patients with hypertension, liver cirrhosis, and HCV non-clearance should be noted the development of hemorrhagic stroke.

The present study was limited by a retrospective cohort trial. Another limitation of the study was that patients were treated with different types of antivirus therapy for different duration. In addition, these patients were treated with different types of drugs for diabetes, hypertension, and dyslipidemia during follow-up. Finally, our cohort contains Japanese subjects only. On the other hand, the strengths of the present study are a long-term follow-up in the large numbers of patients included.

In conclusion, HCV clearance reduced hemorrhagic stroke to about one-fourth in cirrhotic patients.

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Renal dysfunction and hypophosphatemia during long-term lamivudine plus adefovir dipivoxil therapy in patients with chronic hepatitis B

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Abstract
Background Renal dysfunction and Fanconi's syndrome associated with hypophosphatemia caused by long-term administration of low-dose adefovir dipivoxil (ADV) has been reported in recent years. The aim of this retrospective study was to determine the incidence and factors associated with renal dysfunction and hypophosphatemia in patients with hepatitis B infection on long-term treatment with ADV and lamivudine (LAM).
Methods The study subjects were 292 patients treated with 10 mg/day ADV and 100 mg/day LAM for more than 6 months. We evaluated estimated glomerular filtration rate (eGFR), serum creatinine and serum phosphate level at the start of ADV and every 6 months.
Result During a median treatment duration of 64 months, 28 (9.6 %) patients developed renal impairment (defined as eGFR < 50 ml/min/1.73 m^2), and 73 (27.1 %) developed hypophosphatemia, including 14 with persistent hypophosphatemia. The cumulative incidences of renal impairment at 1, 3, and 5 years were 1.4, 7.5, 10.5 %, respectively, and those of hypophosphatemia were 6.8, 20.6, 26.7 %, respectively. Multivariate analysis identified old age, liver cirrhosis and hypertension as determinants of renal impairment, and male sex, HCC, low baseline serum phosphate as determinants of hypophosphatemia. Three of the 14 patients with persistent hypophosphatemia developed Fanconi's syndrome; their serum creatinine level remained normal, but eGFR was lower than at baseline.
Conclusion Long-term treatment of hepatitis B with low-dose (10 mg/day) ADV and LAM can potentially cause renal impairment and hypophosphatemia. We advocate regular monitoring of serum phosphate and evaluation of eGFR, in addition to serum creatinine, in such patients.

Keywords Adefovir dipivoxil · Hepatitis B virus · Renal dysfunction · Hypophosphatemia · Fanconi's syndrome · Osteomalacia

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
CHB	Chronic hepatitis B
CHBI	Chronic hepatitis B infection
CI	Confidence interval
eGFR	Estimated glomerular filtration rate
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
IFN	Interferon
IP	Inorganic phosphate
LC	Liver cirrhosis

Introduction

Hepatitis B virus (HBV) infects more than 350 million people worldwide. Hepatitis B is a leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC)

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[1]. The goal of therapy is to reduce HBV replication to limit progressive liver disease and improve the natural history of chronic HBV infection (CHBI) [2]. Oral nucleotide analogs are used for antiviral therapy of patients with CHBI. Lamivudine (LAM) is the first nucleotide analogue indicated for CHBI [3]. However, long-term LAM therapy is associated with emergence of drug-resistant HBV mutation, and relapse of hepatitis [4–7]. Subsequent studies indicated that adefovir dipivoxil (ADV) alone or in combination with LAM provides effective antiviral therapy in patients with LAM-resistant HBV [8, 9]. However, ADV monotherapy of LAM-resistant HBV resulted in the appearance of virological breakthrough due to acquisition of ADV-resistant mutation [10]. Therefore, the Japanese guidelines recommend the use of the combination of ADV and LAM for patients with LAM-resistant HBV [11].

Renal impairment is one of the most serious side effects of ADV. Nephrotoxicity associated with ADV is dose-dependent. In CHBI phase III trials, significant renal toxicity was not observed during a median follow-up period of 64 weeks in patient treated with ADV at 10 mg/day [12]. However, renal dysfunction associated with long-term use of low-dose ADV has been documented in a few reports published in recent years [13–15]. Moreover, a few case reports also described hypophosphatemia associated with Fanconi's syndrome in association with the use of ADV at 10 mg/day [14, 16, 17].

On the other hand, there are only a few studies on the incidence of renal dysfunction and hypophosphatemia during long-term combination therapy of ADV and LAM. In the present study, we investigated the incidence of renal impairment and hypophosphatemia associated with long-term use of ADV–LAM combination in patients with CHBI and defined the characteristics of those patients who developed the above side effects.

Patients and methods

Patients

The study group comprised 292 Japanese patients who were treated with the combination therapy of ADV and LAM between November 2002 and December 2011 at Toranomon Hospital, Tokyo, Japan. Patients were included in this study if they met the following criteria: (1) patients with LAM-refractory CHBI who commenced ADV add-on LAM at Toranomon Hospital; (2) the starting dose of ADV was 10 mg/day; (3) normal renal function at the commencement of ADV (serum creatinine < 1.2 mg/dl and estimated glomerular filtration rate (eGFR) of ≥ 50 ml/min/1.73 m^2); (4) patients who received the combination therapy for more than 6 months. Furthermore, we excluded patients who had history of treatment with other nucleotide analogs and co-infection with hepatitis C virus or human immunodeficiency virus (HIV).

Study protocol

Patients visited our hospital every 1–3 months after the initiation of ADV treatment, and blood samples were obtained at every visit. We evaluated virological and biochemical markers at the start of ADV and every 6 months thereafter. The eGFR was calculated by the Japanese GFR equation [$194 \times Cr^{1.094} \times age^{0.287}$ ($\times$ 0.739 for females)]. Renal impairment represented a decrease in eGFR to < 50 ml/min/1.73 m^2, while hypophosphatemia was defined by serum phosphate level of < 2.5 mg/dl. The dosing interval of ADV was modified by the attending physician when serum creatinine level increased to > 1.2 mg/dl. Liver cirrhosis was defined by presence of stage 4 fibrosis on histopathological examination and/or clinical evidence of portal hypertension.

The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and approved by the ethics committee of Toranomon Hospital.

Statistical analysis

Descriptive statistics were reported as proportion (%) for categorical variables, and median values (range) for continuous variables. The Mann–Whitney U test was used to compare two continuous variables, and Fisher's exact test or Chi square test was used to compare two categorical variables. The cumulative incidences of renal impairment and hypophosphatemia were calculated using the Kaplan–Meier method and group data were evaluated using the log-rank test. The Cox proportional hazard regression model was used to estimate univariate and multivariate risk factors for renal dysfunction and hypophosphatemia. Wilcoxon rank sum test was used to compare changes in the median values of eGFR and serum phosphate. Statistical significance was defined with two-tailed P value of < 0.05. Statistical analyses were performed using The Statistical Package for Social Sciences (version11; SPSS, Chicago, IL).

Results

Baseline characteristics

Table 1 lists the baseline clinical and laboratory characteristics at the start of ADV. The total duration of the combination therapy of ADV and LAM was 64.3 months (range: 6–118). The median age of the patient was 47 years

Table 1 Baseline characteristics

n	292
Age (years)	47 (25–75)
Male sex	228 (78.1 %)
Body weight (kg)	63 (39.9–92.5)
Body mass index (kg/m^2)	22.2 (15.8–36.9)
Treatment duration (months)	64.3 (6.0–118)
Current cirrhosis	67 (22.9 %)
Current and/or history of HCC	48 (16.4 %)
History of diabetes mellitus	17 (5.8 %)
History of hypertension	42 (14.4 %)
Genotype (A/B/C/others or unknown)	13/15/240/24 (4.5/5.1/82.2/8.2 %)
HBeAg (positive/negative/unknown)	114/176/2 (39.0/60.3/0.7 %)
Serum HBV-DNA (logIU/ml)	6.9 (< 2.1 to ≤9.0)
Total bilirubin (mg/dl)	0.7 (0.2–6.0)
Alanine aminotransferase (IU/ml)	86 (9–3156)
Albumin (g/dl)	3.9 (2.4–4.7)
Platelet($\times 10^4$/mm^3)	16.1 (3.1–45.2)
Creatinine (mg/dl)	0.8 (0.4–1.1)
eGFR (ml/min/1.73 m^2)	85.2 (51.2–179.9)
Inorganic phosphate (mg/dl)	3.2 (1.6–4.6)

Values are expressed as median (range), or number of patient (%)

eGFR estimated glomerular filtration rate, *HCC* hepatocellular carcinoma

(25–75), and patients were mostly men (78.1 %). Sixty-seven (22.9 %) patients had cirrhosis before starting ADV, and 48 patients (16.4 %) had a history of HCC or had HCC at study entry. Forty-two (14.4 %) patients had diabetes mellitus, and 17 (5.4 %) had arterial hypertension. The median body weight was 63 kg (39.9–92.5), and median BMI was 22.2 kg/m^2 (15.8–36.9). Baseline eGFR was 85.2 ml/min/1.73 m^2 (51.2–179.9), and phosphate was 3.2 mg/dl (1.6–4.6).

ADV-induced nephrotoxicity

Frequency of renal impairment

Twenty-eight (9.6 %) patients developed renal impairment during the combination therapy. The eGFR decreased 20–30 % from baseline in 67 (22.9 %) patients, 30–50 % in 54 (18.5 %) patients, and >50 % in 5 (1.7 %) patients. Figure 1 displays the cumulative incidence of renal impairment. Figure 1a shows the time to eGFR of < 50 ml/min/1.73 m^2 (i.e., renal impairment). The 1-, 3-, and 5-year cumulative incidence of renal impairment was 1.4, 7.5, and 10.5 %, respectively. Figure 1b shows the time to reduction in eGFR of ≥30 % from baseline. The 1-, 3-, 5-year

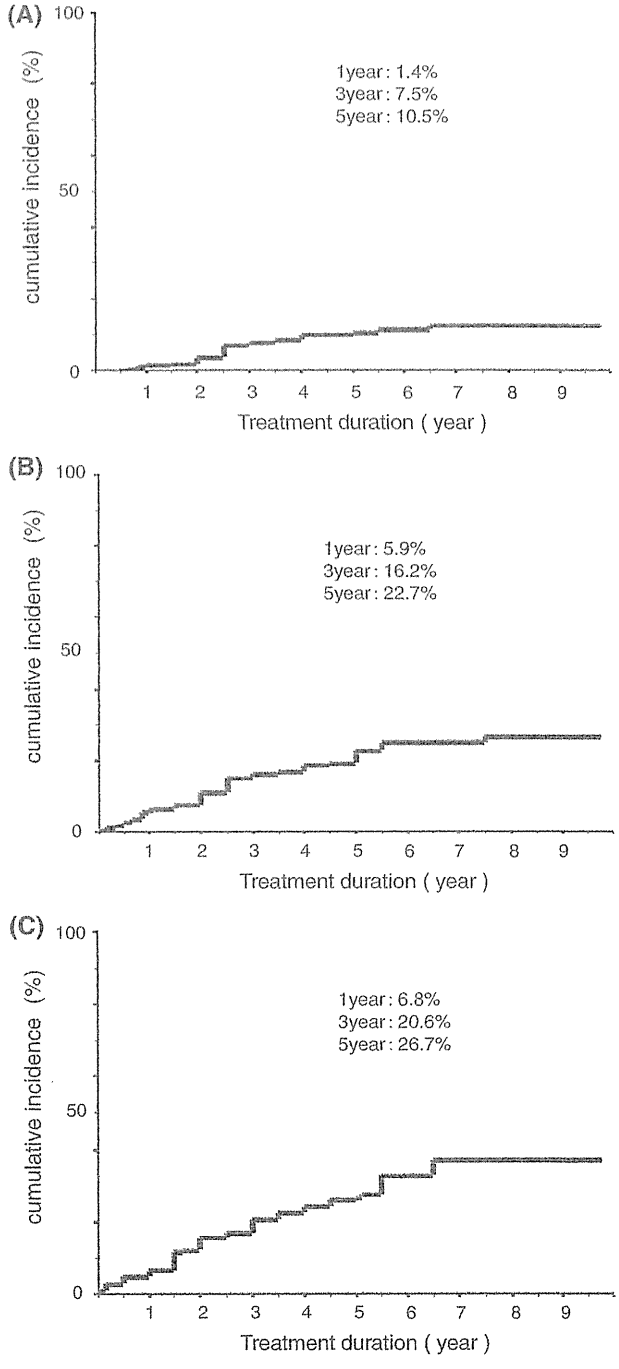

Fig. 1 Cumulative incidence of renal impairment and hypophosphatemia. **a** Cumulative incidence of reduction of eGFR to less than 50 ml/min/1.73 m^2 at 1-, 3-, and 5-years of treatment with ADV and LAM. **b** Cumulative incidence of reduction of eGFR by ≥30 % relative to baseline at 1-, 3-, and 5-years of treatment with ADV and LAM. **c** Cumulative incidence of hypophosphatemia among 269 patients with baseline IP of ≥2.5 mg/dl

cumulative incidence of reduction in eGFR ≥30 % was 5.9, 16.2, 22.7 %, respectively. We also evaluated renal function using serum creatinine. Serum creatinine increased to more

than 1.2 mg/dl in 34 (11.6 %) patients during the study period. The 1-, 3-, and 5-year cumulative incidence of serum creatinine of ≥1.2 mg/dl was 1.4, 6.51, and 11.4 %, respectively. The proportion of patients who developed renal impairment started to increase about 2 years after the commencement of ADV.

Time-course of renal impairment

Figure 2a shows serial changes in the median value of eGFR after the addition of ADV to LAM. We excluded from this analysis those patients in whom the dose of ADV was reduced at the point of modification. The eGFR of 264 patients without renal impairment remained stable throughout the study. On the other hand, the eGFR of 28 patients with renal impairment decreased rapidly within about 2 years after the addition of ADV.

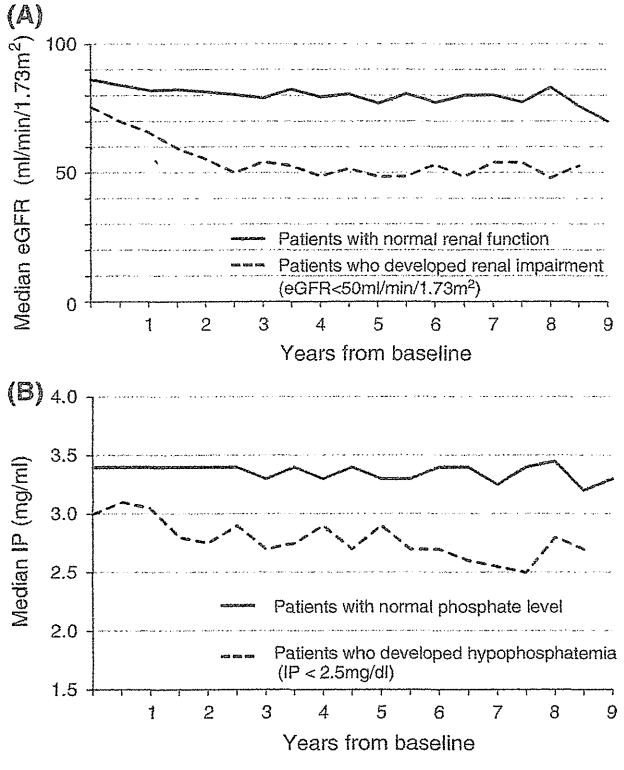

Fig. 2 Clinical course after the addition of ADV to LAM for treatment of chronic hepatitis B infection. **a** Changes in median eGFR level after the addition of ADV to LAM. *Solid line* patients with normal renal function, *broken line* patients who developed renal impairment (excluding patients who required reduction of the dose of ADV at the point). **b** Changes in the median level of serum phosphate after the addition of ADV to LAM. *Solid line* patients with normal phosphate level, broken line: patients who developed hypophosphatemia (excluding patients required reduction of the dose of ADV at the point)

Predictive factors for renal impairment

The results of univariate and multivariate analyses, including the hazard ratio for eGFR to < 50 ml/min/1.73 m^2, are shown in Table 2. Univariate analysis showed that old age (≥ 50 years, $P < 0.0001$), current cirrhosis ($P < 0.0001$), current and/or history of HCC ($P = 0.001$), history of hypertension ($P < 0.0001$), mild renal dysfunction at baseline (eGFR < 80 ml/min/1.73 m^2, $P = 0.001$), and thrombocytopenia (platelet count < 15 × 10^4/mm^3, $P = 0.003$) were associated with the development of nephrotoxicity. Multivariate analysis indicated that old age ($P = 0.006$), cirrhosis ($P = 0.011$), and history of hypertension ($P = 0.005$) were significant predictors of renal impairment.

Univariate and multivariate analyses were also performed for a fall in eGFR of ≥30 % relative to baseline. The results of univariate analysis showed that old age ($P < 0.0001$), female sex ($P = 0.007$), small body weight (< 60 kg, $P = 0.002$), history of diabetes mellitus ($P < 0.0001$), mild renal dysfunction at baseline ($P = 0.018$), hypo-albuminemia ($P = 0.010$), and thrombocytopenia ($P = 0.007$) were associated with decrease in eGFR of ≥30 % relative to baseline. On the other hand, multivariate analysis identified old age ($P < 0.001$), small body weight ($P = 0.015$), history of diabetes mellitus ($P = 0.020$), and mild renal dysfunction at baseline ($P < 0.0001$) as significant predictors of fall in eGFR of ≥30 % relative to baseline.

In either case, old age was a significant contributing factor of ADV-induced renal impairment. History of diabetes mellitus and arterial hypertension were also significant predictors.

Effect of modification of ADV dosing interval on renal impairment

Seventeen (5.8 %) patients required modification of the ADV dosing interval because of renal impairment. The ADV dosing interval was changed from 10 mg every day to 10 mg every other day when creatinine increased to ≥1.2 mg/dl. The clinical characteristics of the 17 patients could be summarized as follows: all were men with a median age of 54 years (35–63), 8 (47.1 %) patients had cirrhosis, 4 (23.5 %) patients had a history of HCC, baseline eGFR was 69.3 ml/min/1.73 m^2 (58.2–89.3), phosphate was 3.3 mg/dl (2.1–3.9), and the median time to modification of ADV dose was 48.5 months (20.7–70.0). Figure 3a shows changes in eGFR and Fig. 3b shows changes in serum phosphate after modification of the ADV dosing interval. The dose modification significantly improved eGFR and serum phosphate as measured at 6 months and 1 year after the modification. Analysis of the

Table 2 Determinants of renal impairment (eGFR less than 50 ml/min/1.73 m^2)

	Univariate analysis		Multivariate analysis	
	HR (95 % CI)	*P* value	HR (95 % CI)	*P* value
Age ≥50 years	7.661 (2.898–20.252)	<0.0001	4.280 (1.505–12.169)	0.006
Male sex	1.227 (0.464–3.236)	0.680		
Body weight < 60 (kg)	1.470 (0.687–3.145)	0.320		
Current cirrhosis	5.344 (2.479–11.518)	<0.0001	2.861 (1.279–6.401)	0.011
Current and/or history of HCC	3.855 (1.788–8.311)	0.001		
History of diabetes mellitus	2.841 (0.982–8.149)	0.054		
History of hypertension	5.116 (2.393–10.938)	<0.0001	3.087 (1.403–6.791)	0.005
Baseline eGFR < 80 (eGFR ≥50)	4.219 (1.786–10.00)	0.001		
Baseline IP < 3.2 mg/dl	1.634 (0.766–3.497)	0.204		
Platelet count < 15 × 10^4/mm^3	3.448 (1.511–7.874)	0.003		

CI confidence interval, *IP* inorganic phosphate, *HCC* hepatocellular carcinoma, *HR* hazard ratio

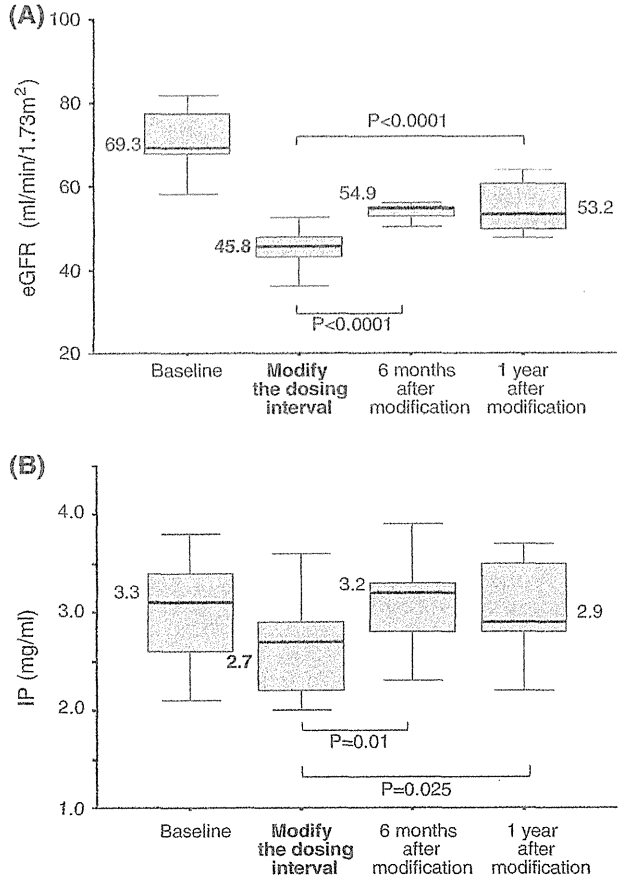

Fig. 3 Changes in eGFR and serum phosphate after modification of the ADV dosing interval. **a** Changes in eGFR. **b** Changes in serum phosphate level

long-term courses of eGFR and phosphate in these 17 patients after modification of ADV showed that the median eGFR after 1-, 2-, and 3- years of modification was 53.2, 56.7, 53.9 ml/min/1.73 m^2, respectively. eGFR remained > 50 ml/min/1.73 m^2 after modification, but never recovered to baseline level. None of the patients required discontinuation of ADV due to renal impairment.

ADV-induced hypophosphatemia

Frequency of hypophosphatemia

Seventy-three (27.1 %) of 269 patients who had normal phosphate at baseline developed hypophosphatemia during the course of the study. Fourteen (19.1 %) of the 73 patients who developed hypophosphatemia continued to show hypophosphatemia until the end of the study. On the other hand, the remaining 59 patients developed transient hypophosphatemia only. The cumulative incidence of hypophosphatemia is shown in Fig. 1c. The 1-, 3-, and 5-year cumulative incidence of hypophosphatemia was 6.8, 20.6, and 26.7 %, respectively. On the other hand, 23 patients had hypophosphatemia at baseline. Seven (30.4 %) of these 23 patients had chronic hypophosphatemia. The phosphate level of 4 (17.4 %) patients reverted spontaneously to normal, while serum phosphate level of the other 12 (52.2 %) patients fluctuated during the study.

Time-course of hypophosphatemia

Figure 2b shows changes in the median serum level of phosphate after the addition of ADV to LAM. We excluded from this analysis those patients in whom the dose of ADV was reduced at the point of modification. The median phosphate level decreased gradually after the addition of ADV in patients who subsequently developed hypophosphatemia.

Predictive factors for hypophosphatemia

Table 3 shows the results of univariate and multivariate analyses, including hazard ratio, of the factors associated

Table 3 Determinants of hypophosphatemia

	Univariate analysis		Multivariate analysis	
	HR (95 % CI)	*P value*	*HR (95 % CI)*	*P value*
Age ≥50 years	1.325 (0.836–2.100)	0.230		
Male sex	3.690 (1.600–8.475)	0.002	2.824 (1.212–6.759)	0.016
Body weight < 60 kg	1.417 (0.850–2.360)	0.181		
Current cirrhosis	1.854 (1.143–3.008)	0.012		
Current and/or history of HCC	1.824 (1.089–3.054)	0.022	1.871 (1.106–3.166)	0.020
History of diabetes mellitus	1.355 (0.546–3.362)	0.513		
History of hypertension	1.558 (0.870–2.791)	0.136		
Baseline eGFR < 80 (eGFR ≥50)	1.264 (0.788–2.029)	0.332		
Baseline IP < 3.2 mg/dl	3.155 (1.965–5.051)	<0.0001	2.833 (1.751–4.032)	<0.0001
Platelet count < 15 × 10^4/mm^3	1.472 (0.925–2.342)	0.103		

Abbreviations as in Table 2

with a fall in serum phosphate level to < 2.5 mg/dl. Patients with baseline serum phosphate of < 2.5 mg/dl ($n = 23$) were excluded from the analysis. Univariate analysis showed that male sex ($P = 0.002$), cirrhosis ($P = 0.012$), current and/or history of HCC ($P = 0.012$), and low baseline phosphate level ($P < 0.0001$) correlated with hypophosphatemia. On the other hand, multivariate analysis identified male sex ($P = 0.016$), current and/or history of HCC ($P = 0.020$), and low baseline serum phosphate level ($P < 0.0001$) as significant determinants of ADV-induced hypophosphatemia.

Further analysis showed that decreases in eGFR of more ≥30 % relative to the baseline value in 2.5 years correlated significantly with hypophosphatemia ($P = 0.007$).

Effect of modification of ADV dosing interval on hypophosphatemia and liver function

The median serum phosphate level after 1-, 2-, and 3- years of modification of ADV dose was 2.9, 3.1, and 3.0 mg/dl, respectively. Serum phosphate level fluctuated even after the dose modification. We also analyzed changes in serum ALT and HBV-DNA. After ADV dose modification, serum ALT level decreased to within the normal range (ALT < 40 IU/L) in 16 of 17 patients. Although serum ALT level of the remaining single case increased transiently after the modification, it normalized 1 year later. The HBV-DNA level was below the detection level at ADV dose modification in 14 of the 17

Table 4 Clinical features of patients with persistent ADV-induced hypophosphatemia

Case no.	Sex	Age (years)	BW (kg)	LC/CH/HCC	Baseline					Min. IP	Max. ALP	Min. UA	Max. Cr	Fall in eGFR (%)	Ostealgia
					IP	ALP	UA	Cr	eGFR						
1	F	63	64.6	LC/HCC	2.7	323	5.4	0.5	93.3	0.8	1081	1.2	0.9	47.9	+
2	F	73	57.2	CH	3.6	285	4.1	0.5	89.3	1.9	1102	2.2	0.8	41.1	+
3	M	35	61.4	CH	3.9	149	4.3	0.8	89.3	2.2	174	3.4	1.2	37.8	–
4	M	57	66.2	LC/HCC	2.9	361	2.8	0.8	77.7	2.2	742	1.7	1.2	37.1	–
5	F	40	60.4	CH	2.9	259	4.9	0.5	105.8	1.1	1012	2.5	0.7	33.1	–
6	M	47	57.4	CH	3.9	203	3.9	0.7	95.1	1.8	241	3.1	1.0	32.3	–
7	M	50	70.2	LC/HCC	3.4	300	5.4	0.6	110.2	1.1	351	5.3	0.8	29.3	–
8	M	41	80.3	LC/HCC	2.7	206	5.3	0.8	85.3	2.0	268	4.3	1.0	23.2	–
9	M	58	73.0	CH	2.6	259	2.9	0.9	67.8	2.2	378	2.2	1.1	20.5	–
10	M	31	89.0	LC	3.4	180	4.4	0.8	92.2	1.6	502	1.8	0.9	17.7	+
11	M	34	62.9	CH	2.7	111	6.4	0.6	123.7	2.2	179	4.6	0.7	16.2	–
12	M	49	83.0	CH	3.1	442	6.1	0.8	80.9	2.2	383	5.0	0.9	14.5	–
13	M	40	83.9	LC/HCC	3.7	216	6.9	0.9	75.4	1.9	383	6.0	1.0	10.9	–
14	M	39	66.0	CH	4.1	144	6.4	1.0	67.7	2.1	179	6.3	1.1	9.9	–

Fall in eGFR represents fall in eGFR relative to the baseline

BW body weight, *IP* inorganic phosphate, *ALP* alkaline phosphatase, *UA* uric acid, *Cr* creatinine, *LC* liver cirrhosis, *CH* chronic hepatitis, *HCC* hepatocellular carcinoma

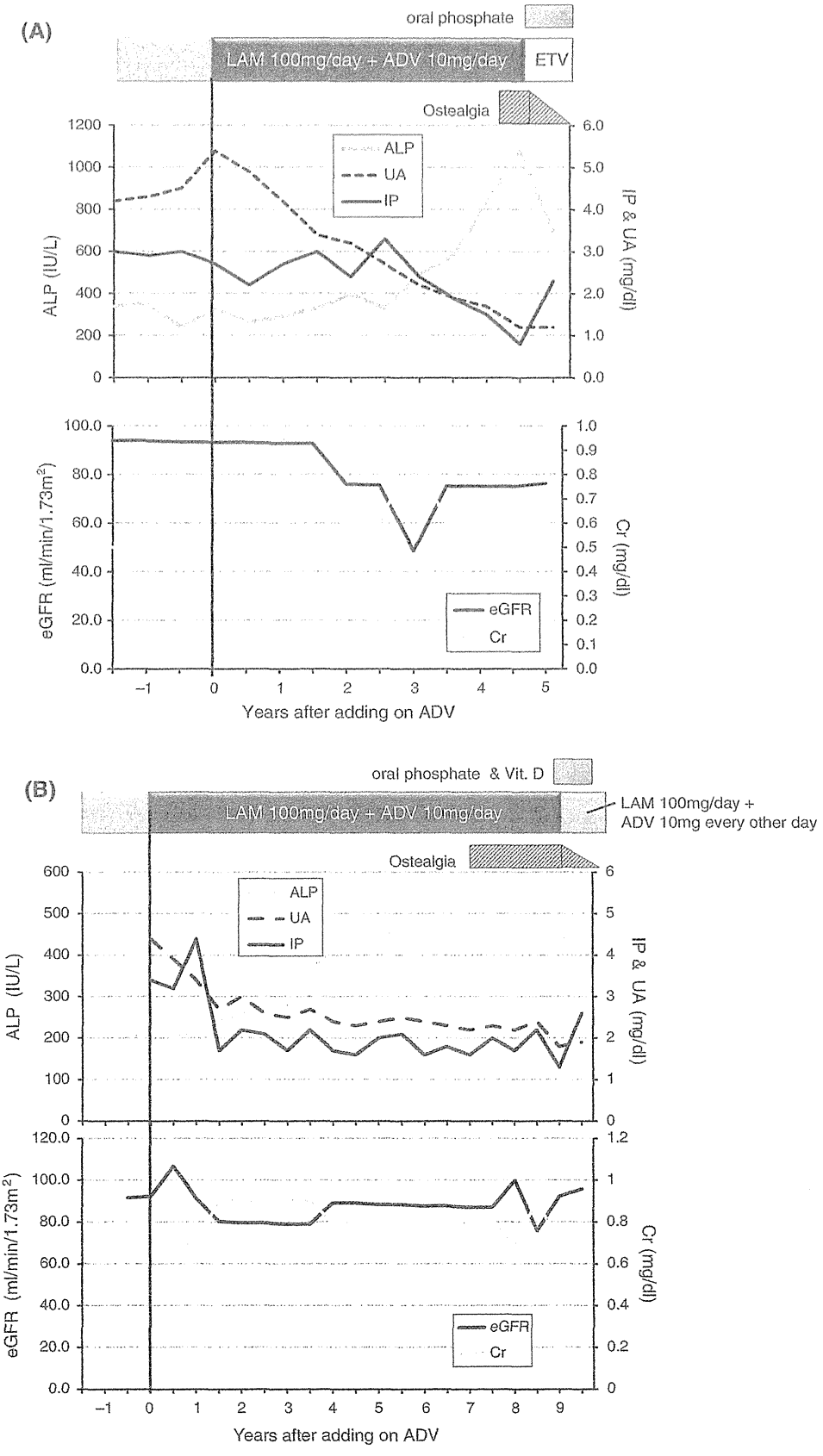

Fig. 4 Two cases who developed Fanconi's syndrome. **a** Case 1: a 63-year-old woman with HBeAg-positive liver cirrhosis. **b** Case 10: a 31 year-old man with HBeAg-positive liver cirrhosis

patients, and the level did not increase after the modification. The remaining three patients with detectable HBV-DNA at modification did not show any change in HBV-DNA.

Patients with persistent hypophosphatemia

Fourteen (5.2 %) patients developed persistent hypophosphatemia. There were no significant differences in clinical features and results of laboratory tests at baseline between patients with transient and persistent hypophosphatemia. Table 4 lists the clinical features of these patients. Three of these patients complained of bone pain during treatment. They had markedly elevated alkaline phosphatase (ALP) and low serum uric acid (UA) levels during the combination therapy. Their serum creatinine level remained normal, but their eGFR decreased relative to baseline. Figure 4 provides a summary of the clinical course of cases 1 and 10.

Case 1 was a 63-year-old woman with HBeAg-positive liver cirrhosis. She was first treated with LAM for chronic hepatitis, but ADV was added 17 months later due to the development of LAM resistance. The laboratory data (serum phosphate, ALP, UA and creatinine) were within normal ranges at baseline, and she had no other health problems. Continuous treatment with ADV for about 3 years resulted in increase in ALP level and decrease in UA and serum phosphate. After 4.5 years, she developed lumbago and right ankle pain. Blood tests showed ALP of 1102 IU/ml, UA of 1.2 mg/dl, and serum phosphate of 0.8 mg/dl. Other laboratory tests demonstrated metabolic acidosis, aminoaciduria, low tubular reabsorption of phosphate (34.8 %; normal value 85–98 %), and high fractional excretion of uric acid (47.6 %; normal value 4–14 %). These results indicated generalized dysfunction of the proximal renal tubules. A technetium bone scan showed increased uptake in bilateral ribs, carpal bones, lumbar spine, and bilateral calcaneus. She was diagnosed with acquired Fanconi's syndrome with hypophosphatemic osteomalacia associated with ADV therapy. ADV was discontinued and replaced with entecavir (ETV) while hypophosphatemia was treated with oral phosphate. Three months after cessation of ADV and oral phosphate supplementation, the patient reported symptomatic improvement and blood tests showed normalization of phosphate level and low ALP level.

Case 10 was a 31-year-old man with HBeAg-positive liver cirrhosis. He was also first treated with LAM, and ADV was added on 16 months later. The laboratory data were within the normal ranges at baseline. Treatment for 1.5 year with ADV resulted in decrease in serum phosphate and UA, and 4-year treatment increased ALP level. After 7 years, the right metatarsal bone broke in an accident. After 9 years of treatment, blood tests showed serum phosphate of 1.3 mg/dl. Detailed clinical examination was conducted at that stage. Other laboratory tests showed aminoaciduria, low tubular reabsorption of phosphate (65.5 %), and high fractional excretion of uric acid (19.1 %). A technetium bone scan showed increased uptake in bilateral ribs, bilateral ankles, tarsal bones, and right metatarsal. He was also diagnosed with acquired Fanconi's syndrome and hypophosphatemic osteomalacia associated with ADV therapy. ADV dosing interval was changed from 10 mg every day to 10 mg every other day, and oral phosphate supplementation and calcitriol were added to the treatment. Treatment for 2 months resulted in improvement of symptoms and normalization of phosphate level.

Discussion

Renal impairment is one of the most serious adverse effects of ADV. The following mechanism is considered to explain ADV-induced nephrotoxicity: the human organic anion transporter-1 (hOAT1) is a renal membrane protein expressed at the basolateral membrane of the proximal tubule cells. hOAT1 can efficiently transport cyclic nucleoside phosphonate, and thus contribute to ADV nephrotoxicity by accumulation of the drug in renal proximal tubules [18, 19].

Previous studies indicated that the ADV-related nephrotoxicity is dose-dependent [12]. In a large-scale clinical trial, 8 % of patients treated with 30 mg/day ADV for 48 weeks had high serum creatinine (≥0.5 mg/dl), relative to baseline. On the other hand, none of the patients treated with 10 mg/day ADV showed increase in creatinine (≥0.5 mg/dl), relative to baseline [20]. Thus, ADV at a dose of 10 mg/day has been used previously for the treatment of patients with CHBI. However, renal dysfunction has been reported even after the use of ADV at this dose, especially after long-term administration [13–15]. For example, in a study of the 10 mg ADV combined with LAM, serum creatinine increased in 38 % of patients following median treatment duration of 38 months [14]. In another retrospective study of 687 patients, during a median treatment period of 27 months, 10.5 % of patients developed renal impairment, which was defined as a decrease in eGFR of more than 20 % relative to the baseline [15]. In our study, 9.6 % of patients developed renal impairment during a median treatment duration of 64.3 months. Our results also showed that 20.2 % of the patients exhibited more than 30 % decrease in eGFR, and a much larger proportion (43.2 %) of the patients showed more than 20 % decrease in eGFR. These rates are higher than those reported previously. Furthermore, as shown in

Fig. 2a, patients with rapid falls in eGFR within the first 2 years of treatment should be carefully monitored for any renal dysfunction. Based on the results of our study, it seems that longer dosing period is associated with higher incidence of renal dysfunction.

We also analyzed the risk factors of renal impairment defined by a decrease in eGFR to less than 50 ml/min/1.73 m^2. Ha et al. [13] reported that age >50 years, mild renal impairment at baseline, hypertension and/or diabetes mellitus, and male sex were significant predictors of renal impairment characterized by decrease in eGFR of ≥20 % relative to baseline. Furthermore, Yu et al. [15] also reported that age ≥50 years was a significant predictor of renal dysfunction in those patients treated with ADV. In our study, age was also identified as a significant and independent determinant of the primary endpoint, together with liver cirrhosis and history of arterial hypertension. Considered together, these data indicate that care should be taken when ADV-based therapy is used for elderly patients with CHBI.

Cross-sectional studies have demonstrated a decline in GFR with age [21, 22]. Moreover, hypertension and diabetes mellitus are also reported to worsen the rate of decline of renal function [23–25]. Renal failure is common and often severe in patients with cirrhosis due to the activation of various vasoconstrictor systems, including the renin–angiotensin system and the sympathetic nervous system [26]. Taken together, eGFR is more likely to decrease during ADV therapy in patients with older age, hypertension, diabetes mellitus, cirrhosis, mild renal dysfunction at baseline.

ADV-induced proximal tubule failure can lead to hypophosphatemia. In a randomized clinical control trial using 120 mg/day ADV for treatment of patients with HIV, hypophosphatemia occurred in 50 % of patients after 48 weeks and in 61 % of patients after 72 weeks of ADV treatment [27]. On the other hand, in another study using 10 mg/day ADV for patients with CHBI, there was no overall change in serum phosphorus level during the 96-week study period [28]. However, in recent years, several reports have described the development of hypophosphatemia in patients treated with ADV at a daily dose of 10 mg [14, 29]. In our study, 27.1 % of patients developed hypophosphatemia during the combination therapy. Although 21.9 % of patients developed transient hypophosphatemia, 5.2 % of patients who had normal phosphate level at baseline developed persistent hypophosphatemia. In this regard, one previous study reported that approximately 2 % of hospitalized patients had hypophosphatemia [30]. Collectively, the above results and our findings indicate that ADV-based treatment is associated with a high incidence of hypophosphatemia. Tamori et al. [14] reported that serum phosphate level decreased to less than 2.5 mg/ml in 16.2 % of their patients during the 38-month combination therapy. Gara et al. [29] reported that 14 % of their patients treated with nucleotide analog therapy (10 mg/day ADV combined with 100 mg/day LAM, or 300 mg/day tenofovir monotherapy) developed persistent hypophosphatemia. Analysis of our data identified male sex, presence and/or history of HCC, and low serum phosphate level at baseline as significant determinants of hypophosphatemia. Furthermore, a decrease in eGFR by ≥30 % relative to baseline within 2.5 years was also associated with the development of hypophosphatemia.

Hepatic insufficiency is associated with impairment in 25-hydroxylation of vitamin D in the liver, which can lead to reduced synthesis of 1, 25 (OH) 2D3, with subsequent worsening of hypophosphatemia based on reduced intestinal absorption of phosphorus [31, 32]. In our study, 73 % of patients with HCC had liver cirrhosis, and the presence and/or history of HCC was a predictor of hypophosphatemia. Another mechanism of hypophosphatemia is protein and calorie malnutrition, which is a common feature of chronic liver disease. Furthermore, invasive treatment of HCC may itself cause hypophosphatemia. The present study also analyzed the relation between gender and hypophosphatemia. In a study that enrolled more than 4500 community-dwelling Italians of broadly diverse age, serum phosphorus levels were similar in males and females until the age of 45 years [33]. Interestingly, serum phosphate level increased in females aged between 45 and 54 years but fell after 55 years of age. The increase in serum phosphate level in females is probably related to menstrual status [33]. In the present study, serum phosphate level was higher in females than in males at baseline (3.51 vs. 3.18, $P < 0.0001$). Thus, male sex was a significant determinant of hypophosphatemia. These findings call for careful monitoring of serum phosphate level in patients treated with ADV, especially male patients, patients with HCC, and patients with renal dysfunction.

Several studies described the development of Fanconi's syndrome and subsequent hypophosphatemic osteomalacia in patients treated with 10 mg/day ADV [14, 16, 17]. Fanconi's syndrome is characterized by generalized transport defect in the proximal tubules, leading to renal losses of glucose, phosphate, uric acid, amino acids, bicarbonate, and other organic compounds [34]. Severe hypophosphatemia seems to cause inadequate mineralization of bone matrix, with subsequent osteomalacia [35, 36]. The electrolyte imbalance and osteomalacia cause symptoms of muscle weakness, fatigue, ostealgia, and bone fractures [37]. Acquired renal tubular defect resulting in Fanconi's syndrome have been described in association with many exogenous agents, including valproate, aminoglycosides, tetracycline, and acyclic nucleoside phosphonates [34].

Various approaches have been used for the treatment of osteomalacia associated with Fanconi's syndrome. Clarke et al. [38] reported successful treatment of osteomalacia associated with acquired Fanconi's syndrome with calcium, phosphate and vitamin D, regardless of the underlying cause of the disease. Eight cases of Fanconi's syndrome with ADV-related hypophosphatemic osteomalacia were reported in the past 5 years [14, 16, 17, 39–41]. Three of the 8 patients were treated with oral phosphate only; while 3 other patients received oral phosphate and vitamin D, and one patient was treated with the combination of oral phosphate, vitamin D and calcium. In all cases, treatment increased serum phosphate level and improved musculoskeletal symptoms. Similar to the eight cases reported in the literature, our 2 patients showed normalization of phosphate level and symptomatic improvement after treatment. Treatment with oral phosphate for ADV-related hypophosphatemic osteomalacia is considered effective.

We also examined the clinical characteristics of the 14 patients who developed persistent hypophosphatemia. Three of the 14 patients developed ostealgia during the treatment. Patients 1 and 10 were diagnosed with acquired Fanconi's syndrome with subsequent hypophosphatemic osteomalacia. Although we could not confirm the diagnosis of Fanconi's syndrome in patient 2 because she was transferred to another hospital, she was considered to have developed Fanconi's syndrome based on the clinical course. Despite persistent hypophosphatemia, serum creatinine remained within the normal range. In addition, 6 of the 14 patients also had low eGFR ($\geq$30 % decrease relative to baseline), and two patients with Fanconi's syndrome showed $\geq$40 % decrease in eGFR, relative to baseline. Based on the above features, patients can develop marked hypophosphatemia and serious complications, such as Fanconi's syndrome, following significant fall in eGFR, irrespective of the level of serum creatinine. In the three patients who developed Fanconi's syndrome, a gradual increase in serum ALP level and simultaneous fall in serum uric acid were noted more than one year before the appearance of ostealgia. Based on the above findings, we recommend reducing the dose or changing medications to other nucleotide analogues in patients who develop hypouricemia, hyper-ALPemia, hypophosphatemia, and low eGFR, to avoid the development of ADV-induced Fanconi's syndrome.

In our study, the dosing interval of ADV was modified by the attending physician following increase in serum creatinine level. Seventeen (5.8 %) patients required such modification, their eGFR and serum phosphate showed significant improvement at 6 and 12 months after the modification, in agreement with previous reports [13, 42]. However, the modification in ADV dosing interval from 10 mg every day to every other day neither affected HBV-DNA level nor the antiviral effect. Therefore, the ADV dose should be modified in patients who show decrease in eGFR and/or serum phosphate.

In conclusion, our results showed that even at low dose of 10 mg/day, long-term combination therapy of ADV and LAM can cause renal impairment and hypophosphatemia, and lead to Fanconi's syndrome in a subgroup of patients. ADV-based treatment tends to reduce eGFR and serum phosphate especially in elderly male patients and those with HCC. We recommend regular monitoring of serum phosphate and evaluation of eGFR, in addition to serum creatinine, in patients treated with ADV. Suspicion of Fanconi's syndrome requires early reduction of ADV dose or switching to other antiviral agents.

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Conflict of interest None.

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Seroclearance rate of hepatitis B surface antigen in 2,112 patients with chronic hepatitis in Japan during long-term follow-up

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Abstract
Background Rate of hepatitis B surface antigen (HBsAg) seroclearance was determined in 2,112 Japanese patients with chronic hepatitis B who were followed up for at least 15 years.
Methods Patients had a median age of 37 years and included 1,431 (67.8 %) men. Median values were AST/ALT, 43/62 IU/L; platelet counts, 182 × 10^3/mm^3; HBsAg, 3,400 IU/mL; and hepatitis B virus (HBV) DNA, 6.2 log copies/mL. Factors influencing HBsAg seroclearance were evaluated by the Cox proportional model and annual rate of HBsAg seroclearance by the Kaplan–Meier life table method.
Results The overall annual rate of HBsAg seroclearance was 1.75 % in 2,112 patients; it was 1.65 % in 1,130 untreated and 2.05 % in 982 treated patients ($p = 0.289$). In untreated patients, seroclearance was influenced by age, no HBV infections in third-degree or closer relatives, and HBsAg levels in univariate analysis. Seroclearance was influenced by a median age ≥50 years [relative risk (RR) 1.61 ($p = 0.018$)] and HBsAg ≤2,000 IU/mL [RR 1.77 ($p = 0.014$)] in multivariate analysis. In treated patients, age, male gender, no HBV infections in third-degree or closer relatives, interferon therapy, chronic hepatitis, high AST and γ-GTP levels, low platelet counts, hepatitis B e antigen (HBeAg)-negative status, low HBsAg levels and the wild-type precore sequence significantly influenced HBsAg seroclearance. In multivariate analysis, no family history [RR 2.22 ($p = 0.006$)], interferon treatment [RR 3.15 ($p < 0.001$)], and HBeAg-negative status [RR 3.75 ($p < 0.001$)] significantly influenced HBsAg seroclearance.
Conclusions In this retrospective cohort study, the annual rate of HBsAg seroclearance was 1.65 % in untreated patients and 2.05 % in treated patients.

Keywords Seroclearance · Hepatitis B surface antigen · Hepatitis B virus · Chronic hepatitis B

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ETV	Entecavir
HBeAg	Hepatitis B e antigen
HBcrAg	Hepatitis B core-related antigen
HBV	Hepatitis B virus
HBV DNA	Hepatitis B virus DNA
HBsAg	Hepatitis B surface antigen
IFN	Interferon
LAM	Lamivudine

Introduction

Worldwide, an estimated 400 million people are infected with hepatitis B virus (HBV) persistently. HBV infection is a common disease that can induce a chronic carrier state

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and is associated with the risk of developing progressive disease and hepatocellular carcinoma (HCC) [1–5]. In regions highly endemic for HBV, such as Asia and Africa, the persistent carrier state is established by perinatal transmission or early in infancy. Carriers serve as the reservoir of HBV in the community and can spread the infection to susceptible individuals. The incidence of HCC is decreased extremely by eradicating HBV from the circulation that is responsible for liver damage [6–9]. In Japan, interferon (IFN) was introduced for the treatment of persistent HBV infections, and long-term IFN increased seroclearance of hepatitis B surface antigen (HBsAg) [10]. Since 2000, the effect of long-term nucleot(s)ide analogues, such as lamivudine [11, 12] and entecavir [13], on HBsAg seroclearance has been monitored in Japan.

In the current study, we followed untreated or treated patients for at least 15 years. We evaluated the seroclearance of HBsAg, achieved in both groups of patients, by using highly sensitive assays. Our aim was to determine factors that can lead to HBsAg seroclearance and to elucidate the factors associated with its success.

Patients and methods

Patients

During at least 15 years from 1968, 2,112 consecutive patients, chronically mono-infected with HBV (confirmed by HBsAg-positivity for at least 6 months) were followed at the Department of Hepatology, Toranomon Hospital, in Metropolitan Tokyo. Patients met the following inclusion and exclusion criteria: (1) negativity for hepatitis C antibody and/or hepatitis C virus RNA by polymerase chain reaction (PCR) in the serum; (2) no history of HCC; and (3) no history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, or chronic liver disease other than chronic hepatitis B. Thus, the 2,112 patients were enrolled in this cohort study. A written informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved a priori by the institution's human research committee.

Treatment

Nine hundred and eighty-two patients received antiviral treatments. Of them, 156 patients received prednisolone (PSL) 40 mg daily for 1 week, 30 mg daily for 1 week, 20 mg daily for 1 week, and then 10 mg daily for 1 week until it was abruptly withdrawn (total 700 mg). A total of 428 patients received 100 mg lamivudine (LAM) daily as an initial therapy. In total, 333 patients received 3–12 MU of IFN-α or IFN-β. The durations and regimens of treatment were as follows: daily for 2 or 4 weeks and then 2 or 3 times per week for 26–104 weeks. The median duration of treatment was 26 weeks (range 4–981). There were 190 (57 %) patients who received multiple treatments of IFN.

LAM treatment was continued as a rule; median duration of LAM treatment was 75 months (55–102). LAM-resistant rtM204I/V mutants developed in 151 (35 %) of the 428 patients, and they were provided with adefovir dipivoxil (10 mg) added on LAM, as a rescue therapy. The remaining patients continued to receive LAM monotherapy. In addition, 65 patients received 0.5 mg entecavir (ETV) daily as an initial therapy. ETV treatment was continued as a rule, and median duration of ETV treatment was 45 months (1.0–104).

Markers of HBV infection

Serum HBsAg titers were determined annually using ARCHITECT HBsAg QT assay kits (Abbott Laboratories, Tokyo, Japan), which have a lower limit of detection of 0.05 IU/mL and an upper limit of detection of 250 IU/mL. To expand the upper limit from 250 to 125,000 IU/mL, serum samples going off the scale were diluted stepwise to 1:20 and 1:500 with ARCHITECT diluents following instructions from the manufacturer.

Hepatitis B e antigen (HBeAg) was determined by enzyme-linked immunosorbent assay with a commercial kit (HBeAg EIA; Institute of Immunology, Tokyo, Japan). HBV DNA was quantified using the Amplicor monitor assay (Roche Diagnostics, Tokyo, Japan) with a dynamic range of 2.6–7.6 log copies/mL, or COBAS TaqMan HBV v.2.0 (Roche Diagnostics, Tokyo, Japan) with a dynamic range of 2.1–9.0 log copies/mL. Hepatitis B core-related antigen (HBcrAg) was determined by chemiluminescence enzyme immunoassay (CLEIA) with the HBcrAg assay kit (Fujirebio Inc., Tokyo, Japan). A commercial kit (HBV Genotype EIA; Institute of Immunology, Tokyo, Japan) was used to serologically determine HBV genotypes by the combination of epitopes expressed on the pre-S2 region product, which is specific for each of the 7 major genotypes (A–G).

Statistical analysis

Baseline data were obtained on the day of the first visit in untreated patients. In patients who received antivirals, baseline data were obtained at the start of the first day of treatment. Categorical data were compared between groups by chi-squared or Fisher's exact tests. Continuous variables with a nonparametric distribution were analyzed by Mann–Whitney U tests, whereas those with a parametric distribution were analyzed by the Student's t test. Cox

regression analyses were used to assess variables that were significantly associated with HBsAg seroclearance. All baseline factors that were found to be significantly associated with HBsAg seroclearance by univariate analysis were entered into a multivariate analysis. Independent baseline factors associated with the seroclearance of HBsAg were evaluated using a stepwise Cox regression analysis. We then performed a time-dependent Cox regression to analyze independent factors associated with HBsAg seroclearance while on-treatment factors and independent baseline factors had been adjusted.

Cumulative HBsAg seroclearance rates were analyzed using the Kaplan–Meier method; differences in the resulting curves were evaluated using log-rank tests. Significance was defined as $p < 0.05$ for all two-tailed tests. Data analysis was performed with the SPSS software package version 11.0.1 J (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics in the 2,112 patients

The baseline characteristics of studied patients are shown in Table 1. They had a median age of 37 years (range 1–81), included 1,431 (67.8 %) men, and 2,031 (96.2 %) of them had chronic hepatitis. Their baseline values were AST/ALT, 43 (3–2,192)/62 (2–3,020 IU/L); γ-GTP, 27 (4–1,494) IU/L; platelet counts, 182 (40–483) × 10^3/mm^3; and HBV markers were HBsAg, 3,400 (0.06–27,700) IU/mL; and HBV DNA, 6.2 (<2.1 to >9.1) log copies/mL. HBeAg was not detectable in 5.4 % of studied patients, and the distribution of genotypes A/B/C/others was 4.5:15.6:79.6:0.3 %.

The HBsAg seroclearance rate analyzed by the Kaplan–Meier method was 9 % in 5 years, 17 % in 10 years, 27 % in 15 years, 35 % in 20 years, 44 % in 25 years, and 54 % in 30 years. The annual rate of HBsAg seroclearance was 1.75 % during 20 years (Fig. 1).

In the 2,112 patients, factors influencing HBsAg seroclearance in univariate analysis by the Cox regression analyses were cirrhosis [relative risk (RR) 2.40 ($p = 0.014$)]; HBeAg negative [RR 3.01 ($p = 0.001$)]; and HBsAg ≤2,000 IU/mL [RR 2.13 ($p = 0.004$)]. In multivariate analyses, only 2 factors contributed to HBsAg seroclearance: HBeAg negative [RR 1.81 ($p < 0.001$)]; and HBsAg ≤2,000 IU/mL [RR 2.60 ($p < 0.001$)] (Table 2).

Untreated patients and treated patients

Differences in the baseline characteristics between 1,130 untreated and 982 treated patients are shown in Table 3: age [31 years vs. 36 ($p < 0.001$)]; male gender [62.4 vs. 71.9 % ($p < 0.001$)]; AST [median 27 vs. 56 IU/L ($p < 0.001$)]; ALT [median 28 vs. 96 IU/L ($p < 0.001$)]; γ-GTP [median 20 vs. 45 IU/L ($p < 0.001$)]; total bilirubin

Table 1 Baseline characteristics 2,112 patients infected with HBV followed for longer than 15 years

Features at the baseline	Patients ($n = 2,112$)
Demographic data	
Age (years)	37 (1–81)
Men	1,431 (67.8 %)
Liver disease	
Chronic hepatitis	2,031 (96.2 %)
Cirrhosis	81 (3.8 %)
Laboratory data	
AST (IU/L)	43 (3–2,192)
ALT (IU/L)	62 (2–3,020)
γ-GTP (IU/L)	27 (4–1,494)
Total bilirubin (mg/dL)	0.7 (0.1–21.2)
Albumin (g/dL)	4.3 (1.1–5.8)
Platelets (×10^3/mm^3)	182 (40–483)
α-Fetoprotein (μg/L)	4 (1–2,060)
HBV markers	
HBeAg-negative status	1,169 (55.4 %)
HBsAg (IU/mL)	3,400 (0.06–277,000)
HBcrAg (log U/mL)	5.4 (<3.0 to >6.8)
Genotypes (A/B/C/others)	4.5 %/15.6 %/79.6 %/0.3 %
HBV DNA (log copies/mL)	6.2 (<2.1 to >9.1)

Median values with the range in parentheses or numbers with the percentage in parentheses are given

HBV hepatitis B virus, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

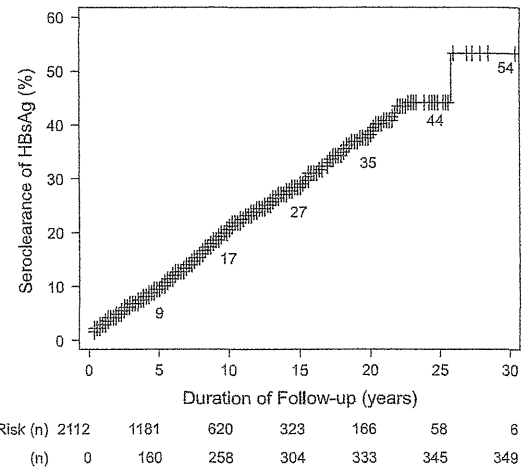

Fig. 1 Seroclearance of HBsAg in the 2,112 patients studied. Numbers of patients at risk and those of patients who lost HBsAg are indicated below each time point

Table 2 Factors influencing the seroclearance of HBsAg in 2,112 patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	*p* value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	*p* value
Age ≥50 years	1.06 (0.64–1.76)	0.824		
Male gender	1.15 (0.69–1.90)	0.594		
No HBV infection in family	1.55 (0.93–2.57)	0.092		
Treatment	1.26 (0.72–2.19)	0.413		
Cirrhosis	2.40 (1.20–4.83)	0.014		
AST ≥50 IU/L	1.30 (0.66–2.57)	0.454		
ALT ≥50 IU/L	1.81 (0.89–3.70)	0.104		
γ-GTP ≥20 IU/L	1.26 (0.72–2.23)	0.418		
Total bilirubin ≥1 mg/dL	1.39 (0.69–2.79)	0.358		
Albumin ≥4 g/dL	1.03 (0.58–1.81)	0.927		
Platelets >150 × 10^3/mm^3	1.22 (0.68–2.18)	0.501		
α-Fetoprotein ≤10 μg/L	1.06 (0.59–1.89)	0.845		
Genotype A or B, C	1.55 (0.86–2.76)	0.142		
HBeAg-negative status	3.01 (0.79–2.07)	0.001	1.81 (1.30–2.77)	<0.001
HBV DNA ≥5 log copies/mL	1.17 (0.64–2.15)	0.612		
HBsAg ≤2,000 IU/mL	2.13 (1.27–3.56)	0.004	2.60 (1.94–3.50)	<0.001
HBcrAg ≥4 log U/mL	1.11 (0.61–2.03)	0.731		
Wild-type precore sequence	0.98 (0.59–1.53)	0.964		
Wild-type core promoter sequence	2.74 (0.80–9.30)	0.104		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

[median 0.5 vs. 0.7 mg/dL ($p < 0.001$)]; albumin [median 4.4 vs. 4.3 g/dL ($p < 0.001$)]; platelets [median 202 vs. 181 × 10^3/mm^3 ($p < 0.001$)]; α-fetoprotein [median 4 vs. 4 μg/L ($p < 0.001$)]; HBeAg-negative status [75.8 vs. 31.8 % ($p < 0.001$)]; HBsAg levels [median 2,240 vs. 5,270 IU/mL ($p < 0.001$)]; HBcrAg [median 3.6 vs. >6.8 log U/mL ($p < 0.001$)]; distribution of genotypes A/B/C/others (5.7/20.0/72.6/1.7 vs. 3.4/11.1/84.9/0.5 %, $p < 0.001$); and HBV DNA [median 4.7 vs. 8.0 log copies/mL ($p < 0.001$)].

The rate of HBsAg seroclearance in treated patients was 8 % in 5 years, 20 % in 10 years, 28 % in 15 years, 41 % in 20 years, 49 % in 25 years, and 49 % in 30 years, with an annual HBsAg seroclearance rate of 2.05 % (Fig. 2). The rate in untreated patients was 9 % in 5 years, 18 % in 10 years, 26 % in 15 years, 33 % in 20 years, 42 % in 25 years, and 56 % in 30 years, with an annual HBsAg seroclearance rate of 1.65 %. No differences in the annual HBsAg seroclearance rate were noted between treated and untreated patients ($p = 0.289$).

HBsAg seroclearance in untreated patients

In the 1,130 untreated patients, HBsAg persisted in 930 (82.3 %), whereas HBsAg seroclearance occurred in 200 (17.7 %). In the baseline characteristics, significant differences were found for age ($p < 0.001$), male gender ($p = 0.003$), chronic hepatitis ($p = 0.020$), γ-GTP ($p < 0.001$), albumin ($p = 0.004$), HBV genotypes ($p < 0.001$), HBeAg-negative status ($p < 0.001$), HBV DNA ($p < 0.001$), HBsAg level ($p < 0.001$), HBcrAg ($p < 0.001$), precore wild-type ($p < 0.001$), and core promoter wild-type ($p = 0.001$) (Table 4).

Factors contributing to HBsAg seroclearance in untreated patients

In the 1,130 untreated patients, factors influencing HBsAg seroclearance in univariate analysis by the Cox regression analyses were age ≥50 [RR 1.63 ($p = 0.002$)]; no family history in third-degree or closer relatives [RR 1.38 ($p = 0.037$)]; and HBsAg ≤2,000 IU/mL [RR 1.87 ($p < 0.006$)].

In multivariate analyses, only 2 factors contributed to HBsAg seroclearance: age ≥50 [RR 1.61 ($p = 0.018$)] and HBsAg ≤2,000 IU/mL [RR 1.77 ($p = 0.014$)] (Table 5).

HBsAg seroclearance in treated patients

In the 982 treated patients, HBsAg persisted in 833 (84.8 %). HBsAg seroclearance occurred in 149 (15.2 %). In the baseline characteristics, significant difference were found for male gender ($p = 0.004$), no family history in third-degree or closer relatives ($p = 0.010$), chronic hepatitis ($p = 0.001$), AST ($p = 0.010$), γ-GTP ($p = 0.023$), platelet counts ($p < 0.001$), HBeAg-negative status

Table 3 Baseline characteristics in untreated and treated patients

Features at the baseline	Untreated (n = 1,130)	Treated (n = 982)	Differences p value
Age (years)	31 (1–81)	36 (6–75)	<0.001
Men	705 (62.4 %)	726 (71.9 %)	<0.001
Chronic hepatitis	1,094 (96.8 %)	937 (96.4 %)	0.079
Cirrhosis	36 (3.2 %)	45 (3.6 %)	
AST (IU/L)	27 (3–1,776)	56 (6–2,192)	<0.001
ALT (IU/L)	28 (2–3,020)	96 (8–2,740)	<0.001
γ-GTP (IU/L)	20 (4–1,494)	45 (4–1,278)	<0.001
Total bilirubin (mg/dL)	0.5 (0.1–20.1)	0.7 (0.2–21.2)	<0.001
Albumin (g/dL)	4.4 (2.2–5.8)	4.3 (1.1–5.4)	<0.001
Platelets ($\times 10^3$/mm^3)	202 (40–443)	181 (40–483)	<0.001
α-Fetoprotein (μg/L)	4 (1–2,060)	4 (1–1,610)	<0.001
HBeAg-negative status	857 (75.8 %)	312 (31.8 %)	<0.001
HBsAg (IU/mL)	2,240 (0.06–141,000)	5,270 (0.09–277,000)	<0.001
HBcrAg (log U/mL)	3.6 (<3.0 to >6.8)	> 6.8 (<3.0 to >6.8)	<0.001
Genotypes [A/B/C/others (%)]	5.7/20.0/72.6/1.7	3.4/11.1/84.9/0.5	<0.001
HBV DNA (log copies/mL)	4.7 (<2.1 to >9.1)	8.0 (<2.1 to >9.1)	<0.001

Median values with the range in parentheses or numbers with the percentage in parentheses are given

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

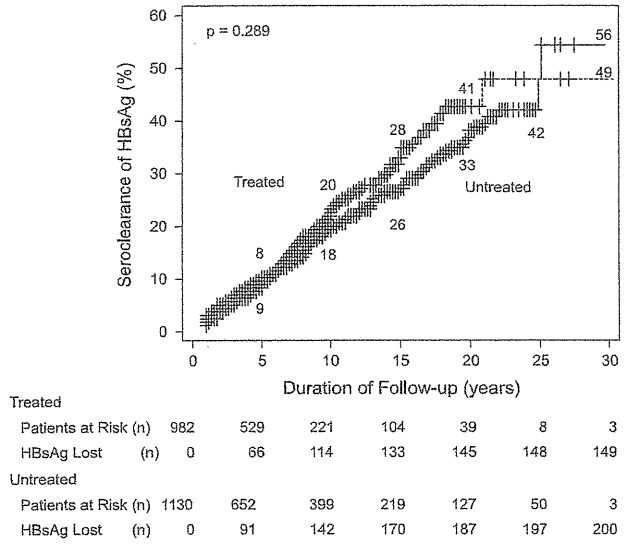

Fig. 2 Comparison of HBsAg seroclearance rates between 982 treated and 1,130 untreated patients. Numbers of patients at risk and those of patients who lost HBsAg are indicated below each time point

($p < 0.001$), HBV DNA ($p = 0.002$), HBsAg ($p < 0.001$), HBcrAg ($p = 0.003$), and precore wild-type ($p = 0.013$) (Table 6).

Factors contributing to HBsAg seroclearance in treated patients

In the 982 treated patients, factors influencing HBsAg seroclearance in univariate analysis by the Cox regression analyses were age ≥50 [RR 1.91 ($p = 0.001$)]; male gender [RR 2.14 ($p = 0.001$)], no family history in third-degree or closer relatives [RR 1.58 ($p = 0.005$)]; previous treatment with interferon [RR 2.13 ($p < 0.001$)]; chronic hepatitis [RR 3.12 ($p < 0.001$)]; AST ≥50 IU/L [RR 1.47 ($p = 0.031$)]; γ-GTP ≥20 IU/L [RR 1.87 ($p = 0.001$)]; platelets ≤150 × 10^3/mm^3 [RR 2.10 ($p < 0.001$)]; HBeAg-negative status [RR 2.53 ($p < 0.00$)]; HBV DNA ≤5 log copies/mL [RR 2.07 ($p = 0.001$)]; HBsAg ≤2,000 IU/mL [RR 2.29 ($p < 0.001$)]; HBcrAg ≤4 log U/mL [RR 2.28 ($p = 0.003$)]; and the wild-type precore sequence [RR 2.04 ($p = 0.011$)].

In multivariate analysis, only 3 factors contributed to HBsAg seroclearance: no family history in third-degree or closer relatives [RR 2.22 ($p = 0.006$)]; previous treatments with interferon [RR 3.15 ($p < 0.001$)]; and HBeAg-negative status [RR 3.75 ($p < 0.001$)] (Table 7).

Discussion

In Japan, perinatal materno-fetal transmission was the main route of HBV infection, but this transmission has been prevented since 1986 by the national campaign to prevent it by immunoprophylaxis with combined passive-active immunization of babies born to HBeAg-positive carrier mothers. However, HCC develops in about 10 % of the patients who have established chronic HBV infection by materno-fetal infection or through child-to-child transmission. Hence, HBsAg seroclearance is crucially required for preventing the development of cirrhosis followed by HCC.

In the present study, we analyzed 2,112 patients with persistent HBV infection to establish the factors

Table 4 Differences between the baseline characteristics of 917 untreated patients in whom HBsAg persisted and 213 those who lost HBsAg

Features at the baseline	HBsAg persisted ($n = 917$)	HBsAg lost ($n = 213$)	Differences p value
Age (years)	37 (1–81)	44 (0–80)	<0.001
Men	553 (60.3 %)	152 (71.4 %)	0.003
HBV in family members	349 (38.1 %)	76 (35.7 %)	0.509
Chronic hepatitis	893 (97.4 %)	201 (94.4 %)	0.020
AST (IU/L)	27 (3–1,144)	25 (6–1,776)	0.283
ALT (IU/L)	28 (6–1,960)	27 (6–3,020)	0.389
γ-GTP (IU/L)	22 (1–1,494)	29 (4–1,092)	<0.001
Total bilirubin (mg/dL)	0.6 (0.2–20.1)	0.7 (0.1–4.0)	0.257
Albumin (g/dL)	4.3 (2.0–5.3)	4.4 (1.6–5.7)	0.004
Platelets ($\times 10^3$/mm^3)	203 (40–443)	203 (33–417)	0.473
α-Fetoprotein (μg/L)	3 (1–2,060)	1 (1–478)	0.373
Genotypes [A/B/C/others (%)]	5.7/19.0/73.3/1.9	5.5/24.7/69.2/0.7	<0.001
HBeAg-negative status	663 (72.3 %)	194 (91.1 %)	<0.001
HBV DNA (log copies/mL)	4.9 (<2.1 to >9.1)	3.8 (<2.1 to >9.1)	<0.001
HBsAg (IU/mL)	3,100 (1.94–141,000)	149 (0.06–88,800)	<0.001
HBcrAg (log U/mL)	3.9 (<3.0 to >6.8)	2.9 (<3.0 to >6.8)	<0.001
Wild-type precore sequence	441 (48.1 %)	160 (75.0 %)	<0.001
Wild-type core promoter sequence	320 (34.9 %)	47 (22.0 %)	0.001

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Table 5 Factors influencing the seroclearance of HBsAg in untreated patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	p value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	p value
Age ≥50 years	1.63 (1.19–2.23)	0.002	1.61 (1.09–2.37)	0.018
Male gender	1.08 (0.79–1.48)	0.618		
No HBV infection in family	1.38 (1.02–1.86)	0.037		
Cirrhosis	1.19 (0.73–1.93)	0.484		
AST ≥50 IU/L	1.01 (0.70–1.45)	0.979		
ALT ≥50 IU/L	0.93 (0.68–1.27)	0.633		
γ-GTP ≥20 IU/L	1.17 (0.85–1.61)	0.330		
Total bilirubin ≥1 mg/dL	1.41 (0.80–2.49)	0.239		
Albumin ≥4 g/dL	0.78 (0.51–1.18)	0.239		
Platelets >150 × 10^3/mm^3	0.99 (0.67–1.46)	0.946		
α-Fetoprotein ≤10 μg/L	0.84 (0.48–1.47)	0.543		
Genotype A or B	1.17 (0.81–1.69)	0.410		
HBeAg-negative status	0.78 (0.79–2.07)	0.314		
HBV DNA ≥5 log copies/mL	0.84 (0.58–1.24)	0.383		
HBsAg ≤2,000 IU/mL	1.87 (1.19–2.91)	0.006	1.77 (1.12–2.77)	0.014
HBcrAg ≥4 log U/mL	0.85 (0.50–1.45)	0.555		
Wild-type precore sequence	0.99 (0.60–1.52)	0.967		
Wild-type core promoter sequence	0.78 (0.35–1.73)	0.538		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

contributing to HBsAg seroclearance. The overall rate of HBsAg seroclearance was 1.75 % annually. The annual seroclearance rates of HBsAg are reported to be 1.7 % in Korea [14] and 1.6 % in Taiwan [15–17], as well as 2.5 % in Goto Islands of Japan, where HBV infections are very prevalent [18]. In 1,271 natives in Alaska, the rate of HBsAg seroclearance was 0.7 % annually [19]. These differences could be ascribed, in part, to HBV genotypes distinct among Asian countries and Alaska. Since treatment with IFN and/or nucleot(s)ide analogues has suppressive effects on the development of HCC [6, 20], they may influence HBsAg seroclearance.

Table 6 Differences in baseline characteristics between the 833 treated patients in whom HBsAg persisted and 149 those who lost HBsAg

Features at the baseline	HBsAg persisted (n = 833)	HBsAg lost (n = 149)	Differences p value
Age (years)	41 (13–88)	43 (17–71)	0.285
Men	601 (72.2 %)	124 (83.2 %)	0.004
HBV in family members	496 (59.6 %)	72 (48.3 %)	0.010
Chronic hepatitis	802 (96.3 %)	134 (89.9 %)	0.001
AST (IU/L)	54 (6–2,192)	78 (7–888)	0.010
ALT (IU/L)	93 (8–2,740)	118 (8–1,700)	0.117
γ-GTP (IU/L)	44 (4–1,278)	46 (4–1,278)	0.023
Total bilirubin (mg/dL)	0.7 (0.2–21.2)	0.7 (0.3–8.4)	0.273
Albumin (g/dL)	4.3 (1.1–5.4)	4.5 (1.4–5.3)	0.281
Platelets ($\times 10^3/mm^3$)	182 (40–483)	171 (50–391)	<0.001
α-Fetoprotein (μg/L)	4 (1–1,610)	4 (1–765)	0.682
Genotypes [A/B/C/others (%)]	3.2/10.7/85.1/1.0	5.1/12.4/81.6/0.9	0.565
HBeAg-negative status	230 (27.6 %)	79 (53.0 %)	<0.001
HBV DNA (log copies/mL)	7.8 (<2.1 to >9.1)	8.3 (<2.1 to >9.1)	0.002
HBsAg (IU/mL)	7,880 (0.04–277,000)	1,380 (0.04–188,000)	<0.001
HBcrAg (log U/mL)	6.9 (<3.0 to >6.8)	5.9 (<3.0 to >6.8)	0.003
Wild-type precore sequence	554 (66.6 %)	61 (41.2 %)	0.013
Wild-type core promoter sequence	274 (32.9 %)	67 (45.0 %)	0.836

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Table 7 Factors influencing the seroclearance of HBsAg in treated patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	p value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	p value
Age ≥50 years	1.91 (1.32–2.77)	0.001		
Male gender	2.14 (1.37–3.33)	0.001		
No HBV infection in family	1.58 (1.15–2.19)	0.005	2.22 (2.32–3.94)	0.006
Treatments (interferon vs. others)	2.13 (1.53–2.98)	<0.001	3.15 (1.69–5.87)	<0.001
Chronic hepatitis	3.12 (2.05–4.74)	<0.001		
AST ≥50 IU/L	1.47 (1.04–2.09)	0.031		
ALT ≥50 IU/L	1.29 (0.82–1.92)	0.201		
γ-GTP ≥20 IU/L	1.87 (1.30–2.70)	0.001		
Total bilirubin ≥1 mg/dL	1.35 (0.87–2.08)	0.179		
Albumin ≥4 g/dL	1.11 (0.66–1.86)	0.688		
Platelets ≤150 × $10^3/mm^3$	2.10 (1.49–2.96)	<0.001		
α-Fetoprotein ≤10 μg/L	1.33 (0.92–1.92)	0.136		
Genotype A or B vs. others	1.16 (0.74–1.82)	0.529		
HBeAg-negative status	2.53 (1.83–3.50)	<0.001	3.75 (2.09–6.74)	<0.001
HBV DNA ≤5 log copies/mL	2.07 (1.37–3.13)	0.001		
HBsAg ≤2,000 IU/mL	2.29 (1.52–3.47)	<0.001		
HBcrAg ≤4 log U/mL	2.28 (1.31–3.97)	0.003		
Wild-type precore sequence	2.04 (1.18–3.55)	0.011		
Wild-type core promoter sequence	1.18 (0.63–2.21)	0.608		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A176.2/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Therefore, we went on to extend our analysis to untreated patients and those treated with IFN or nucleotide analogues separately. Criteria for upper or lower levels of each parameter were set, taking into consideration the median value or a cutoff value with the lowest p value of the entire 2,112-patient cohort (Table 1), and unified for untreated and treated patients (Tables 5, 7).

Firstly, in the univariate analysis, age, no family history of HBV infection in third-degree or closer relatives, and decreased HBsAg levels lowered the annual rate of HBsAg seroclearance significantly. In multivariate analysis, age $\geq$50 years (RR 1.61, $p = 0.018$) and HBsAg $\leq$2,000 IU/mL (RR 1.77, $p = 0.014$) decreased the annual rate of HBsAg seroclearance significantly. Kato et al. [18] reported high HBsAg seroclearance rates in patients over 40 or over 50 years; in our patients, also, age $\geq$50 years increased RR to 1.61 ($p = 0.018$). As for HBsAg and HBV DNA, low HBsAg and HBV DNA levels increased the HBsAg seroclearance rate to 37.7 %, and therefore, low HBsAg levels are an important factor. In actuality, HBsAg levels $\leq$2,000 IU/mL increased the rate of HBsAg seroclearance with RR 1.77 ($p = 0.014$).

In treated patients, by contrast, age, the male gender, no HBV infections in third-degree or closer relatives, treatment with IFN, chronic hepatitis, high AST levels, high γ-GTP levels, low platelet counts, HBeAg-negative status, low HBsAg levels, low HBcrAg levels and the wild-type precore sequence were significant factors in univariate analysis. In multivariate analysis, no HBV infections in third-degree or closer relatives (RR 2.22, $p = 0.006$), interferon treatments (RR 3.15, $p < 0.001$), and HBeAg-negative status (RR 3.75, $p < 0.001$) were significant factors.

Thus, there were differences in factors predictive of the HBsAg loss between untreated and treated patients. Remarkably, age and HBsAg titer were independent factors in untreated patients, whereas family history and negative HBeAg were independent factors in treated patients. Since this work studied patients who were followed for a long time (>15 years), age and HBsAg titer were factors for clearance of HBsAg in untreated patients. Treated patients, in contrast, would have included more patients with HBeAg, with a good response to antiviral treatment, as well as those without family history who would have been infected with HBV with a sorter duration than those with family history. In other words, most untreated patients were those with favorable clinical course, in whom HBsAg titer gradually decreased and eventually lost it with time. In fact, there would be many such patients, the majority of whom do not visit hospitals and are unaware of HBV infection, who may have unapparent liver disease. Treated patients, on the other hand, would have had higher risks for cirrhosis and HCC, owing to elevated ALT/AST levels; this risk is especially high for patients with a family history of HBV [21]. Therefore, patients with family history would not be able to easily lose HBsAg.

In treated patients, IFN led to HBsAg loss more effectively than other treatments [RR 2.13, $p < 0.001$ (Table 7)]. The immunomodulatory activity of IFN, which is not shared by nucleot(s)ide analogues, would have accelerated the immune response to HBV required for the seroclearance of HBsAg. Of the 333 patients who received IFN, 190 (57 %) were treated with IFN multiply. In them, seroclearance of HBsAg was achieved in 49 of the 190 (26 %) patients with multiple IFN treatments in comparison with 41 of the 143 (29 %) with single IFN treatment. Owing to indications for IFN, patients who received IFN tended to be younger, without previous treatments and higher HBV DNA as well as ALT levels. They might have increased the rate of HBsAg loss that was higher with IFN than other treatments.

Since this is a retrospective cohort study of patients visiting our hospital for more than 15 years, and there has been so much innovation in the treatment of chronic hepatitis B during that period, treated and untreated patients have different backgrounds at the baseline. Hence, treated patients had higher ALT and HBV DNA levels with severer liver disease than untreated patients (Table 3). This might have been responsible, at least in part, for the failure in finding differences in the rate of HBsAg loss between untreated and treated patients (Fig. 2). Future studies will be aimed at analyzing contributing factors in treated and matched controls. This will allow us to analyze factors contributing to HBsAg seroclearance in the treatment of patients with chronic hepatitis B.

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