on adherence to medication

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**Abstract**

***Objective.*** Currently, five nucleos(t)ide analogues (NUCs) are available for the treatment of chronic hepatitis B in the world. We examined the prevalence of hepatitis B virus (HBV) DNA and alanine aminotransferase normalization in patients receiving entecavir (ETV) and the frequency of ETV-resistant mutations during an approximately 27-month use of ETV in chronic hepatitis B patients in an urban hospital in Japan. ***Materials and methods.*** A retrospective analysis of 81 NUC-naive chronic hepatitis B patients who received 0.5 mg of ETV daily was performed. HBV DNA was measured and sequence analysis of HBV DNA was performed in virological breakthrough patients. ***Results.*** Hepatitis B e antigen (HBeAg)-positive patients with HBV DNA 5.0–7.0 log IU/mL group and all HBeAg-negative patients achieved serum HBV DNA negativity by 12 months. Four patients experienced virological breakthrough during ETV therapy. Two patients had no genotypic mutations, and medical interviews revealed that they had poor adherence to ETV. ***Conclusions.*** We found that some of the HBV virological breakthroughs during ETV treatment were related to poor adherence to medication, highlighting that clinicians should pay attention to the emergence of resistant mutants as well as adherence to ETV.

**Key Words:** *Adherence, entecavir, HBV, resistant mutants, virological breakthrough*

## Introduction

Two billion people have been exposed to hepatitis B virus (HBV), and 350–400 million people remain chronically infected worldwide. In Japan, the prevalence of HBV carriers is estimated at ~1% of the population, but HBV is one of the major health issues because it leads to acute hepatitis, chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [1–5].

Recent studies have shown that the serum HBV DNA level is one of the most potent risk factors for the development of cirrhosis or HCC, and it seems that suppressing the serum HBV viral load is essential for improving the prognosis of HBV carriers [6,7]. Currently, there are five approved nucleos(t)ide analogues (NUCs) for the treatment of chronic hepatitis B [8]. At present, the Japanese national health insurance system approves entecavir (ETV) as the first-line therapy for chronic hepatitis B, although some patients are treated with standard interferon-alfa. ETV is an NUC belonging to a new subgroup, cyclopentane [9], and has been shown to be highly effective in suppressing HBV replication to an undetectable level and normalizing alanine aminotransferase (ALT), although NUCs do not eradicate the virus. Most patients therefore require long durations of treatment, but prolonged treatment is associated with increasing rates of drug resistance. There was

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also a report that the 3-year cumulative probability of resistance was 1.7% for 0.5 mg/day ETV therapy in NUC-naive Japanese patients [10].

In the present study, we examined the prevalence of HBV DNA and ALT normalization in patients receiving ETV as well as the frequency of ETV-resistant mutations during an approximately 27-month use of ETV in chronic hepatitis B patients in an urban hospital in Japan. We found a relationship between some of the HBV virological breakthroughs during ETV treatment with poor adherence to medication, and clinicians need to focus on the possible emergence of resistant mutants as well as the adherence to ETV.

## Patients and methods

### *Patients*

A retrospective analysis of NUC-naive chronic hepatitis B patients ($n = 81$) receiving 0.5 mg of ETV daily at Chiba University Hospital between May 2003 and December 2009 was performed. The patients were divided into three groups based on their HBV DNA level just before starting ETV according to the Japanese Ministry of Health, Labor and Welfare Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis B [11]: HBV DNA <5.0 log IU/mL ($n$ = 14, 17.3%), 5.0–7.0 log IU/mL ($n$ = 30, 37.0%), and >7.0 log IU/mL ($n$ = 37, 45.7%) (Table I). All patients had serum hepatitis B surface antigen (HBsAg) detectable for at least 6 months, regardless of their hepatitis B e antigen (HBeAg) status. They were negative for hepatitis C virus and HIV antibodies. They were followed up at least every 3 months to examine physical status and monitor liver biochemistry and virology. Adherence to ETV was assessed during each visit to the clinic. This study was approved by the Ethics Committee of Chiba University, Graduate School of Medicine.

### *Serological examination*

All clinical laboratory tests including hematological data, biochemical data, and HBV serologies were performed at the Central Laboratory of Chiba University Hospital. HBsAg, HBeAg, and anti-HBe antibody were determined by ELISA (Abbott, Chicago, IL, USA) or CLEIA (Fujirebio, Tokyo, Japan) [12]. HBV genotype was determined from patients' sera by ELISA (Institute of Immunology, Tokyo, Japan) as reported by Usuda et al. [13]. HBV DNA was measured by Roche Amplicor™ PCR assay (detection limits: 2.6 log IU/mL; Roche Diagnostics, Tokyo, Japan). The clinical efficacy of ETV was assessed as the proportion of patients achieving HBV DNA negativity, which is defined as an HBV DNA level of <2.6 log IU/mL and that of patients achieving ALT normalization (normal range: 8–42 IU/L). Using generally available biological parameters, the aspartate aminotransferase (AST) to platelets ratio index (APRI), and serum liver fibrosis score, was calculated according to the following formula: AST/35 × 100/platelet count [14,15].

### *Sequence analysis of HBV DNA*

Sera obtained from patients were stored at -20°C until analysis. HBV polymerase/reverse transcriptase (RT) substitutions were analyzed for all patients who had experienced virological breakthrough (>1 log IU/mL

Table I. Baseline characteristics of patients.

| | HBV DNA (log IU/mL) | | | | HBeAg | |
|---|---|---|---|---|---|---|
| | Total | <5.0 | 5.0–7.0 | >7.0 | Positive | Negative |
| Number of cases | 81 | 14 | 30 | 37 | 40 | 41 |
| Age (years) | 49.7 ± 12.2 | 55.7 ± 13.1[a] | 50.9 ± 11.3 | 46.4 ± 11.8[a] | 44.7 ± 10.3[e] | 54.5 ± 12.0[e] |
| Gender (male/female) | 55/26 | 7/7 | 22/8 | 26/11 | 28/12 | 27/14 |
| HBeAg (+/-) | 40/41 | 0/14[b] | 11/19[b] | 29/8[b] | | |
| Genotype (B/C/N.D.) | 2/33/46 | 0/2/12 | 2/14/14 | 0/17/20 | 1/23/16 | 1/10/30 |
| ALT (IU/L) | 169 ± 186 | 85.1 ± 115 | 185 ± 192 | 189 ± 197 | 179 ± 190 | 159 ± 184 |
| AST (IU/L) | 108 ± 113 | 62.6 ± 87.5 | 128 ± 149 | 109 ± 81.1 | 104 ± 83.6 | 111 ± 136 |
| Platelets ($\times 10^4/mm^3$) | 16.2 ± 8.2 | 21.4 ± 15.5[c] | 14.9 ± 5.0[c] | 15.5 ± 5.9 | 15.7 ± 5.5 | 16.8 ± 10.2 |
| APRI (<0.50/0.50–1.50/>1.50) | 14/35/32 | 7/5/2[d] | 4/11/15[d] | 3/19/15[d] | 2/24/14[f] | 12/11/18[f] |

Abbreviations: ALT = alanine aminotransferase; APRI = AST to platelets ratio index; AST = aspartate aminotransferase; HBeAg = hepatitis B e antigen; HBV = hepatitis B virus; N.D = not determined; SD = standard deviation. APRI, AST/35 × 100/platelet count [15];
Data are expressed as mean ± SD. [a]$p = 0.036$ between <5.0 group and >7.0 group; [b]$p < 0.001$ among three groups; [c]$p = 0.041$ between <5.0 group and 5.0–7.0 group; [d]$p = 0.0049$ among three groups; [e]$p < 0.001$ between HBeAg-positive and HBeAg-negative groups; [f]$p = 0.0020$ between HBeAg-positive and HBeAg-negative groups.

increase in serum HBV DNA level from nadir) using ETV on-treatment sera. Briefly, HBV DNA was extracted from 100 μL of sera using SepaGene (Sanko Junyaku, Tokyo, Japan). Nested PCR was performed using LA Taq polymerase (Takara Bio, Otsu, Shiga, Japan) under the following conditions: 5-min denaturation at 94°C, 35 cycles with denaturation at 94°C for 40 s, annealing at 58°C for 1 min, and extension at 68°C for 1.5 min [5]. An 862 base-pair fragment (nt 242–1103) containing the polymerase RT domain was amplified on PCR Thermal Cycler Dice Model TP600 (Takara Bio). The primers for the second round of PCR were 5′-CAG AGT CTA GAC TCG TGG-3′ (sense, nt 242–258) and 5′-GGC GAG AAA GTG AAAGCC-3′ (antisense, nt 1103–1086). The PCR product was sequenced using the primers: 5′-TGG CTC AGT TTA CTAGTG CC -3′ (nt 668–687), 5′-GGC ACT AGT AAA CTGAGC CA-3′ (nt 687–668), and the primers for the second round of PCR. To prepare the sequence template, PCR products were treated with ExoSAP-ITR (Affymetrix, Inc., Santa Clara, CA, USA) and then sequenced using a BigDye(R) Terminator v3.1 Cycle Sequencing Kit (Life Technologies, Tokyo, Japan). Sequences were analyzed using Applied Biosystems 3730 × 1 (Life Technologies) [16].

### *Statistical analysis*

Statistical analyses were performed using Microsoft Excel 2010 for Windows™ 7. Continuous variables were expressed as mean ± standard deviation and were compared by one-factor analysis of variance. Categorical variables were compared by Chi-square test. Baseline was taken as the date when the first dose of ETV was taken. Statistical significance was considered at $p < 0.05$.

## Results

### *Baseline characteristics of the patients*

Eighty-one patients (67.9% male) were included in this study. The group with HBV DNA >7.0 log IU/mL was younger than that with HBV DNA <5.0 log IU/mL ($p = 0.036$) (Table I). Treatment duration was not significantly different among these three groups (21.4 ± 9.8, 27.8 ± 16.3, and 33.1 ± 24.6 months; $p = 0.16$). The status of HBeAg differed significantly among these three groups ($p < 0.001$). There was a statistically significant difference in platelet counts between the HBV DNA <5.0 log IU/mL and 5.0–7.0 log IU/mL groups ($p = 0.041$). This suggested that patients with an HBV DNA level <5.0 log IU/mL were likely not to progress to liver fibrosis because APRI tended to be lower and HBeAg was negative. To compare HBeAg-positive and HBeAg-negative cases, the patients were divided into two groups based on their HBeAg status just before starting ETV. The HBeAg-positive cases were younger than the HBeAg-negative cases ($p < 0.001$).

### *Virological response*

The numbers (proportions) of patients achieving serum HBV DNA negativity at 3, 6, 12, 18, 24, and 36 months, respectively, in the three groups are shown in Figure 1. At 3 months, HBV DNA negativity of the HBV DNA <5.0 log IU/mL group was higher than that of the HBV DNA 5.0–7.0 log IU/mL group ($p = 0.0040$). At 3 and 6 months, HBV DNA negativity of the HBV DNA <5.0 log IU/mL group was higher than that of the HBV DNA >7.0 log IU/mL group ($p < 0.001$ and $p = 0.018$, respectively) (Figure 1A). At 12 and 24 months, HBV DNA negativity of the HBV DNA 5.0–7.0 log IU/mL group was higher than that of the HBV DNA >7.0 log IU/mL group ($p = 0.034$ and 0.035, respectively).

The patients with HBV DNA <5.0 log IU/mL all achieved serum HBV DNA negativity throughout the duration. The patients with HBV DNA 5.0–7.0 log IU/mL all achieved serum HBV DNA negativity by 12 months after starting treatment. Only one patient in this group experienced an increase in HBV DNA, at 29 months, but the duration of serum HBV DNA detectability was only 4 months, when he had poor adherence. From then, after discussions with his physician, he understood the importance of taking ETV for suppression of HBV replication and strictly adhered to the treatment schedule, and HBV DNA became undetectable again (Patient 3 in Table II). On the other hand, in the HBV DNA >7.0 log IU/mL group, the proportions of patients achieving serum HBV DNA negativity were 84.8%, 88.0%, 76.2%, and 69.2% at 12, 18, 24, and 36 months, respectively. In three patients of this group, HBV DNA increased at 28, 26, and 12 months, respectively (Patients 1, 2, and 4 in Table II). When we investigated the negativity of HBV DNA with or without HBeAg at baseline, HBeAg-positive patients with HBV DNA 5.0–7.0 log IU/mL group (Figure 1B) and all HBeAg-negative patients achieved serum HBV DNA negativity by 12 months (Figure 1C).

The proportions of patients achieving serum HBeAg negativity in HBeAg-positive patients with HBV DNA 5.0–7.0 log IU/mL group were 0%

(0/11), 9.1% (1/11), 9.1% (1/11), 22.2% (2/9), 20% (1/5), and 25% (1/4) at 3, 6, 12, 18, 24, and 36 months, respectively. On the other hand, in the HBV DNA >7.0 log IU/mL HBeAg positive-group, the proportions of patients achieving serum HBeAg negativity were 5.1% (2/28), 11.1% (3/27), 23.1% (6/26), 30.0% (6/20), 38.9% (7/18), and 60.0% (6/10) at 3, 6, 12, 18, 24, and 36 months, respectively.

*Biochemical response*

At 3 months, the proportion of ALT normalization of the HBV DNA 5.0–7.0 log IU/mL group was higher than that of the HBV DNA >7.0 log IU/mL group (24/28 vs. 20/35, $p = 0.014$). When we investigated the normalization of ALT with or without HBeAg at baseline, HBeAg-positive patients seemed

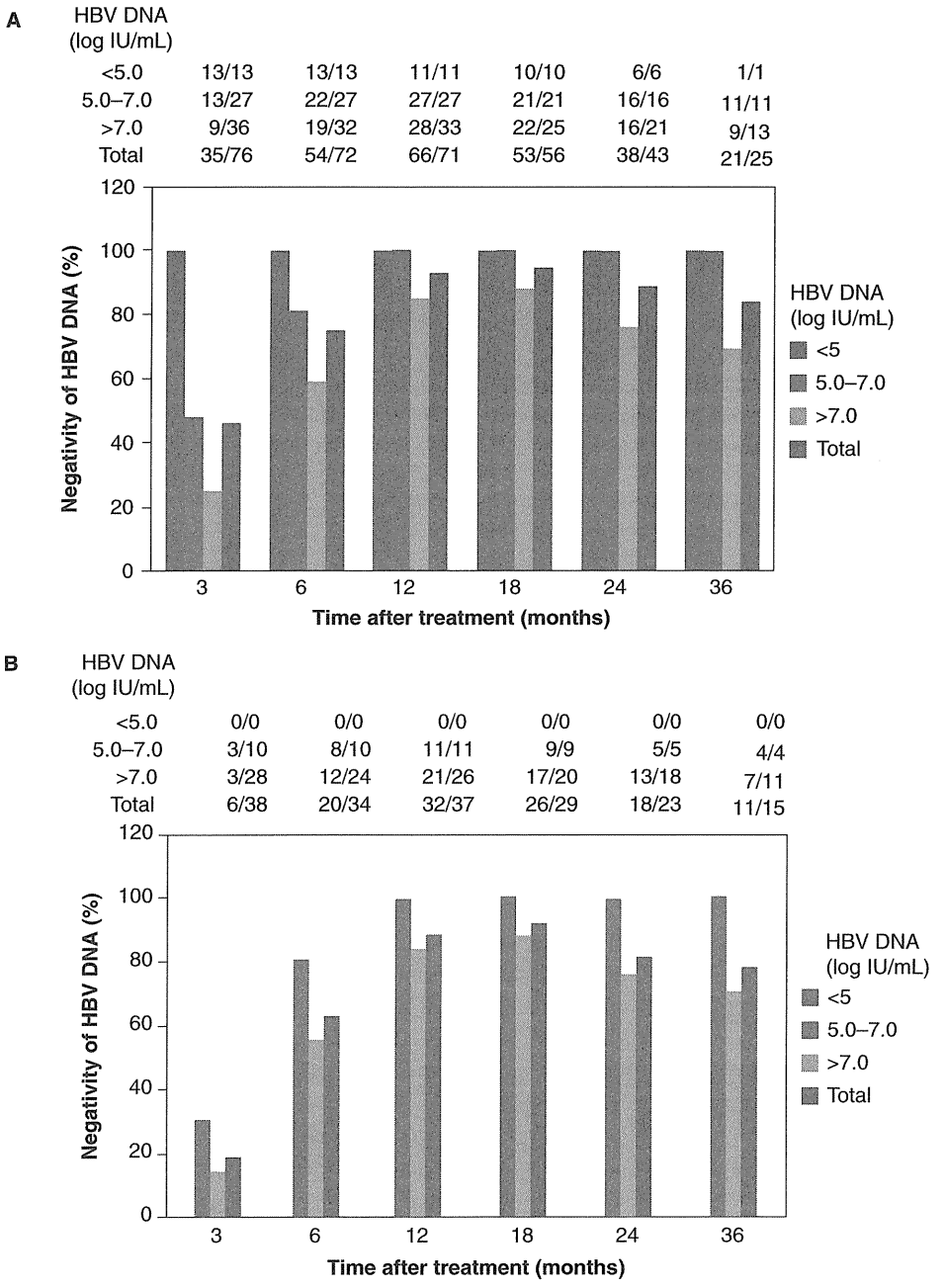


Figure 1. Negativity of HBV DNA (%) during ETV treatment. (A) A total of 81 patients (violet bars) were included in this study. Patients were divided into three groups: HBV DNA <5.0 log IU/mL group ($n = 14$, 17.3%) (blue bars), 5.0–7.0 log IU /mL group ($n = 30$, 37.0%) (red bars), and >7.0 log IU/mL group ($n = 37$, 45.7%) (green bars). (B) HBeAg-positive patients ($n = 40$). (C) HBeAg-negative patients ($n = 41$). ETV = entecavir; HBeAg = hepatitis B e antigen; HBV = hepatitis B virus.

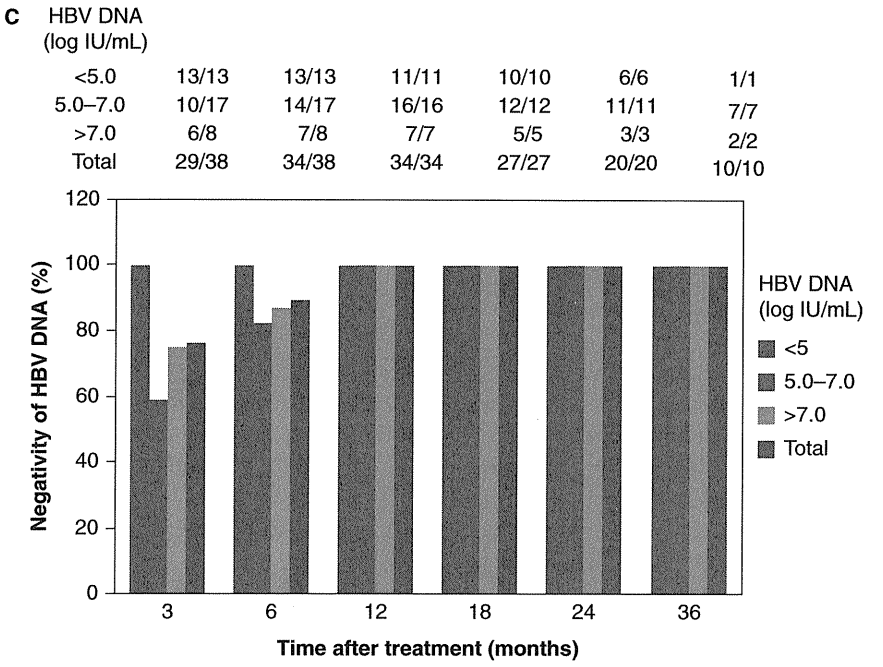


Figure 1. (*Continued*).

to have a slower response to ETV than HBeAg-negative patients (data not shown).

*Sequence analysis*

Four patients, one with HBV DNA 5.0–7.0 log IU/mL and three with HBV DNA >7.0 log IU/mL, experienced virological breakthrough during ETV therapy and were all analyzed to clarify whether genotypic mutations were acquired or not (Table II). Two of the three patients with HBV DNA >7.0 log IU/mL acquired genotypic mutations resistant to ETV. One had rtS202G (Patient 1 in Table III) and the other rtT184A (Patient 2 in Table III), both accompanied by lamivudine (3TC)-resistant substitutions (rtL180M and rtM204V). The other two patients had no genotypic mutations (Patients 3 and 4 in Table III). The physicians who had seen these patients at the time of virological breakthrough performed medical interviews to ask about their adherence, adding the information to their medical charts, and their poor adherence to ETV was revealed.

## Discussion

In the present study, an approximately 27-month ETV treatment for NUC-naive patients resulted in an optimized outcome, in line with previous reports [10,17]. Early on-treatment virological response leads to optimized long-term outcome. We found two HBeAg-positive patients with ETV-resistant mutations. One had rtS202G (Patient 1 in Table III) and the other rtT184A (Patient 2 in Table III), accompanied by 3TC-resistant substitutions (rtL180M and rtM204V) [17–19]. These two patients discontinued ETV and then received a combination therapy of 100 mg 3TC and 10 mg adefovir-dipivoxil daily, as previously reported [20]. As ETV-resistance can be

Table II. Characteristics of patients with HBV virological breakthrough.

| Patient | Age (years)/gender | HBV genotype | Baseline HBeAg | Baseline HBV DNA (log IU/mL) | Baseline ALT (IU/L) | Duration of treatment before VB (months) | Adherence to ETV |
|---|---|---|---|---|---|---|---|
| 1 | 49/M | C | + | 7.3 | 107 | 28 | Good |
| 2 | 57/M | C | + | >7.6 | 55 | 26 | Good |
| 3 | 38/M | C | + | 6.9 | 59 | 29 | Poor |
| 4 | 46/F | C | + | >7.6 | 85 | 12 | Poor |

Abbreviations: ALT = alanine aminotransferase; ETV = entecavir; HBeAg = hepatitis B e antigen; HBV = hepatitis B virus; VB = virological breakthrough.

Table III. Amino acid mutations of HBV DNA polymerase sequences in patients with virological breakthrough.

| Amino acid No | 180 | 184 | 202 | 204 | 250 |
|---|---|---|---|---|---|
| Wild sequences | L | T | S | M | M |
| Patient 1 | **M** | T | **G** | **V** | M |
| 2 | **M** | **A** | S | **V** | M |
| 3 | L | T | S | M | M |
| 4 | L | T | S | M | M |

Abbreviations: HBV = hepatitis B virus; **Bold**, amino acid mutations. Numbers of top line indicate amino acid positions [5].

relatively easily diagnosed, it is important to perform both the HBV DNA and the resistance tests. The mechanism by which ETV-resistant substitutions can induce virological breakthrough during ETV therapy is largely known [21]. We also found two patients with poor adherence to ETV. Multiple drug-resistant strains were also reported, and so monitoring NUC-resistant mutations is essential. In such a case, we have to pay attention to patients with poor adherence as well [19].

In Japan, HBV genotype C is predominant. HBV genotype C is reported to be associated with delayed HBe seroconversion, more advanced liver disease, and increased probability of HCC development [22,23]. No statistical difference was observed in response to ETV among patients with different genotypes [24], although HBV genotype A and B patients were reported to respond to standard interferon-alfa better than HBV genotype C and D patients [23]. In this study, HBeAg-negative patients achieved serum HBV DNA negativity by 12 months. On the other hand, HBeAg-positive patients tended to have poor response to ETV. One HBeAg-negative patient of the total 81 patients became negative for HBsAg (data not shown). These observations might suggest important pathogenic differences in HBV genotypes.

It is well known that non-adherence results in non-control of other common diseases such as diabetes mellitus [25] and hypertension [26]. Poor medication adherence among HIV-infected adults leads to neuropsychological dysfunction [27] as well as increase of HIV RNA [28]. Recently, Ha et al. [29] also reported that medication non-adherence was likely to be a more important contributor to treatment failure than antiviral resistance, especially with new anti-HBV agents such as ETV and tenofovir. Although the number of patients in our study is small, our results highlight the importance of making efforts to ensure medication adherence for HBV-positive patients and providing support to improve poor adherence to control HBV replication.

In the present study, ETV resulted in less ETV-resistant mutations in NUC-naive Japanese patients than in previous reports with other drugs such as 3TC [5,18]. In conclusion, attention should be paid to patients with poor adherence as well as emerging ETV-resistant mutations in HBV during ETV-treatment.

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＜短　報＞

# B型急性肝炎の経過予測におけるHBs抗原定量の有用性

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はじめに：GenotypeAによるB型急性肝炎（AH-B）は高率な遷延化または慢性化が知られるが，その予測は困難である．我々はHBs抗原定量系を用いて，AH-BにおけるHBs抗原量の推移と臨床転帰の関連を検討した．

対象と方法：2001年12月から2010年3月に受診した，AH-B 143例中抗ウイルス療法が導入された10例（Genotype A/B/C：4/1/5，重症化阻止/慢性化阻止：8/2）を除く，133例（男：女＝104：29，平均年齢32.8±10.0歳）を対象とした．AH-Bの診断は，1）急性の肝障害を認め，肝障害の既往がない，2）以前のHBs抗原陰性が確認されている，以前のHBs抗原が不明の場合IgM-HBc抗体が高力価（>10 S/CO）陽性である，或いは経過でHBs抗原が消失している，3）他の肝障害の原因が血清学的に否定されている，の3点により行った．

HBs抗原は全期間を通じHISCL-2000i®によるCLEIA法；（シスメックス社：log IU/m*l*）を測定した．Genotypeはジェノタイプ特異的プローブアッセイ（スマイテストHBVジェノタイプ判定キット；ゲノムサイエンス社）により測定し，119例で決定可能であった．転帰はHBs抗原消失時期により3カ月までの群（I群），6カ月までの群（II群），6カ月以上の経過で消失した遷延化群（III群），12カ月以上消失のない慢性化群（IV群）の4群に区分した．データは平均値±標準偏差で示し，統計学的処理はMann-Whitney *U* testを用い，両側検定にてp<0.05を有意差とした．本研究は本学倫理委員会の承認を受け（No865），対象者全員へ十分に説明後同意を得て行われた．

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＜受付日2011年1月25日＞＜採択日2011年5月9日＞

結果：1）Genotypeの内訳（119例）：A：66例（55%），B：16例（14%），C：37例（31%）であった．2）各群でのGenotypeの内訳I群；A/B/C：28/8/24，II群；A/B/C：28/7/7，III群；A/B/C：5/0/6，IV群；A/B/C：5/1/0であった．IV群の83.3%（5/6）がGenotype Aであった．3）HBs抗体陽性化は133例中37例（27.8%）（I/II/III群；20/14/3）で確認された．4）各群のHBs抗原量の推移をTable 1に示す．HBs抗原量は臨床転帰により早期から異なる推移を示し，I群では0週に比し2週までに有意に低下した（$p<0.001$）．一方，III群及びIV群では0週に比し4週までは同等の値を示し，8週においても高値であった．特にIV群では2週から4週にかけて再上昇を示す傾向にあった（Fig. 1）．

考察：本邦では首都圏を中心に慢性化率の高いGenotype AによるAH-Bが増加している[1)2)]．このため，Genotype AのHBVキャリアの増加やこれに伴う肝硬変・肝細胞癌症例の増加が危惧され，新規感染予防及び慢性化の阻止が急務となっている．新規感染予防策としてはユニバーサルHBワクチンの導入が検討されている．一方，慢性化阻止策としては肝炎の遷延化例，特にGenotype A感染に対して，抗ウイルス療法介入による肝炎鎮静化が試みられるが[3)]，適切な治療介入時期に関しては一定の見解が得られていない．

近年開発されたHISCL-2000i®を用いたCLEIA法によるHBs抗原測定系は，簡便かつ迅速なHBs抗原定量系であり，HBV DNAに比べて早く測定結果を把握することができ，肝炎の遷延化及び慢性化の予測に重要なウイルス動態のリアルタイムなモニタリングに有用である．HBs抗原量はI群では早期より低下したが，III群またはIV群では4週ないし8週においても0週とほぼ同等の高値であった．従って，4週ないし8週のHBs抗原量は，肝炎遷延化及び慢性化の予測に有用と考えられ，特に早期再上昇を示す症例では慢性化する可能性が示された．肝炎の遷延化及び慢性化阻止目的の抗

**Table 1** Quantitative evaluation of HBsAg in the course of acute hepatitis B.

| Weeks from the initial visit | HBsAg (IU/ml) | | | |
|---|---|---|---|---|
| | Group I | Group II | Group III | Group IV |
| 0 | 3.46 ± 1.44 | 4.21 ± 0.32 # | 4.56 ± 0.44 | 4.67 ± 0.42 |
| 2 | 1.59 ± 2.23 | 3.43 ± 0.50 ## | 4.24 ± 0.85* | 3.70 ± 1.64 |
| 4 | 0.73 ± 2.09 | 2.95 ± 0.54 ## | 4.15 ± 0.68 ## | 4.08 ± 1.25 ## |
| 8 | −1.23 ± 1.38 | 1.58 ± 1.08** | 2.95 ± 0.99** | 4.04 ± 1.14*. ** |
| 12 | | −0.39 ± 1.16 | 2.19 ± 0.63* | 4.97 ± 0.19†. †† |

#: $p<0.05$ vs. Group I, ##: $p<0.01$ vs. Group I, *: $p<0.05$ vs. Group II,
**: $p<0.001$ vs. Group I, †: $p<0.01$ vs. Group II, ††: $p<0.05$ vs. Group III

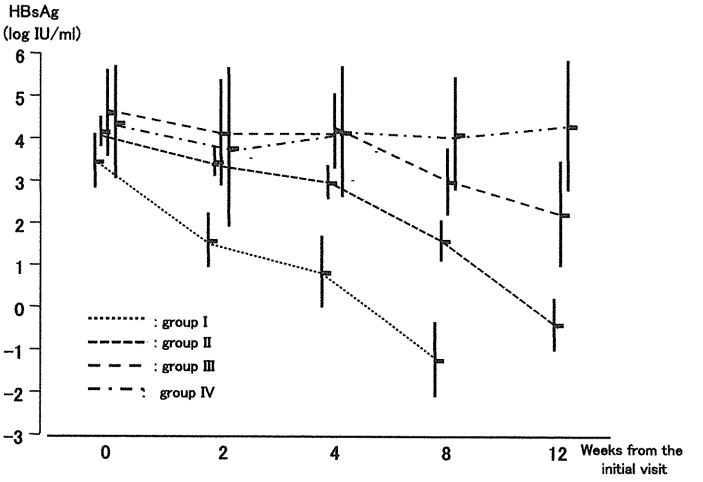


**Fig. 1** Changes in quantitative HBsAg in the course of acute hepatitis B.
An abrupt decrease in HBsAg levels was observed and HBsAg levels at 2 weeks were significantly lower than those at 0 weeks in Group I ($p<0.001$). On the other hand, levels of HBsAg remained high at 4 weeks and 8 weeks in Groups III and IV. Furthermore, HBsAg levels showed an increase from 2 weeks to 4 weeks in Group IV.
Abbreviations: HBsAg, hepatitis B surface antigen

ウイルス療法介入の有用性に関しては，今後慎重な検証を重ねる必要はあるが，4週ないし8週でのHBs抗原量の低下に乏しい症例では，遷延化及び慢性化が予測されるため，抗ウイルス療法介入を念頭に置いた治療戦略の検討が必要であると考えられた．

結語：AH-Bにおいて発症後4週ないし8週のHBs抗原量は，遷延化及び慢性化の予測に有用であり，抗ウイルス療法介入の指標となり得ることが示唆された．

索引用語：B型急性肝炎，HBs抗原定量，慢性化

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## 英文要旨

## Usefulness of quantitative HBsAg in outcome prediction of acute hepatitis B

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We evaluated the usefulness of quantitative evaluation of HBsAg in outcome prediction among 133 patients with acute hepatitis B (AH-B). Patients were classified into 4 groups according to duration of HBsAg positivity as follows: less than 3 months (Group I); less than 6 months (Group II); over 6 months (Group III); and over 12 months (Group IV). An abrupt decrease in HBsAg levels was observed and HBsAg levels at 2 weeks were significantly lower than those at the initial visit in Group I ($p<0.001$). On the other hand, levels of HBsAg remained high at 4 weeks and 8 weeks in Groups III and IV. Furthermore, HBsAg levels showed an increase from 2 weeks to 4 weeks in Group IV. These results suggest that quantitation of HBsAg at 4 or 8 weeks after initial visit may be useful in the prediction of clinical outcome in AH-B.

**Key words**: acute hepatitis B, quantitative HBsAg, chronicity

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＜原　著＞

# 当院および関連施設におけるB型肝炎ワクチン接種の有用性に関する検討

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要旨：2004年から2008年までにB型肝炎（HB）ワクチン接種が完遂された601名を対象とし，HBワクチンの有用性を検討した．全体でのHBs抗体陽転率は92.2%であった．年齢層別の検討では，20～29歳の群でHBs抗体陽転率が86.0%と他の群に比較し低値であり，19歳以下の群と有意差を認めたが（$P<0.0001$），全体としては高齢化に伴い低下する傾向を示した．性差による検討では，男性と女性のHBs抗体陽転率はそれぞれ78.7%と97.5%で，女性に比較して男性のHBs抗体陽転率が有意に低値であった（$P<0.0001$）．男性は20歳以上でHBs抗体陽転率が低下する傾向を有し，特に19歳以下の群に比較して20～29歳の群では有意に低値であった（$P=0.0411$）．HBワクチン接種により，効率的にHBs抗体を獲得するためには，特に男性においては，20歳以前の接種が望ましいと考えられた．

索引用語：　B型肝炎ウイルス　B型肝炎ワクチン　HBs抗体　性差　年齢

## はじめに

本邦におけるB型肝炎ウイルス（hepatitis B virus：HBV）持続感染症例（HBVキャリア）は，行政によるB型肝炎母子感染防止事業や日本赤十字社による血液製剤の高感度スクリーニング検査である核酸増幅検査の導入の結果，母子感染や輸血後肝炎がほぼ制御可能となり，症例数が大きく減少している[1)2)]．

一方，成人の水平感染によるB型急性肝炎は増加傾向にあり[3)]，特に首都圏においては感染経路の変遷により，genotype AのHBV感染による急性肝炎の増加が明らかになっている[4)～7)]．Genotype Aによる急性肝炎は慢性化率が高いことが報告されており[8)9)]，今後の本邦におけるgenotype AのHBVキャリア増加による感染拡大が危惧される．このため現在では感染防止策としてB型肝炎ワクチン（HBワクチン）のuniversal vaccinationとしての導入が提唱されている[7)]．

このように，現在HBV感染を取り巻く社会情勢の変化から，HBワクチンの重要性が再び注目を集めている．しかし，本邦におけるHBワクチン接種後のHBs抗体陽転率やHBワクチンに対する反応性に寄与する因子に関する検討は，HBワクチン創成期の報告が主であり[10)～15)]，現状に関する評価は不十分である．

そこで我々はHBワクチンの有用性に関する検討を最近のHBワクチン接種完遂者について行った．

## 対象と方法

対象：

2004年から2008年までに聖マリアンナ医科大学病院（以下施設A），川崎市立多摩病院（以下施設B），静山会　清川病院（以下施設C）および四日市看護医療大学（以下施設D）にて酵素免疫抗体法あるいは化学発光免疫測定法によるHBs抗原および化学発光免疫測定法によるHBs抗体陰性が確認され，HBワクチン接種に関するインフォームド・コンセントを取得し，3回のHBワクチン接種が完遂された601名（男性122名，女性479名）を対象とした．内訳は医学部学生160名，病院職員86名，歯科衛生士学生258名および看護学生97名であった．詳細をTable 1に示す．

方法：

HBワクチンは施設AではヘプタバックスⓇ（万有製

1）聖マリアンナ医科大学消化器肝臓内科
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**Table 1** Summary of studied person

| Facilities | A | B | C | D |
|---|---|---|---|---|
| Number of subjects | 160 | 86 | 258 | 97 |
| Male/Female (%Male) | 92/68 (57.5%) | 17/69 (19.8%) | 0/258 (0%) | 13/84 (13.4%) |
| Average age | 20.3±1.5 | 35.1±9.1 | 20.3±3.0 | 18.5±2.7 |
| Vaccine | Heptavax-II | Bimmugen | Bimmugen | Bimmugen |
| Route of administration | subcutaneous injection | subcutaneous injection | subcutaneous injection | subcutaneous injection |

薬株式会社，東京）を用い，施設B，CおよびDではビームゲン®（化学及血清療法研究所，熊本）を用いた．投与方法は10 μg/0.5 mLの容量で初回，1カ月後および6カ月後の計3回の投与を皮下注射で行い，HBs抗体陽転率を検討した．HBs抗体の確認は施設A，CおよびDでは3回目の投与終了後6カ月目に，施設Bでは3回目の投与終了後2カ月目に行い，受身赤血球凝集反応によるHBs抗体価が16倍以上または化学発光免疫測定法によるHBs抗体価が10 mIU/mL以上を陽性とした．なお，いずれの施設においてもHBワクチン接種による重篤な副作用は認めなかった．

統計学的解析：

データは平均値±標準偏差で表示し，有意差検定はMann-Whitney *U* testおよびChi-square testを用いた．両側検定にて$P<0.05$をもって有意差とした．

## 結　果

### 1. 全施設および各施設でのHBs抗体陽転率

全施設でのHBs抗体陽転率は92.2%（554/601）であった．施設A，B，CおよびDにおけるHBs抗体陽転率は，それぞれ84.5%（133/160），91.9%（79/86），96.5%（249/258）および95.9%（93/97）であった．施設Aは施設Bに比較して低値である傾向を有し，施設CおよびDに比較しては有意差をもって低値であった（施設A vs 施設C；$P<0.0001$，施設A vs 施設D；$P=0.0044$，Chi-square test）．

### 2. 対象者年齢とHBs抗体陽転率との関連

施設A，B，CおよびDにおける平均年齢は各施設間で明らかな差異を有しており（$P<0.0001$，Mann-Whitney *U* test）（Table 1），今回の検討では各施設でのHBs抗体陽転率と平均年齢に関連を認めなかった．次に，対象者の年齢層別HBs抗体陽転率を検討した．19歳以下，20～29歳，30～39歳，40～49歳および50歳以上のHBs抗体陽転率は，それぞれ96.1%（317/330），86.0%（178/207），94.4%（34/36），89.5%（17/19）および88.9%（8/9）であった．20～29歳の群でのHBs抗体陽転率は他の群に比較し低値であり，19歳以下の群と明らかな差異を有していたが（$P<0.0001$，Chi-square test），全体としては高齢化に伴いHBs抗体陽転率は低下する傾向を有していた．さらにこれらを39歳以下と40歳以上の2群に分け比較したところ，HBs抗体陽転率は39歳以下の群では92.3%（529/573），40歳以上の群では89.3%（25/28）と，若干ではあるが40歳以上の群で低値となる傾向を示していた．

### 3. 対象者性差とHBs抗体陽転率との関連

施設A，B，CおよびDにおける男性の比率は，それぞれ57.5%，19.8%，0%および13.4%（男性/女性；92/68，17/69，0/258および13/84）と各施設間で明らかな差異を有していたが，特に施設Aにおいて顕著に高値であった（$P<0.0001$，Chi-square test）．対象者の性差とHBs抗体陽転率を検討した結果，男性および女性のHBs抗体陽転率はそれぞれ78.7%（96/122）および97.5%（467/479）と，女性に比較して男性でのHBs抗体陽転率は有意に低値であった（$P<0.0001$，Chi-square test）．加えて，全例が女性であった施設Cを除く施設A，BおよびDにおける各施設での対象者の性差とHBs抗体陽転率の検討を行った．結果，施設AおよびBでは男性でのHBs抗体陽転率が女性に比較し有意に低値を示し（施設A；$P=0.0336$，施設B；$P=0.0361$，Chi-square test），施設Dにおいても有意差は認めないものの男性でのHBs抗体陽転率が低値である傾向を有した（Fig. 1）．また，施設A，B，CおよびDにおける女性でのHBs抗体陽転率の比較では，各施設間に有意差は認めなかった．一方，男性での検討では各施設間に明らかな差異は認めないものの施設Dにおいて高値である傾向を有していた．なお，施設Dの男性対象者は全例が19歳以下であった．

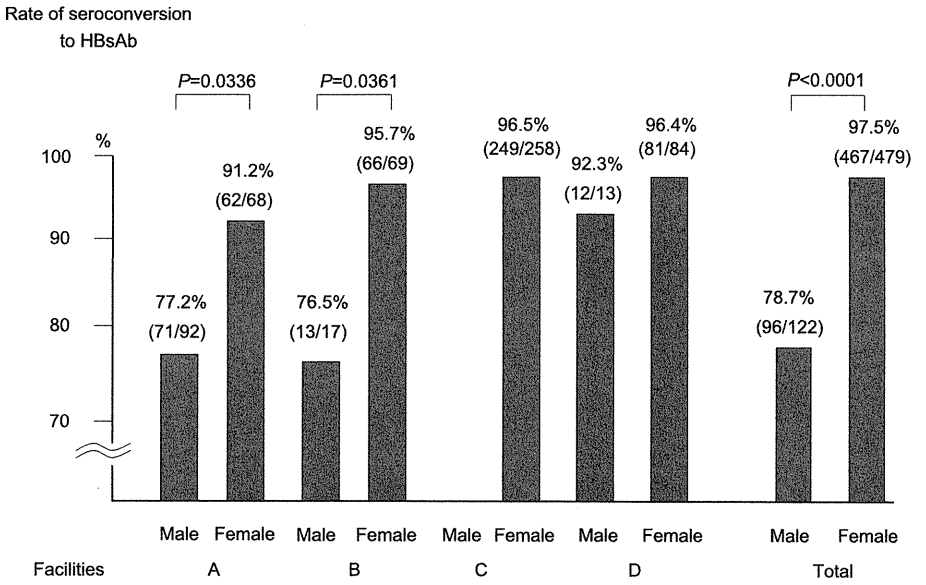


**Fig. 1** According to sex difference, total rate of seroconversion to HBsAb in male was significantly lower than that in female (78.7% vs. 97.5%; $P<0.0001$, Chi-square test).
Abbreviation: HBsAb; hepatitis B surface antibody

**Table 2** Correlation between background of studied person and rate of seroconversion to HBsAb

| Age | Sex | Number of studied person | %Male | Number of person who acquired HBsAb | Rate of seroconversion to HBsAb (%) |
|---|---|---|---|---|---|
| ～19 | Male | 36 | 10.9 | 33 | 91.7 |
| | Female | 294 | | 284 | 96.6 |
| 20～29 | Male | 69 | 33.3 | 50 | 72.5 |
| | Female | 138 | | 128 | 92.8 |
| 30～39 | Male | 9 | 25.0 | 7 | 77.8 |
| | Female | 27 | | 27 | 100 |
| 40～49 | Male | 5 | 26.3 | 4 | 80.0 |
| | Female | 14 | | 13 | 92.9 |
| 50～ | Male | 3 | 33.3 | 2 | 66.7 |
| | Female | 6 | | 6 | 100 |

Abbreviation: HBsAb; hepatitis B surface antibody

**4. 対象者年齢分布および性差と HBs 抗体陽転率との関連**

各年齢層の男女別 HBs 抗体陽転率を比較，検討した．19 歳以下の群での HBs 抗体陽転率は性差を問わずほぼ同等であったが，20 歳以上の群では各年齢層ともに女性に比較して男性は低値を示し，特に 20～29 歳の群では男女間に有意差を認めた（$P=0.0002$, Chi-square test）．男性は 20 歳以上で HBs 抗体陽転率が低下する傾向を有し，特に 19 歳以下の群に比較して 20～29 歳の群では有意に低値であった（$P=0.0411$，Chi-square test）．一方，女性においては各年齢層で若干の相違は認めるものの，明らかな差異は認めなかった（Table 2）．

## 考　察

本邦では，1986 年より血漿由来の HB ワクチンの市販が開始され，現在では第 2 世代の組換え沈降 HB ワク

チンが使用されている．現在，本邦ではヘプタバックス®およびビームゲン®の2種類の組換え沈降HBワクチンが使用可能である．いずれのHBワクチンも酵母由来の製剤であるが，ヘプタバックス®はsubtype：adw[11]，ビームゲン®はsubtype：adrと，異なるHBs抗原のsubtypeに対応している[12]．ただし，それぞれのHBワクチン接種によって獲得し得たHBs抗体は，対応するHBs抗原のsubtypeに関わらずHBVの感染防御効果を有するとされている[10]．HBワクチン接種後のHBs抗体陽性基準に関しては，本邦では主として受身赤血球凝集反応によるHBs抗体価16倍をHBV感染防御最小抗体価として用いてきた．一方，国際的にはWHO reference preparationを基準に検定された10 mIU/mLをHBV感染防御最小抗体価としている[16]．近年では，本邦においても10 mIU/mLを採用する施設が増加している．

本邦での標準的投与法である3回のHBワクチン接種により，90～95％でHBs抗体の獲得が可能であるが，その反応には各個体の年齢や性差および投与経路などの関与が示唆されている[10)～15)]．

HBワクチン反応性と性差との関連について，飯野の検討では，3回の組換え沈降HBワクチン接種後1カ月目のHBs抗体陽転率は，男性に比較し女性で高率であり，男女別かつ投与経路別（筋肉内注射または皮下注射）HBs抗体価（mIU/ml）の比較にて，女性・筋肉内注射，男性・筋肉内注射，女性・皮下注射および男性・皮下注射におけるHBs抗体価が，それぞれ140.9 mIU/mL，81.1 mIU/mL，91.6 mIU/mLおよび47.7 mIU/mLであった．有意差検定はなされていないものの，得られたHBs抗体価は女性・筋肉内注射で最も高く，男性・皮下注射で最も低い値を示した[10)11)]．また，矢野の検討でも，HBs抗体陽転率は，女性が98.2％と，男性の92.7％に比較して有意に高率であり，獲得抗体価も女性が624.4 mIU/mLと男性の305.9 mIU/mLに比較して有意に高値であったと報告している[12)]．従って，女性は男性に比較してHBs抗体陽転率および獲得抗体価が高いことが示されている．

また，年齢もHBワクチン反応性における重要な因子である．飯野の報告によれば，3回の組換え沈降HBワクチン接種後1カ月目の年齢層別かつ投与経路別HBs抗体価の比較にて，10代・筋肉内注射および皮下注射では，それぞれ211.7 mIU/mLおよび187.4 mIU/mLであるのに対し，50代・筋肉内注射および皮下注射では，それぞれ17.5 mIU/mLおよび38.8 mIU/mLと低値であった[10)11)]．一方，矢野による年齢層別HBs抗体陽転率および獲得抗体価の検討においてもHBワクチン接種による10代のHBs抗体陽転率および獲得抗体価は，それぞれ100％および939.6 mIU/mLであったのに対し，40代以降では，それぞれ90.7％および277.7 mIU/mLと，いずれも高齢化に伴い低下する傾向を示した．特に10代と20代，20代と30代でHBs抗体陽転率および獲得抗体価ともに有意差をもって低下したと報告しており[12)]，高齢層に比較して若年層においてHBs抗体陽転率および獲得抗体価が高いことが示されている．この報告の中で，矢野は19歳以上の対象者について投与経路別のHBs抗体陽転率および抗体価についても検討をしている．筋肉内注射でのHBs抗体陽転率および獲得抗体価は，それぞれ94.6％および917.9 mIU/mLであったのに対し，皮下注射では，それぞれ96.0％および473.4 mIU/mLとHBs抗体陽転率は同等であったが，獲得抗体価は有意差がないものの筋肉内注射で高値である傾向を有した[12)]．

以上の検討から，HBワクチン接種に対する反応は，若年，女性および筋肉内注射において良好であることが明らかとなっている[10)～12)]．

今回の検討においては，全体でのHBs抗体陽転率は従来の報告とほぼ同等の値であったが[10)～15)]，施設Aにおいては84.5％と低値を示した．施設Aの特徴として，対象者における男性の比率が顕著に高値であったことが挙げられる．全施設における対象者性別とHBs抗体陽転率との検討では，女性に比較して男性でのHBs抗体陽転率が有意に低値を示した．従って今回の我々の検討からも，特にHBワクチンに対する反応性は女性に比較して男性で劣ることが示唆された．

対象者年齢とHBs抗体陽転率との関連について今回の検討からは，対象者の高齢化に伴いHBs抗体陽転率は低値となる傾向が認められた．他の群に比較して20～29歳の群でHBs抗体陽転率が低値であったが，その要因はこの群における男性の比率が高かったことにあると思われる．飯野は対象者年齢の高齢化に伴いHBs抗体陽転率は低下し，特に40歳以上においてはその傾向が顕著になるとしているが[10)11)]，今回の我々の検討結果からも同様の結論が導きだせる．

高齢者においてHBワクチンに対する反応性が低下する一つの要因として，加齢に伴う免疫能低下，すなわち免疫老化の関与が推測される．高齢者では免疫老化により，自然免疫系および獲得免疫系の双方が機能低下を示す[17)]．従って，高齢者の獲得免疫系においてはT細胞およびB細胞の細胞数減少や機能低下が[18)]，自

然免疫系においては抗原提示細胞の抗原提示能の低下が認められる[19]．この免疫老化が，高齢者におけるHBワクチンに対する反応性の低下の要因であることが示唆される．また，女性に比較して男性におけるHBワクチンに対する反応性が乏しい一つの要因としては，性差による免疫応答能の差異の関与が示唆されている．従来から，女性は男性に比較し優れた抗体産生能を有することが報告されてきた[20][21]．その理由としてKenny JFらは，雄マウスにestradiol-17βを添加し，熱処理した大腸菌の投与を行った結果，大腸菌に対する抗体産生能が増加したことから，estradiolが免疫応答性を高める作用を有し，血清中estradiol濃度が高値である女性において優れた免疫応答能を獲得する可能性を挙げている[22]．HBワクチン反応性と年齢および性差との関連については，より効率的に抗体を獲得するために重要な検討課題であり，今後もさらなる検証が必要である．

一方，使用するHBワクチンの種類によっても，HBs抗体陽転率に差異を有することが示唆されている[11][12][23]．今回，施設Aではヘプタバックス®を，施設B，CおよびDではビームゲン®を使用したが，臨床第III相試験におけるヘプタバックス®およびビームゲン®のHBs抗体非陽転率は，それぞれ7.6%および3.6%と若干の差異が認められた[11][12]．今回の検討では，対象者背景や対象者数に差異を認めることから，異なるHBワクチンでのHBs抗体陽転率の比較検討は困難であるが，ヘプタバックス®を使用した施設Aにおいて，HBs抗体陽転率が最も低値であったことは重要である可能性があり，今後多施設での検証が必要である．

現在，職業感染防止目的のHBワクチンは皮下注射で行われることが一般的である．従って今回の検討では，皮下注射と筋肉内注射での抗体獲得率の差異に関しては検討がなされていない．上述の通り，HBワクチンの投与を筋肉内注射にすることは，抗体獲得率を改善させるとされているため，抗体率が低いと想定される者に対しては最初から筋肉内注射を行うことを考慮する必要がある．また，現在使用されているHBワクチンが沈降ワクチンであることを認識し，必ず十分に攪拌し沈降成分を再浮遊させた後に接種するという基本的事項を忠実に施行することも重要である．

我々の今回の成績からは男性，特に20歳以降の男性の抗体獲得率は低値であった．また，従来の検討においてもHBs抗体陽転率および獲得抗体価ともに10代に比較して20代において有意に低下することが示されている[12]．従って，HBワクチン接種により，効率的にHBs抗体を獲得するためには，特に男性においては，20歳以前の接種が望ましいと考えられる．

現在使用されている組換え沈降HBワクチンでは，5～10%の不応または反応不良例が存在するとされていたが[10]～[15]，今回の検討ではさらに高い割合でHBワクチン不応者が存在することが示唆された．HBワクチン不応者に対しては，①2倍量を6カ月以内に追加接種する，②通常量を1カ月間隔で2回追加接種する，③1年後に再度通常量を3回接種する，④ワクチンの種類を変更する，などの工夫がなされているが[13]～[15]，何れの策が最も効率的にHBs抗体を獲得し得るのかは明らかになっておらず，今後の検討課題と考えられる．アジュバントを添加した免疫原性の高いワクチンや免疫応答の高い混合ワクチンの開発，実用化も，今後必要になると考えられる．

近年genotype Aによる急性肝炎の増加および慢性化例からの感染拡大が危惧され[4]～[7]，またHBV感染の既往と認識される症例からの，免疫抑制・化学療法による予後不良な重症肝炎（いわゆるde novo B型肝炎）の発症が増加傾向を示している[24]～[26]．このように，現在ではHBV感染を取り巻く社会情勢に大きな変化が認められる．従って，HBワクチンの有用性を再認識し，より効率的な感染防止策を講じる必要がある．性感染症のハイリスク群である年齢層[7]においてHBs抗体獲得率が低いことを考慮すると，若年でのuniversal vaccinationの導入による感染防止策を講じることが望ましいと考えられた．

## 終わりに

本論文を聖マリアンナ医科大学　消化器肝臓内科前教授，静山会　清川病院　前院長の飯野四郎先生（故人）に捧げる．

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# Evaluation of usefulness of hepatitis B vaccination at St. Marianna University School of Medicine Hospital and related facilities

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The Immunogenicity of hepatitis B (HB) vaccine was evaluated among 601 persons who were inoculated with HB vaccine. Seroconversion (SC) to HB surface antibody (HBsAb) was observed in 92.2% of the persons. Rate of SC tended to decrease with aging, and significant difference was observed between group aged 20-29 and group aged 19 or under (86.0% versus 96.1%: $P<0.0001$). Rate of SC in male was significantly lower than that in female (78.7% versus 97.5%: $P<0.0001$). In male, rate of SC in the group aged 20-29 was significantly lower than that in the group aged 19 or under (72.5% versus 91.7%: $P=0.0411$), while no obvious difference with aging was found in female. These results suggested that response to HB vaccination may be impaired in male and aged persons. Thus, HB vaccination should be done before 20 years old, especially in male, to acquire sufficient amount of HBsAb.

**Key words:** hepatitis B virus (HBV)　hepatitis B vaccine　HBsAb　sex　age

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