Biochemical tests were conducted at each examination together with regular check-up. Four hundred fifty patients were lost to follow-up. The final date of follow-up in 452 patients with loss of follow-up was regarded as last consulting day.

Patients with either of the following criteria during follow-up were regarded as censored data in statistical analysis [Fleming et al., 1984]: (1) they were retreated with IFN (N = 949); (2) they had new onset of carcinogenesis (N = 645); and (3) they had been given anticoagulant and antiplatelet drugs (N = 28). The final date of follow-up in these patients with censored data was regarded as the time of the initiation of criteria described above. The mean follow-up period was 6.7 [standard deviation (SD) 4.3] years in 452 patients with loss of follow-up and 7.4 (SD 4.7) years in 1,722 patients who had censored data. Patients with loss of follow-up and censored data were counted in the analysis.

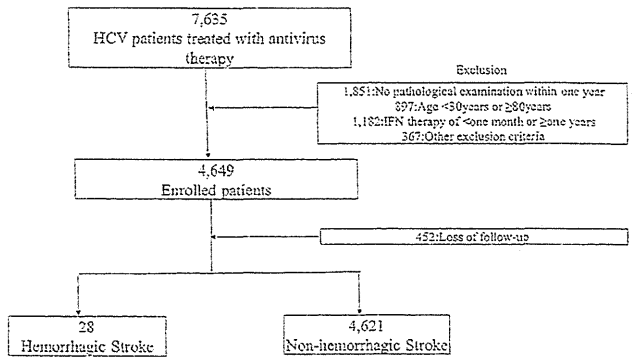


Fig. 1. An algorithm of the study population.

Statistical Analysis

Clinical differences between patients with hemorrhagic stroke and those without events were evaluated using Mann–Whitney test. The cumulative incidence of hemorrhagic stroke were calculated by using the Kaplan–Meier technique, and differences in the curves were tested using the log-rank test [Kaplan and Meier, 1958; Harrington and Fleming, 1983]. Independent risk factors associated with hemorrhagic stroke were studied using the stepwise Cox regression analysis [Cox, 1972]. The following

TABLE I. Clinical Backgrounds at the Initiation of Follow-Up in Enrolled Patients

	Total	Hemorrhagic stroke group	Without events group	*P*-value
N	4,649	28	4,621	
Age (years)	51.9 ± 11.8	60.4 ± 6.7	51.8 ± 11.9	<0.001
Gender (M/F)	2,966/1,883	16/12	2,950/1,871	0.781
Height (cm)	163.1 ± 9.2	159.5 ± 9.4	163.2 ± 9.2	0.171
Weight (kg)	61.4 ± 12.8	57.9 ± 8.0	61.4 ± 12.7	0.113
BMI	22.7 ± 3.1	23.4 ± 2.8	22.7 ± 3.1	0.582
BP (systolic, mmHg)	128 ± 18	140 ± 20	127 ± 18	0.007
BP (diastolic, mmHg)	77 ± 13	86 ± 15	77 ± 13	0.001
Total alcohol intake (kg)[a]	95 ± 92	148 ± 105	94 ± 92	0.002
Smoking index[a]	6.5 ± 9.5	11.8 ± 12.4	6.4 ± 9.4	<0.001
AST (IU/L)	41 ± 43	48 ± 28	41 ± 43	<0.001
ALT (IU/L)	44 ± 53	53 ± 38	43 ± 52	0.004
GGT (IU/L)	53 ± 60	59 ± 47	52 ± 61	0.078
Albumin (g/dl)	4.0 ± 0.3	3.5 ± 0.4	4.0 ± 0.3	0.110
Triglyceride (mg/dl)	101 ± 52	108 ± 46	100 ± 52	0.097
Cholesterol (mg/dl)	170 ± 31	171 ± 27	170 ± 31	0.893
HDL-C (mg/dl)	48 ± 14	45 ± 12	48 ± 14	0.002
LDL-C (mg/dl)	104 ± 29	108 ± 37	103 ± 29	0.049
Fasting plasma glucose (mg/dl)	99 ± 22	103 ± 23	100 ± 22	0.093
HbA_{1C} (%)	5.7 ± 1.1	5.9 ± 1.2	5.7 ± 1.1	0.024
Platelet ($\times 10^4/mm^3$)	17.2 ± 5.2	14.1 ± 6.2	17.3 ± 5.4	0.001
Staging (cirrhosis/non-cirrhosis)[b]	485/4,164	12/16	473/4,148	<0.001
HCV genotype (1b/2a/2b/other)[b]	2,859/1,109/497/184	22/5/1/0	2,837/1,104/496/184	0.104
HCV RNA (log IU/ml)[b]	6.07 ± 1.05	6.03 ± 1.03	6.08 ± 1.05	0.387
IFN monotherapy/combination therapy[c]	3,000/1,649	24/4	2,976/1,645	<0.001
Efficacy (HCV; clearance/non-clearance)	2,103/2,546	5/23	2,098/2,523	0.006

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; GGT, gamma-glutamyltransferase; HbA_{1C}; hemoglobin A_{1C}; HCV, hepatitis C virus; HDL, high density lipoprotein; IFN, interferon.
Data are number of patients or mean ± standard deviation.
[a]Smoking index is defined as package per day × year; total alcohol intake and smoking index indicate the sum before and after first consultation.
[b]Value before IFN treatment.
[c]Outbreak of IFN monotherapy: recombinant IFN alpha 2a, 238 cases; recombinant IFN alpha 2b, 183 cases; natural IFN alpha, 1,750 cases; natural IFN beta, 750 cases; total dose of IFN = 554 ± 164 MU. Outbreak of peg IFN monotherapy: peg IFN alpha 2a, 93 cases, total dose of peg IFN = 7.54 ± 2.20 mg.
Outbreak of combination therapy: recombinant IFN alpha 2b + ribavirin, 335 cases, total dose of IFN = 508 ± 184 MU, total dose of ribavirin = 160 ± 68 g; natural IFN beta + ribavirin, 127 cases, total dose of IFN = 502 ± 177 MU, total dose of ribavirin = 155 ± 67 g; peg IFN alpha 2b + ribavirin, 1,173 cases, total dose of peg IFN = 4.12 ± 1.10 mg, total dose of ribavirin = 205 ± 58 g.

variables were analyzed for potential covariates for incidence of primary outcome: (1) age, gender, type 2 diabetes mellitus, hypertension, BMI at the initiation time of follow-up, (2) HCV genotype, HCV load, and hepatic fibrosis before IFN therapy, (3) average value of aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and platelet during follow-up, (4) sum value of smoking and alcohol before, during, and after the IFN therapy, (5) efficacy of IFN therapy, combination of ribavirin, type of IFN, and total dose of IFN. A P-value of less than 0.05 was considered statistically significant. Data analysis was performed using SPSS 11.5 for Windows (SPSS, Chicago, IL).

RESULTS

Patients Characteristics

Figure 1 shows the algorithm of the study population. For the mean observation period of 8.0 years, 28 of 4,649 patients developed hemorrhagic stroke. Table I shows the baseline characteristics of the enrolled 4,649 patients at the initiation of follow-up. The patients are divided into two groups of patients with hemorrhagic stroke and without event. There are significant differences in several baseline characteristics between the two groups. The HCV clearance rate was 34.7% (1,042/3,000) in IFN monotherapy and 64.3% (1,061/1,649) in combination therapy of IFN and ribavirin. Thus, the number of patients with HCV clearance was 2,103. The mean follow-up was 8.0 (SD 5.0) years. The 28-day vascular disease-related mortality rate was 33% (10/28) in hemorrhagic stroke.

Predictive Factors for the Development of Intracerebral Hemorrhagic Stroke

The cumulative incidence of intracerebral hemorrhagic stroke was 0.3% at 5 years, 0.8% at 10 years, and 1.7% at 15 years (Fig. 2A). The factors associated with the development of intracerebral hemorrhagic stroke are shown in Table II. Intracerebral hemorrhagic stroke occurred when patients had age increments of 10 years [hazard ratio: 2.77; 95% confidence interval (CI) 1.48–5.18; $P=0.001$], hypertension

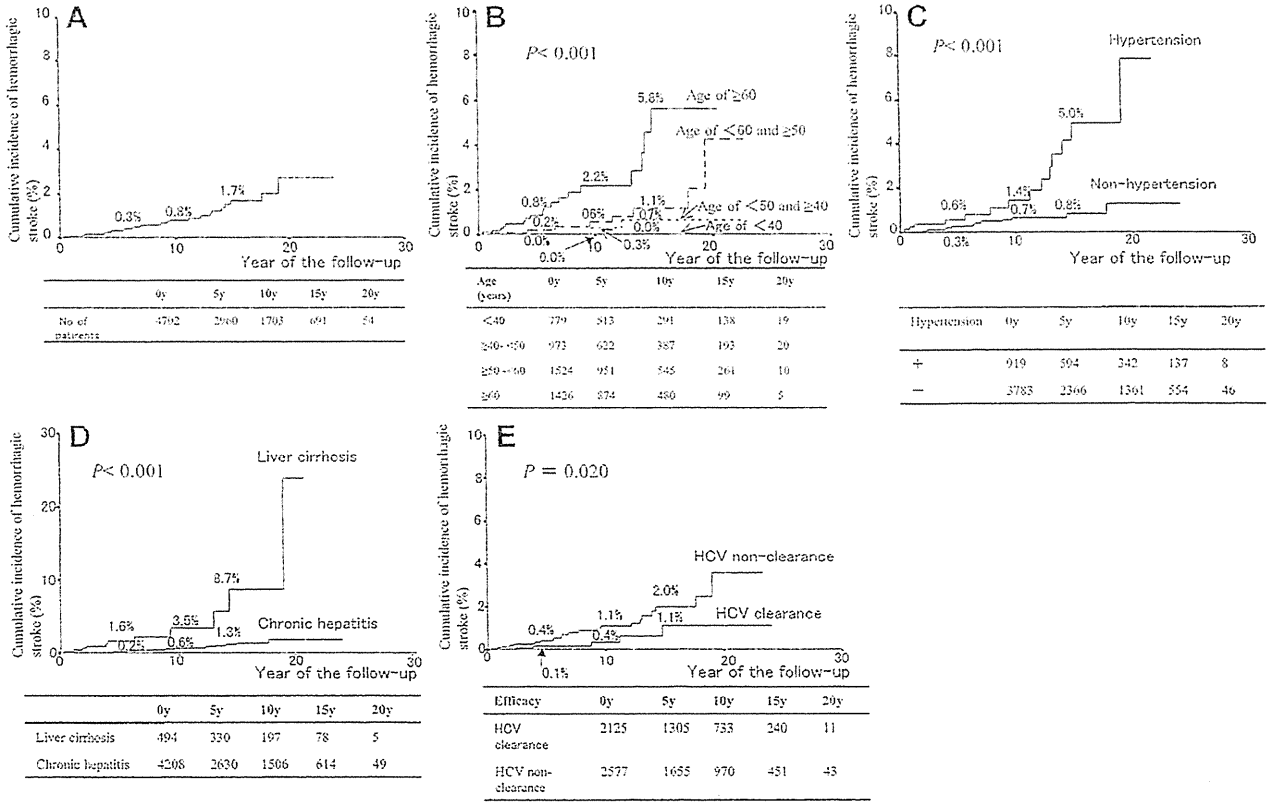


Fig. 2. **Panel A**: Cumulative development rate of intracerebral hemorrhagic stroke in total HCV patients treated with IFN therapy. **Panel B**: Cumulative development rate of intracerebral hemorrhagic stroke based on difference of age. **Panel C**: Cumulative development rate of ischemic stroke based on the difference of blood pressure. **Panel D**: Cumulative development rate of intracerebral hemorrhagic stroke based on difference of liver fibrosis. **Panel E**: Cumulative development rate of intracerebral hemorrhagic stroke based on difference of interferon efficacy.

TABLE II. Predictive Factors for the Development of Intracerebral Hemorrhagic Stroke

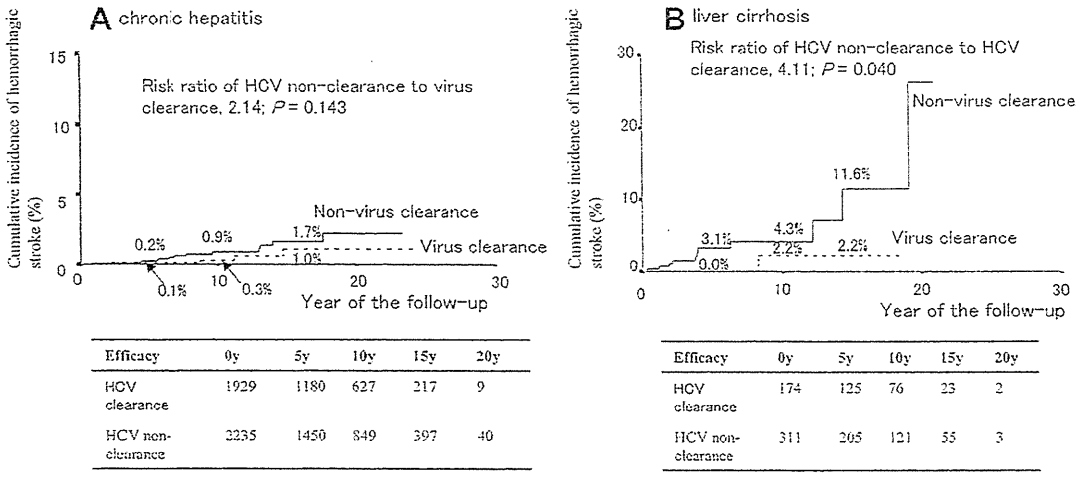
	Univariate analysis		Cox regression	
Variables	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (years, per 10)	3.55 (1.96–6.43)	<0.001	2.77 (1.48–5.18)	0.001
Gender (M/F)	1.26 (0.65–2.44)	0.334		
BMI (≥22/<22)	0.97 (0.75–1.24)	0.767		
Diabetes (+/−)	3.40 (1.26–9.15)	0.015		
Hypertension (+/−)	4.07 (1.94–8.54)	<0.001	2.30 (1.09–4.83)	0.021
Smoking index (≥20/<20)[a]	2.12 (0.95–4.76)	0.068		
Total alcohol intake (kg, ≥200/<200)[a]	1.10 (0.53–4.37)	0.138		
AST (IU/L, ≥34/<34)	2.79 (1.17–6.66)	0.020		
ALT (IU/L, ≥36/<36)	2.68 (1.14–6.29)	0.023		
GGT (IU/L, ≥109/<109)	1.28 (0.610–1.89)	0.655		
Albumin (g/dl, <3.9/≥3.9)	2.96 (1.24–7.09)	0.015		
Triglyceride (mg/dl, ≥100/<100)	1.19 (0.83–1.49)	0.283		
Total cholesterol (mg/dl, <150/≥150)	1.06 (0.48–1.91)	0.936		
HDL-C (mg/dl, ≥40/<40)	0.96 (0.38–2.50)	0.960		
LDL-C (mg/dl, ≥120/<120)	0.81 (0.50–2.51)	0.572		
Platelet ($\times 10^4$/mm^3, <15/≥15)	3.22 (1.41–7.35)	0.005		
Histological diagnosis (cirrhosis/non-cirrhosis)	7.40 (3.30–16.77)	<0.001	4.50 (2.07–9.78)	<0.001
Combination of ribavirin (+/−)	0.80 (0.25–2.54)	0.701		
Type of IFN (α/β)	1.29 (0.65–2.33)	0.116		
Total dose of IFN (MU, ≥500/<500)	0.87 (0.39–1.99)	0.744		
HCV genotype (1/2)	1.53 (0.62–3.80)	0.360		
HCV RNA (log IU/ml, ≥5/<5)	1.35 (1.02–1.79)	0.035		
Efficacy (HCV: non-clearance/clearance)	2.98 (1.13–6.59)	0.020	3.22 (1.22–8.53)	0.018

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; GGT, gamma-glutamyltransferase; HCV, hepatitis C virus; IFN, interferon.
[a]Smoking index is defined as package per day × year; total alcohol intake and smoking index indicate the sum before and after first consultation.

(hazard ratio: 2.30; 95% CI 1.09–4.83; $P=0.021$), liver cirrhosis (hazard ratio: 4.50; 95% 2.07–9.78; $P<0.001$), and HCV non-clearance (hazard ratio: 3.22; 95% CI 1.22–8.53; $P=0.018$). Figure 2B–E shows the cumulative incidence of hemorrhagic stroke based on difference of age, blood pressure, liver fibrosis, and efficacy of IFN therapy.

Hemorrhagic Stroke Based on the Difference of Liver Fibrosis and Efficacy

Figure 3A,B shows the cumulative incidence of intracerebral hemorrhagic stroke based on the difference of liver fibrosis and efficacy of IFN therapy. As shown in Figure 3B, HCV clearance reduced



Efficacy	0y	5y	10y	15y	20y
HCV clearance	1929	1180	627	217	9
HCV non-clearance	2235	1450	849	397	40

Efficacy	0y	5y	10y	15y	20y
HCV clearance	174	125	76	23	2
HCV non-clearance	311	205	121	55	3

Fig. 3. **Panel A**: Cumulative development rate of intracerebral hemorrhagic stroke based on difference of efficacy after interferon treatment in HCV patients with chronic hepatitis. **Panel B**: Cumulative development rate of intracerebral hemorrhagic stroke based on the difference of efficacy after interferon treatment in HCV patients with liver cirrhosis.

TABLE III. Comparison in Clinical Backgrounds Between HCV Clearance and HCV Non-Clearance in Patients With Liver Cirrhosis

	HCV clearance group	HCV non-clearance group	*P*-value
N	174	311	
Age (years)	56.7 ± 9.6	57.0 ± 9.9	0.721
Gender (M/F)	108/66	184/127	0.562
BMI	23.8 ± 3.7	23.6 ± 3.5	0.479
BP (systolic, mmHg)	132 ± 18	131 ± 17	0.791
BP (diastolic, mmHg)	80 ± 11	79 ± 12	0.775
Total alcohol intake (kg)[a]	112 ± 97	128 ± 101	0.057
Smoking index[a]	6.2 ± 10.7	5.9 ± 10.2	0.129
AST (IU/L)	33 ± 20	73 ± 47	<0.001
ALT (IU/L)	34 ± 28	79 ± 61	<0.001
GGT (IU/L)	24 ± 26	61 ± 65	<0.001
Albumin (g/dl)	3.7 ± 0.4	3.5 ± 0.4	0.149
Triglyceride (mg/dl)	110 ± 47	104 ± 45	0.243
Cholesterol (mg/dl)	157 ± 29	161 ± 31	0.373
HDL-C (mg/dl)	42 ± 12	45 ± 12	0.257
LDL-C (mg/dl)	96 ± 26	95 ± 30	0.748
Fasting plasma glucose (mg/dl)	104 ± 22	109 ± 26	0.085
HbA_{1C} (%)	5.7 ± 1.2	6.0 ± 1.3	0.024
Platelet ($\times 10^4/mm^3$)	14.1 ± 6.2	17.3 ± 5.4	0.097
HCV genotype (1b/2a/2b/other)[b]	75/72/24/3	209/54/15/33	<0.001
HCV RNA (log IU/ml)[b]	5.32 ± 1.12	6.38 ± 1.00	<0.001
IFN monotherapy/combination therapy[c]	110/64	232/79	0.012

Data are number of patients or mean ± standard deviation, ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; GGT, gamma-glutamyltransferase; HbA_{1C}, hemoglobin A_{1C}; HCV, hepatitis C virus; HDL, high density lipoprotein; IFN, interferon.
[a]Smoking index is defined as package per day × year; total alcohol intake and smoking index indicate the sum before and after first consultation.
[b]Value before IFN treatment.
[c]Outbreak of IFN monotherapy: natural IFN alpha, 252 cases; natural IFN beta, 90 cases; total dose of IFN = 518 ± 156 MU.
Outbreak of combination therapy: natural IFN beta + ribavirin, 41 cases, total dose of IFN = 490 ± 171 MU, total dose of ribavirin = 151 ± 64 g; peg IFN alpha 2b + ribavirin, 102 cases, total dose of peg IFN = 3.96 ± 1.03 mg, total dose of ribavirin = 188 ± 51 g.

hemorrhagic stroke to one-fourth in cirrhotic patients. Table III shows the clinical backgrounds between HCV clearance and HCV non-clearance in patients with liver cirrhosis. There are significant differences in AST, ALT, GGT, HCV genotype, HCV RNA, and HbA_{1C} between HCV clearance group and HCV non-clearance group. However, there are no significant differences in age and hypertension between HCV clearance group and HCV non-clearance group.

DISCUSSION

The incidence of hemorrhagic stroke after the termination of IFN therapy in HCV patients has been described in the present study. The strengths of the present study are a prolonged follow-up in the large numbers of patients included.

The present study shows several findings with regard to the cumulative incidence and predictive factors for hemorrhagic stroke after IFN therapy for HCV patients. First, intracranial hemorrhagic stroke occurred significantly when patients had advanced age of ≥60 years, hypertension, liver cirrhosis, and HCV non-clearance. Several authors have reported that the most common risk factor for hemorrhagic stroke is aging, high levels of blood pressure [Turin et al., 2010; O'Donnell et al., 2010; Naidech, 2011; Cervera et al., 2012]. In addition, antiplatelet and anticoagulant medications also increase the risk of hemorrhagic stroke [Cervera et al., 2012]. Our results evaluated hemorrhagic stroke in HCV patients agreed with these reports concerning aging and hypertension.

Second, HCV clearance reduced hemorrhagic stroke to about one-fourth in cirrhotic patients. In general, patients with advanced liver fibrosis have often the hemorrhagic tendency due to prothrombin deficit and platelets diminution. Thus, our result suggests that the HCV clearance prevent the aggravation of prothrombin deficit and platelets diminution. Our previous reports have indicated that HCV clearance reduces type 2 diabetes mellitus [Arase et al., 2009], bone fracture [Arase et al., 2010], and chronic kidney disease [Arase et al., 2011]. In the present study, HCV clearance reduced the incidence of intracerebral hemorrhagic stroke. In particular, HCV clearance reduced intracerebral hemorrhagic stroke to about one-fourth in cirrhotic patients.

A hemorrhagic stroke is the rapid loss of brain function due to hemorrhage. As a result, a hemorrhagic stroke is a medical emergency and can cause permanent neurological damage and death. Recently, the life span has been long in Japan. Thus, in near the future, a large number of patients with HCV will be >60 years of age. A hemorrhagic stroke might be increasing in HCV positive patients in aging society. Our results show that physicians in charge of HCV

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.

ACKNOWLEDGMENT

We thank Thomas Hughes for editorial assistance.

REFERENCES

Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, Hu PY, Miller JK, Gerber MA, Sampliner RE. 1992. The natural history of community acquired hepatitis C in the United States. N Engl J Med 327:1899–1905.

American Diabetes Association. 2010. Diagnosis and classification of diabetes mellitus. Diabetes Care 33:S62–S69.

Arase Y, Suzuki F, Suzuki Y, Akuta N, Kobayashi M, Kawamura Y, Yatsuji H, Sezaki H, Hosaka T, Hirakawa M, Saitoh S, Ikeda K, Kobayashi M, Kumada H. 2009. Sustained virological response reduces incidence of onset of type 2 diabetes in chronic hepatitis C. Hepatology 49:739–744.

Arase Y, Suzuki F, Suzuki Y, Akuta N, Kobayashi M, Sezaki H, Hosaka T, Kawamura Y, Yatsuji H, Hirakawa M, Ikeda K, Hsieh SD, Oomoto Y, Amakawa K, Kato H, Kazawa T, Tsuji H, Kobayashi T, Kumada H. 2010. Virus clearance reduces bone fracture in postmenopausal women with osteoporosis and chronic liver disease caused by hepatitis C virus. J Med Virol 82:390–395.

Arase Y, Suzuki F, Kawamura Y, Suzuki Y, Kobayashi M, Matsumoto N, Akuta N, Sezaki H, Hosaka T, Ogawa K, Imai N, Seko Y, Saito S, Ikeda K, Kobayashi M, Kumada H. 2011. Development rate of chronic kidney disease in hepatitis C virus patients with advanced fibrosis after interferon therapy. Hepatol Res 41:946–954.

Arase Y, Kobayashi M, Suzuki F, Suzuki Y, Kawamura Y, Akuta N, Imai N, Kobayashi M, Sezaki H, Matsumoto N, Saito S, Hosaka T, Ikeda K, Kumada H, Ohmoto Y, Amakawa K, Hsieh SD, Ogawa K, Tanabe M, Tsuji H, Kobayashi T. 2012. Difference in malignancies of chronic liver disease due to non-alcoholic fatty liver disease or hepatitis C in Japanese elderly patients. Hepatol Res 42:264–272.

Arase Y, Kobayashi M, Suzuki F, Suzuki Y, Kawamura Y, Akuta N, Kobayashi M, Sezaki H, Saito S, Hosaka T, Ikeda K, Kumada H, Kobayashi T. 2013. Effect of type 2 diabetes on risk for malignancies included hepatocellular carcinoma in chronic hepatitis C. Hepatology 57:964–973.

Cervera A, Amaro S, Chamorro A. 2012. Oral anticoagulant-associated intracerebral hemorrhage. J Neurol 259:212–224.

Cox DR. 1972. Regression models and life tables. J R Stat Soc 34:248–275.

Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Sheuer PJ. 1994. Classification of chronic hepatitis: Diagnosis, grading and staging. Hepatology 19:1513–1520.

Donnan GA, Fisher M, Macleod M, Davis SM. 2008. Stroke. Lancet 371:1612–1623.

Dusheiko G, Schmilovitz-Weiss H, Brown D, McOmish F, Yap PL, Sherlock S, McIntyre N, Simmonds P. 1994. Hepatitis C virus genotypes: An investigation of type-specific differences in geographic origin and disease. Hepatology 19:13–18.

Fleming TR, Harrington DP, O'Brien PC. 1984. Designs for group sequential tests. Control Clin Trials 5:348–361.

Harrington DP, Fleming TR. 1983. A class of rank test procedures for censored survival data. Biometrika 62:205–209.

Hasan F, Jeffers LJ, De Medina M, Reddy KR, Parker T, Schiff ER, Houghton M, Choo QL, Kuo G. 1990. Hepatitis C-associated hepatocellular carcinoma. Hepatology 12:589–591.

Ikeda K, Saitoh S, Koida I, Arase Y, Tsubota A, Chayama K, Kumada H, Kawanishi M. 1993. A multivariate analysis of risk factors for hepatocellular carcinogenesis: A prospective observation of 795 patients with viral and alcoholic cirrhosis. Hepatology 18:47–53.

Iso H, Sato S, Kitamura A, Imano H, Kiyama M, Yamagishi K, Cui R, Tanigawa T, Shimamoto T. 2007. Metabolic syndrome and the risk of ischemic heart disease and stroke among Japanese men and women. Stroke 38:1744–1751.

Kaplan EL, Meier P. 1958. Nonparametric estimation for incomplete observation. J Am Stat Assoc 53:457–481.

Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, Iijima A, Urushihara A, Kiyosawa K, Okuda M, Hino K, Okita K. 1998. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. Hepatology 27:1394–1402.

Kew MC, Houghton M, Choo QL, Kuo G. 1990. Hepatitis C virus antibodies in southern. African blacks with hepatocellular carcinoma. Lancet 335:873–874.

Kiyosawa K, Furuta S. 1991. Review of hepatitis C in Japan. J Gastroenterol Hepatol 6:383–391.

Naidech AM. 2011. Intracranial hemorrhage. Am J Respir Crit Care Med 184:998–1006.

O'Donnell MJ, Xavier D, Liu L, Zhang H, Chin SL, Rao-Melacini P, Islam S, Pais P, McQueen MJ, Mondo C, Damasceno A, Lopez-Jaramillo P, Hankey GJ, Dans AL, Yusoff K, Truelsen T, Diener HC, Sacco RL, Ryglewicz D, Czlonkowska A, Weimar C, Wang X, Yusuf S, INTERSTROKE Investigators. 2010. Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): A case-control study. Lancet 376:112–123.

Truelsen T, Mähönen M, Tolonen H, Asplund K, Bonita R, Vanuzzo D, WHO MONICA Project. 2003. Trends in stroke and coronary heart disease in the WHO MONICA Project. Stroke 34:1346–1352.

Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, Fujimoto I, Inoue A, Yamazaki H. 1993. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 328:1797–1801.

Turin TC, Kokubo Y, Murakami Y, Higashiyama A, Rumana N, Watanabe M, Okamura T. 2010. Lifetime risk of stroke in Japan. Stroke 41:1552–1554.

Yoshida H, Arakawa Y, Sata M, Nishiguchi S, Ya M, Fujiyama S, Yokosuka O, Shiratori Y, Omata M. 2002. Interferon therapy prolonged life expectancy among chronic hepatitis patients. Gastroenterology 123:483–491.

J Gastroenterol (2014) 49:470–480
DOI 10.1007/s00535-013-0779-0

The Japanese Society of Gastroenterology

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

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Received: 26 November 2012 / Accepted: 12 February 2013 / Published online: 26 March 2013

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(× 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

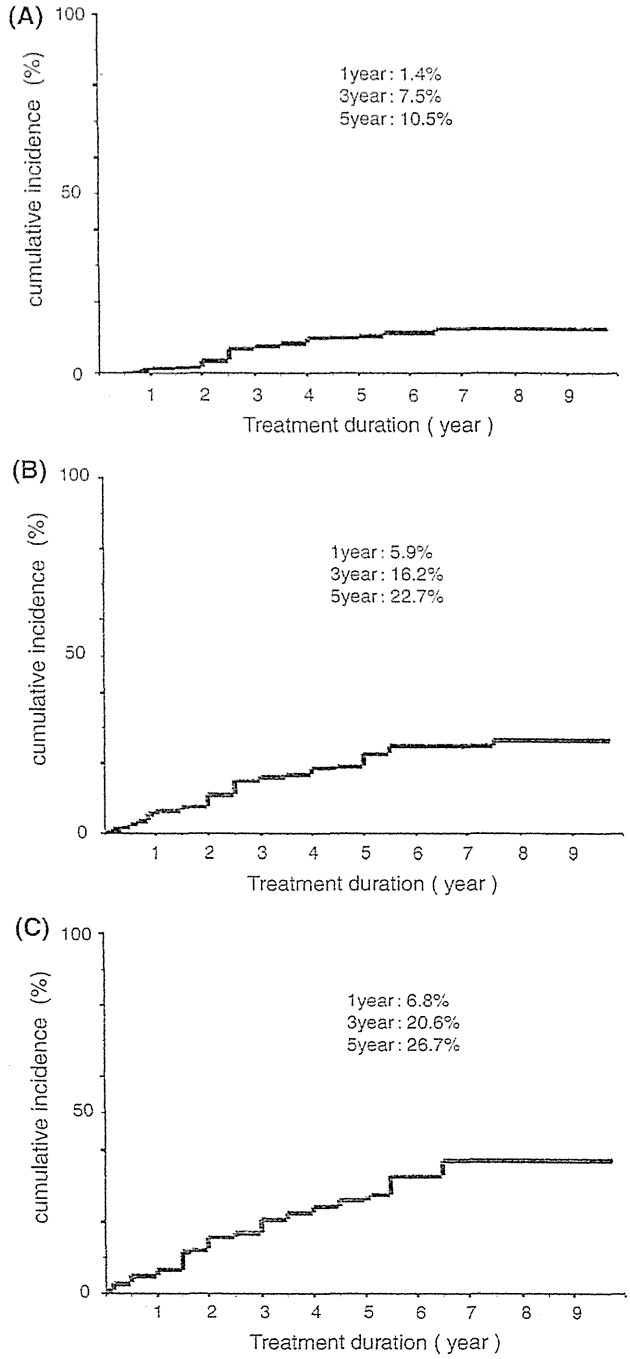

Fig. 1 Cumulative incidence of renal impairment and hypophosphatemia. a Cumulative incidence of reduction of eGFR to less than 50 ml/min/1.73 m^2 at 1-, 3-, and 5-years of treatment with ADV and LAM. b Cumulative incidence of reduction of eGFR by ≥30 % relative to baseline at 1-, 3-, and 5-years of treatment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

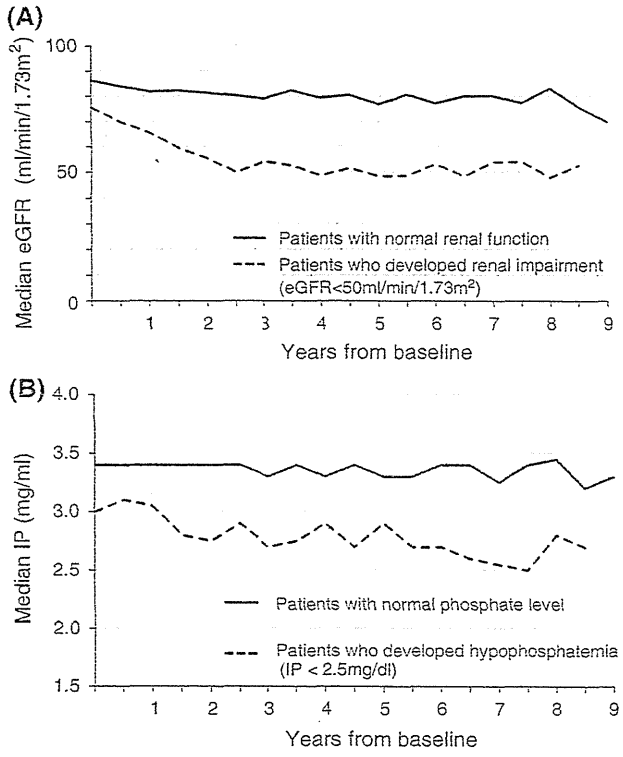

Fig. 2 Clinical course after the addition of ADV to LAM for treatment of chronic hepatitis B infection. **a** Changes in median eGFR level after the addition of ADV to LAM. *Solid line* patients with normal renal function, *broken line* patients who developed renal impairment (excluding patients who 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

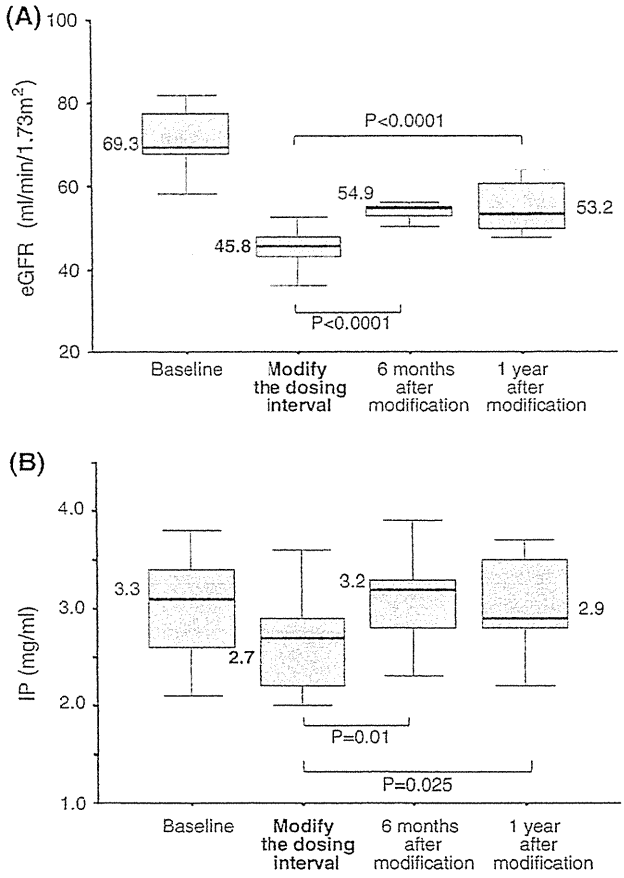

Fig. 3 Changes in eGFR and serum phosphate after modification of the ADV dosing interval. **a** Changes in eGFR. **b** Changes in serum phosphate level

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

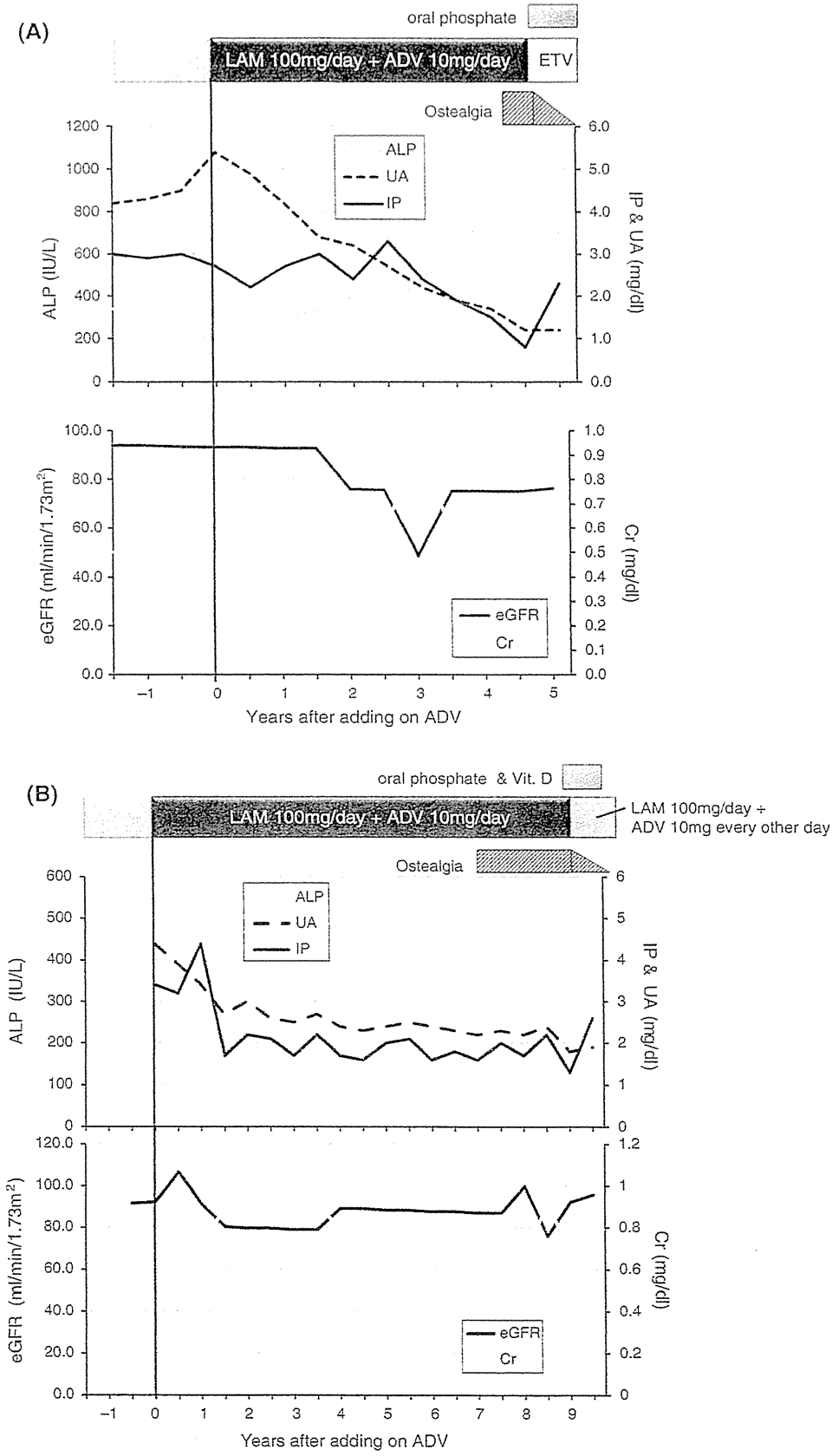

Fig. 4 Two cases who developed Fanconi's syndrome. a Case 1: a 63-year-old woman with HBeAg-positive liver cirrhosis. b Case 10: a 31 year-old man with HBeAg-positive liver cirrhosis

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 (≥30 % decrease relative to baseline), and two patients with Fanconi's syndrome showed ≥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.

Acknowledgments This study was supported in part by a Grant-in-aid from the Ministry of Health, Labor and Welfare of Japan.

Conflict of interest None.

References

1. Lee WM, Hepatitis B. Virus infection. N Engl J Med. 1997;337:1733–45.
2. Dienstag JL, Hepatitis B. Virus infection. N Engl J Med. 2008;359:1486–500.
3. Lai C-L, Chien R-N, Leung NWY, Chang TT, Guan R, Tai D-I, et al. A one-year trial of lamivudine for chronic hepatitis B. N Engl J Med. 1998;339:61–8.
4. Chen CH, Lee CM, Lu SN, Wang JH, Tung HD, Hung CH, et al. Comparison of clinical outcome between patients continuing and discontinuing lamivudine therapy after biochemical breakthrough of YMDD mutants. J Hepatol. 2004;41:454–61.
5. Suzuki F, Suzuki Y, Tsubota A, Akuta N, Someya T, Kobayashi M, et al. Mutations of polymerase, precore and core promoter gene in hepatitis B virus during 5-year lamivudine therapy. J Hepatol. 2002;37:824–30.
6. Suzuki F, Tsubota A, Arase Y, Suzuki Y, Akuta N, Hosaka T, et al. Efficacy of lamivudine therapy and factors associated with emergence ofresistance in chronic hepatitis B virus infection in Japan. Intervirology. 2003;46:182–9.
7. Hashimoto Y, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, et al. Clinical and virological effects of long-term (over 5 years) lamivudine therapy. J Med Virol. 2010;82:684–91.
8. Peters MG, Hann HH, Martin P, Heathcote EJ, Buggisch P, Rubin R, et al. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. Gastroenterology. 2004;126:91–101.
9. Hosaka T, Suzuki F, Suzuki Y, Saitoh S, Kobayashi M, Someya T, Sezaki H, Akuta N, Arase Y, Ikeda K, Kumada H. Factors associated with the virological response of lamivudine-resistant hepatitis B virus during combination therapy with adefovir dipivoxil plus lamivudine. J Gastroenterol. 2007;42:368–74.
10. Rapti I, Dimou E, Mitsoula P, Hadziyannis SJ. Adding-on versus switching-to adefovir therapy in lamivudine-resistant HBeAg-negative chronic hepatitis B. Hepatology. 2007;45:307–13.
11. Kumada H, Okanoue T, Onji M, Moriwaki H, Izumi N, Tanaka E, et al. Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis B virus infection for the fiscal year 2008 in Japan. Hepatol Res. 2010;40:1–7.

12. Izzedine H, Hulot JS, Launay-Vacher V, Marcellini P, Hadziyannis SJ, Currie G, et al. Renal safety of adefovir dipivoxil in patients with chronic hepatitis B: two double-blind, randomized, placebo-controlled studies. Kidney Int. 2004;66:1153–8.
13. Ha NB, Ha NB, Garcia RT, Trinh HN, Vu AA, Nguyen HA, et al. Renal dysfunction in chronic hepatitis B patients treated with adefovir dipivoxil. Hepatology. 2009;50:727–34.
14. Tamori A, Enomoto M, Kobayashi S, Iwai S, Morikawa H, Sakaguchi H, et al. Add-on combination therapy with adefovir dipivoxil induces renal impairment in patients with lamivudine-refractory hepatitis B virus. J Viral Hepat. 2010;17:123–9.
15. Kim YJ, Cho HC, Sinn DH, Gwak GY, Choi MS, Koh KC, et al. Frequency and risk factors of renal impairment during long-term adefovir dipivoxil treatment in chronic hepatitis B patients. J Gastroenterol Hepatol. 2012;27:306–12.
16. Jung YK, Yeon JE, Choi JH, Kim CH, Jung ES, Kim JH, et al. Fanconi's syndrome associated with prolonged adefovir dipivoxil therapy in a hepatitis B virus patient. Gut Liver. 2010;4:389–93.
17. Law ST, Li KK, Ho YY. Nephrotoxicity, including acquired Fanconi's syndrome, caused by adefovir dipivoxil—is there a safe dose? J Clin Pharm Ther. 2012;37:128–31.
18. Izzedine H, Launay-Vacher V, Isnard-Bagnis C, Deray G. Drug-induced Fanconi's syndrome. Am J Kidney Dis. 2003;41: 292–309.
19. Cihlar T, Ho ES, Lin DC, Mulato AS. Human renal organic anion transporter 1 (hOAT1) and its role in the nephrotoxicity of antiviral nucleotide analogs. Nucleosides, Nucleotides Nucleic Acids. 2001;20:641–8.
20. Marcellin P, Chang TT, Lim SG, Tong MJ, Sievert W, Shiffman ML, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med. 2003; 348:808–16.
21. Davies DF, Shock NW. Age changes in glomerular filtration rate, effective renal plasma flow, and tubular excretory capacity in adult males. J Clin Invest. 1950;29:496–507.
22. Lewis WH, Alvey AS. Changes with age in the renal function in adult men. I. Clearance of urea. Am J Physiol. 1938;123:500–15.
23. Wollom GL, Gifford RW. The kidney as a target organ in hypertension. Geriatrics. 1976;31:71–9.
24. Lindeman RD, Tobin JD, Shock NW. Association between blood pressure and the rate of decline in renal function with age. Kidney Int. 1984;26:861–8.
25. Dronavalli S, Duka I, Bakris GL. The pathogenesis of diabetic nephropathy. Nat Clin Pract Endocrinol Metab. 2008;4:444–52.
26. Ginès P, Schrier RW. Renal failure in cirrhosis. N Engl J Med. 2009;361:1279–90.
27. Kahn J, Lagakos S, Wulfsohn M, Cherng D, Miller M, Cherrington J, et al. Efficacy and safety of adefovir dipivoxil with antiretroviral therapy: a randomized controlled trial. JAMA. 1999;282:2305–12.
28. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, et al. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B. N Engl J Med. 2005;352:2673–81.
29. Gara N, Zhao X, Collins MT, Chong WH, Kleiner DE, Jake Liang T, et al. Renal tubular dysfunction during long-term adefovir or tenofovir therapy in chronic hepatitis B. Aliment Pharmacol Ther. 2012;35:1317–25.
30. Amanzadeh J, Jr. Reilly RF. Hypophosphatemia: an evidence-based approach to its clinical consequences and management. Nat Clin Pract Nephrol. 2006;2:136–48.
31. Bushinsky DA, Monk RD. Electrolyte quintet: calcium. Lancet. 1998;352:306–11.
32. Weisinger JR, Bellorín-Font E. Magnesium and phosphorus. Lancet. 1998;352:391–6.
33. Cirillo M, Ciacci C, De Santo NG. Age, renal tubular phosphate reabsorption, and serum phosphate levels in adults. N Engl J Med. 2008;359:864–6.
34. Izzedine H, Launay-Vacher V, Isnard-Bagnis C, Deray G. Drug-induced Fanconi's syndrome. Am J Kidney Dis. 2003;41:292–309.
35. Kim DH, Sung DH, Min YK. Hypophosphatemic osteomalacia induced by low-dose adefovir therapy: focus on manifestations in the skeletal system and literature review. J Bone Miner Metab. 2012;14.
36. Carpenter TO. The expanding family of hypophosphatemic syndromes. J Bone Miner Metab. 2012;30:1–9.
37. Laing CM, Toye AM, Capasso G, Unwin RJ. Renal tubular acidosis: developments in our understanding of the molecular basis. Int J Biochem Cell Biol. 2005;37:1151–61.
38. Clarke BL, Wynne AG, Wilson DM, Fitzpatrick LA. Osteomalacia associated with adult Fanconi's syndrome: clinical and diagnostic features. Clin Endocrinol. 1995;43:479–90.
39. Wong T, Girgis CM, Ngu MC, Chen RC, Emmett L, Archer KA, et al. Hypophosphatemic osteomalacia after low-dose adefovir dipivoxil therapy for hepatitis B. J Clin Endocrinol Metab. 2010;95:479–80.
40. Girgis CM, Wong T, Ngu MC, Emmett L, Archer KA, Chen RC, Seibel MJ. Hypophosphataemic osteomalacia in patients on adefovir dipivoxil. J Clin Gastroenterol. 2011;45:468–73.
41. Kim DH, Sung DH, Min YK. Hypophosphatemic osteomalacia induced by low-dose adefovir therapy: focus on manifestations in the skeletal system and literature review. J Bone Miner Metab. 2012: Epub ahead of print.
42. Ozeki I, Karino Y, Akaike J, Kimura R, Arakawa T, Nakashima T, et al. Renal dysfunction in chronic hepatitis B patients treated with adefovir dipivoxil. Kanzo. 2011;52:102–11.

J Gastroenterol (2014) 49:538–546
DOI 10.1007/s00535-013-0821-2

The Japanese Society of Gastroenterology

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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Received: 5 February 2013 / Accepted: 18 April 2013 / Published online: 20 June 2013

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

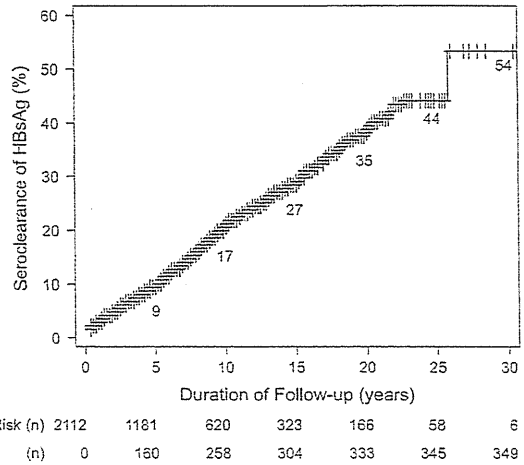

Fig. 1 Seroclearance of HBsAg in the 2,112 patients studied. Numbers of patients at risk and those of patients who lost HBsAg are indicated below each time point

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