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Case report

Sustained virological response in a patient with chronic hepatitis C treated by monotherapy with the NS3-4A protease inhibitor telaprevir

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ABSTRACT

Here, we describe for the first time a case of sustained virological response (SVR) achieved in a patient with chronic hepatitis C (CH-C) by monotherapy with a NS3-4A protease inhibitor, telaprevir, without interferon therapy. A 59-year-old treatment-naïve Japanese man was enrolled in a phase II trial of telaprevir by repeat oral administration at a dose of 750 mg every 8 h for 24 weeks. At the start of treatment, he exhibited a low-level viremia with genotype 1b of the hepatitis C virus (HCV). After the first week of treatment with telaprevir, serum HCV RNA was undetectable, and negativity remained until the end of treatment. Moreover, he was evaluated as having a SVR after the post-treatment 24-week follow-up program. Two characteristics may explain the strong antiviral activity of telaprevir in the present case. First, although pre-treatment PCR-direct sequencing and cloning for the N-terminal in the NS3 region showed a protease inhibitor-resistant variant (T54A) in 1 of 32 independent clones, the T54A substitution has only a low-level resistance to protease inhibitors and his viral load was low. Second, when compared to a consequence sequence of 35 treatment-naïve patients with HCV genotype 1b, R130K and Q195K substitutions were unique to the present case. Although it is presently unknown whether the R130K and Q195K substitutions are related to SVR, this case suggests that long-term telaprevir monotherapy may be effective in CH-C patients with genotype 1 and a low viral load.

1. Introduction

The goals of antiviral treatment in patients with chronic hepatitis C (CH-C) are long-lasting eradication of the virus and a decrease in disease-related hepatic mortality. Standard treatment uses a combination of pegylated interferon and ribavirin (PEG-IFN-RBV), which provides a sustained virological response (SVR) rate of over 50%.[1,2] In Japan, approximately 70% of patients with CH-C are infected with genotype 1b, and those with a high titer of genotype 1b (≥100 KIU/mL [Amplicor; Roche Diagnostics K.K. Tokyo, Japan]) have lower rates of SVR (<50%), even on 48 weeks of PEG-IFN-RBV combination therapy.[3] Further, although treatment for CH-C is currently based on interferon (IFN), use of this agent is associated with serious adverse effects in some patients, such as mental disorders, apathy, and laboratory abnormalities.[1,2,4] Moreover, most CH-C patients in Japan over 70 years of age cannot receive IFN therapy due to either or both co-morbidities and the risk of adverse effects. For these reasons, a new treatment strategy is needed for patients with CH-C that displays high SVR rates and a favorable side-effect profile.

One recently introduced treatment strategy for CH-C is inhibition of the NS3-4A protease in the HCV polyprotein. Potential inhibitors include telaprevir (VX-950; MP-424; Mitsubishi Tanabe Pharma Co., Osaka, Japan), which has been selected as a clinical therapy candidate for the treatment of CH-C.[5] In some patients with genotype 1 and a high viral load, however, the efficacy of telaprevir monotherapy was limited, and combination therapy of telaprevir plus PEG-IFN-RBV is now standard.[6–10] On this background, we therefore report here for the first time a patient with CH-C who achieved a SVR following monotherapy with telaprevir.

2. Case report

A 59-year-old Japanese man was admitted to Toranomon Hospital, Tokyo in July 2007 following a positive result for HCV RNA at general check-up. Laboratory tests before treatment showed mild

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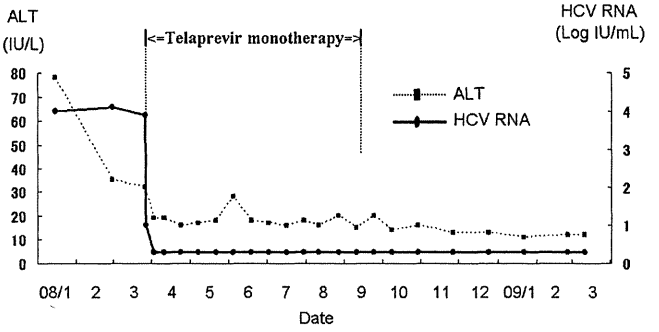

Fig. 1. Clinical course during and after 24 weeks of telaprevir monotherapy.

elevation of ALT (46 IU/L), and persistent HCV infection with genotype 1b and low-level viremia (<5 Log IU/mL [COBAS TaqMan HCV test, Roche Diagnostics K.K. Tokyo]) that continued to remain low until the start of treatment. He was diagnosed with CH-C by peritoneoscopy and liver biopsy (mild hepatitis [A1] and moderate fibrosis [F2]) at our hospital in February 2008. He had not received IFN therapy or any other antiviral drugs, and was enrolled in a phase II trial of telaprevir. Written informed consent was obtained, and the study was conducted in compliance with Good Clinical Practice and the Declaration of Helsinki. Treatment with telaprevir was started in March 2008, at which time serum HCV RNA was 3.9 Log IU/mL. Treatment was by repeat oral administration at a dose of 750 mg every 8 h for 24 weeks. Serum HCV RNA was undetectable after the first week and remained negative until the end of treatment (September 2008), and moreover remains undetectable as of March 2009. He was evaluated as having a SVR after the post-treatment 24-week follow-up program (Fig. 1).

The genome sequence for the N-terminal 609 nucleotides (203 amino acids) in the NS3 region of HCV isolates from the patient was analyzed before treatment with telaprevir. HCV RNA was extracted from 100 μL of serum and the nucleotide sequences were determined by direct sequencing and cloning. The primers used to amplify the NS3 region were NS3-F1 (5′-ACACCGCGGCGTGTGGGGACAT-3′; nucleotides 3295–3316) and NS3-AS2 (5′-GCTCTTGCCGCTGCCAGTGGGA-3′; nucleotides 4040–4019) as the first (outer) primer pair and NS3-F3 (5′-CAGGGGTGGCGGCTCCTT -3′; nucleotides 3390–3407) and NS3-AS2 as the second (inner) primer pair.[11] Thirty-five cycles of first and second amplifications were performed as follows: denaturation for 30 s at 95 °C, annealing of primers for 1 min at 63 °C, extension for 1 min at 72 °C, and final extension was performed at 72 °C for 7 min. PCR-amplified DNA was purified after agarose gel electrophoresis and amplification products of the second-round PCR were ligated with plasmid and transformed in *Escherichia coli* in a cloning kit (TA Cloning; Invitrogen, Carlsbad, CA). Dideoxynucleotide termination sequencing was performed with the BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems Japan, Tokyo). Sequences of 32 independent clones from the sample were determined and analyzed. The pre-treatment analyses by PCR-cloning showed a variant (T54A) resistant to protease inhibitors in 1 of the 32 clones.

We also made a consensus sequence of the NS3 region from the PCR-direct sequences of 35 treatment-naïve Japanese patients with HCV genotype 1b in our hospital (Fig. 2). Compared to the consensus sequence, there were a total of 5 identical substitution variants (V48I, P89S, S122G, R130K, Q195K) within the 32 independent clones from this patient, among which R130K and Q195K were unique to this patient.

3. Discussion

Previous studies showed that telaprevir monotherapy for HCV patients with genotype 1 and a high viral load demonstrated substantial antiviral activity, and the median maximum change was −4.77 Log IU/mL with administration at 750 mg every 8 h for 2 weeks.[6,7] In Reesink et al., HCV RNA decreased below the limit of

```
                1       10        20        30        40        50
CONSENSUS       APITAYSQQT RGLLGCIITS LTGRDKNQVE GEVQVVSTAT QSFLATCVNG
Case clone1     ---------- ---------- ---------- ---------- -------I--
Case clone2     ---------- ---------- ---------- ---------- -------I--
Case clone3     -----H---- ---------- ---------- ---------- -------I--
Case clone4     ---------- ---------- ---------- ---------- -------I--
Case clone5     ---------- ---------- ---------- ---------- -------I--
                51                                                100
CONSENSUS       VCWTVYHGAG SKTLAGPKGP ITQMYTNVDQ DLVGWQAPPG ARSLTPCTCG
Case clone1     ---------- ---------- ---------- --------S- ----------
Case clone2     -----F---- ---------- ---------- --------S- ----------
Case clone3     ---A------ ---------- ---------- --------S- ----------
Case clone4     ---------- ---------- ---------- --------S- -----L----
Case clone5     -----F---- ---------- ---------- --------S- ----------
                101                       130               150
CONSENSUS       SSDLYLVTRH ADVIPVRRRG DSRGSLLSPR PVSYLKGSSG GPLLCPSGHA
Case clone1     ---------- ---------- -G-------K ---------- ----------
Case clone2     ---------- ---------- -G-------K ---------- ----------
Case clone3     ---------- ---------- -G-------K ---------- ----------
Case clone4     ---------- ---------- -G-------K ---------- ----------
Case clone5     ---------- ---------- -G-------K ---------- ----------
                151                                   195  200
CONSENSUS       VGIFRAAVCT RGVAKAVDFI PVESMETTMR SPVFTDNSSP PAVPQTFQVA
Case clone1     ---------- ---------- ---------- ---------- ----K-----  15
Case clone2     ---------- ---------- ---------- ---------- ----K-----  14
Case clone3     ---------- ---------- ---------- ---------- ----K-----   1
Case clone4     ---------- ---------- ---------- ---------- ----K-----   1
Case clone5     ---------- ---------- ---------- ---------- ----K----V   1
```

Fig. 2. Evolution of the HCV NS3 gene sequence at the start of telaprevir monotherapy. Consensus sequence was made from the HCV RNA of 35 treatment-naïve Japanese patients with genotype 1b in our hospital. The number of clones within each sample of identical amino acid sequences is given on the right at the end of each sequence. Dashes indicate identical amino acid sequences.

detection (10 IU/mL) for 2 patients in the group receiving 750 mg every 8 h.[6] In some patients, however, HCV RNA levels increased between days 7 and 14, and mutations that confer resistance to telaprevir were detected. This trial of telaprevir monotherapy was therefore terminated after 2 weeks, and combination therapy of telaprevir plus PEG-IFN-RBV is now used in the USA and Europe.[8–10] Our case may therefore represent an unusual and possibly serendipitous response to long-term telaprevir monotherapy, and the efficacy of monotherapy remains unclear.

To our knowledge, this is the first report of a patient with CH-C achieving SVR by telaprevir monotherapy, without the use of IFN. Three treatment-naïve Japanese patients were enrolled in our hospital for this phase II trial of telaprevir monotherapy over 24 weeks. Before treatment, the 2 non-SVR patients had a high HCV RNA viral load (>5 Log IU/mL), while the viral load in the SVR patient remained low. Further, while HCV RNA decreased below the limit of detection (10 IU/mL) and negativity of HCV RNA remained until the end of treatment in 2 patients, HCV RNA in the other non-SVR patient reappeared after treatment cessation.

The development of drug resistance has been a challenge for treatment strategies in many viral infections. The high replication rate and the error-prone nature of viral RNA polymerases generate a viral quasi-species from which variants resistant to viral inhibitors can be selected. Recently, Kuntzen et al. reported that viral loads were high in the majority of treatment-naïve patients carrying mutations of protease and polymerase inhibitors.[12] Low viral load may therefore be an important factor for achieving SVR by telaprevir monotherapy.

It has recently been reported that CH-C patients never treated with an NS3-4A protease inhibitor may nevertheless possess variants resistant to protease inhibitors involving the HCV RNA NS3 region.[12–14] While there was a resistant variant (T54A) in this case, this mutation exhibits only low-level resistance,[7] and the number of mutant variants may have been few along with substantial suppression of HCV replication by telaprevir. This may also help to explain the effectiveness of telaprevir in this case.

Moreover, two amino acid substitutions (R130K and Q195K) were unique to this patient. We therefore checked the nucleotide sequence data in the DDBJ/EMBL/GenBank databases and found a previous report by Franco et al. on the R130K substitution (EF013801, EF013863, EF013867, EF013869).[15] Interestingly, although only a minor clone (4% of total) in that study, the viral load of the patient with the R130K substitution was also low (2364 IU/mL). To date, however, the Q195K substitution has not been reported. Their presence in this case may indicate that telaprevir has a stronger antiviral activity against HCV with these substitutions.

The NS3-4A protease targeted by protease inhibitors is required for viral polyprotein processing, an essential step in viral replication, but is also responsible for disrupting IFN responses to the infection.[16] Previous studies have shown that high concentrations of protease inhibitors may restore retinoic acid-inducible gene I (RIG-I) signaling in HCV replicon cells, [16–18] and Liang et al. also recently reported that protease inhibitors could restore interferon regulatory factor 3 (IRF-3) signaling in HCV-infected cells.[19] In our patient, telaprevir may have therefore rescued the NS3-4A-mediated blockade of IRF-3 signaling *in vivo*.

Further studies are required, such as sequencing analyses of the HCV NS3 region, and research into the rescue of IFN-β signaling through the RIG-I pathway. It is foreseeable in the future for CH-C patients to be treated by one or a combination of two or more oral drugs with high efficacy and genetic barriers to resistance and low side-effect profiles. Telaprevir may hold promise for being one of these drugs, even if only within a subset of patients, and further studies into telaprevir monotherapy or combination therapy with other oral drugs is therefore warranted. Although still an isolated response, based on our current molecular understanding of HCV infection and pharmacotherapy, this case suggests that long-term telaprevir monotherapy may be effective in other CH-C patients with genotype 1 and a low viral load.

Conflict of interest

The authors have no commercial or other associations that may pose a conflict of interest.

Acknowledgments

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Virus Clearance Reduces Bone Fracture in Postmenopausal Women With Osteoporosis and Chronic Liver Disease Caused by Hepatitis C Virus

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Osteoporosis is often present in postmenopausal women. The aim of this retrospective cohort study was to assess the cumulative incidence and predictive factors for bone fracture after cessation of interferon (IFN) in postmenopausal women with osteoporosis and chronic liver disease caused by hepatitis C virus (HCV). A total of 420 postmenopausal women treated with IFN monotherapy were enrolled. The mean observation period was 7.2 years. The primary goal was the development of bone fracture. Evaluation was carried out by using the Kaplan–Meier method and the Cox proportional hazards analysis. Thirty-one out of 420 patients sustained bone fracture. The cumulative development rate of bone fracture was 3.6% at 5th year, 9.2% at 10th year, and 17.4% at 15th year. Multivariate Cox proportional hazards analysis showed that bone fracture after cessation of IFN therapy occurred when histological staging of the liver was advanced (hazard ratio (HR): 2.54; 95% confidence interval (CI) = 1.21–5.31; $P = 0.013$), serum albumin level was < 3.5g/dl (HR: 2.25; 95% CI = 1.10–4.59; $P = 0.026$), and virus clearance was not achieved (HR: 3.65; 95% CI = 1.11–12.05; $P = 0.033$). The results indicate that virus clearance causes a reduction of two-thirds in the risk of bone fracture after cessation of IFN therapy in postmenopausal women with osteoporosis and chronic liver disease caused by HCV. ***J. Med. Virol. 82:390–395, 2010.***

KEY WORDS: chronic hepatitis C; osteoporosis; interferon; bone fracture

INTRODUCTION

Hepatitis C virus (HCV) is one of the more common causes of chronic liver disease in all countries. Chronic hepatitis C is a progressive form of liver disease that progresses relentlessly but silently to cirrhosis and/or hepatocellular carcinoma (HCC) over a period of 10–30 years [Kiyosawa and Furuta, 1991; Alter et al., 1992; Ikeda et al., 1993; Tsukuma et al., 1993]. Additionally, chronic infection due to HCV has been associated with a variety of extrahepatic complications such as essential mixed cryoglobulinemia, membranoproliferative glomerulonephritis, autoimmune thyroiditis, and sialadenitis [Johnson et al., 1993; Gumber and Chopra, 1995; Pawlotsky et al., 1995].

Bone disease is one of the major complications of chronic liver disease. The rate of bone fracture is increased in chronic liver disease, especially in postmenopausal women [Rouillard and Lane, 2001; Shiomo et al., 2002; Arase et al., 2008]. Although there is growing evidence to support the concept that HCV infection is a risk factor for bone fracture, there have been a few interventional studies confirming this issue. This

Abbreviations used: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; IFN, interferon.

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requires confirmation by a long-term follow-up of patients with a high risk of developing bone fracture.

The present retrospective cohort study was, therefore, undertaken to determine the cumulative incidence and risk factors of bone fracture after cessation of interferon (IFN) monotherapy among postmenopausal women with osteoporosis and chronic liver disease caused by HCV.

MATERIALS AND METHODS

Patients

The number of patients who were diagnosed with chronic HCV infection and treated with IFN between April 1994 and March 2007 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan was 6,003. Out of these, 420 postmenopausal women with the following criteria were enrolled in this retrospective cohort study. The enrollment criteria were: age of 55–75 years; postmenopausal osteoporosis; features of chronic hepatitis or cirrhosis diagnosed by laparoscopy, liver biopsy, ultrasonography clinical features, and/or laboratory tests; positive for HCV-RNA; treatment with IFN monotherapy, negative for hepatitis B surface antigen, antinuclear antibodies, or antimitochondrial antibodies in serum, as determined by radioimmunoassay or spot hybridization; no evidence of HCC nodules as shown by ultrasonography and/or computed tomography; no underlying systemic disease, such as systemic lupus erythmatosus, rheumatic arthritis. The diagnosis of osteoporosis was based to X-ray evidence of vertical trabecular and/or loss bone mineral density of spine or femur (AP spine by dual-energy X-ray absorptiometry, DEXA) >2 SD of young adult mean. A total of 234 patients were diagnosed by standard X-ray examination. The remaining 186 patients were diagnosed by standard X-ray and DEXA. Patients with either of the following criteria were excluded from the study: (1) malignant tumor, (2) advanced and decompensated stage of cirrhosis with encephalopathy, icterus, or refractory ascites.

In the present study, predictive factors for bone fracture after cessation of IFN therapy were assessed. All of the studies were performed retrospectively by collecting and analyzing data from the patient records. This study had been approved by Institutional Review Board of the hospital.

Viral Markers of HCV

Diagnosis of HCV infection was based on detection of serum HCV antibodies and HCV RNA. Anti-HCV antibodies were detected using a second-generation enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, v2.0, Roche Molecular Systems, Inc., Branchburg, NJ).

Evaluation of the Stage of Liver Disease

The stage of liver disease was determined partly on the basis of peritoneoscopy and/or liver biopsy. The 291 patients out of 420 were diagnosed by peritoneoscopy and/or liver biopsy. Liver biopsy specimens were obtained using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin–eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas [Desmet et al., 1994]. The remaining patients were diagnosed by clinical features, laboratory tests, and ultrasonographic findings.

Follow-Up

Patients were followed-up monthly to tri-monthly after the cessation of IFN therapy at the Toranomon hospital. Physical examination and biochemical tests were conducted at each examination together with a regular follow-up using abdominal ultrasonography and/or computed tomography imaging in each patient. When a patient had any symptoms relating to bone fracture, the physicians in charge explored further the possibility of bone fracture. Forty-seven patients were lost to follow-up. Because bone fracture and death were not identified in these 47 patients, they were regarded as withdrawals at the time of the last visit at the Toranomon hospital in statistical analysis [Harrington and Fleming, 1983].

Statistical Analysis

The cumulative development rate of bone fracture was calculated from the time of cessation of IFN therapy by using the Kaplan–Meier method. Differences in the development of bone fracture were tested using the log rank test. Independent factors associated with the development of bone fracture were analyzed by the Cox proportional hazard model. The following 12 variables were analyzed for potential covariates for development of bone fracture: age, body mass index, state of liver disease (chronic hepatitis or liver cirrhosis), HCV load, HCV-genotype, platelet count, albumin, total-cholesterol, alanine aminotransferase (ALT), kind of IFN, total dose of IFN, and efficacy of IFN therapy. A P-value of <0.05 in the two-tailed test was considered significant. Data analysis was performed using the computer program SPSS version 11.0.

RESULTS

Characteristics of the Patients

Table I shows the characteristics of the 420 women with postmenopausal osteoporosis and type C chronic liver disease. The number of patients with virus clearance was 111 (26.4%). The observation period (mean ± standard deviation) was 7.2 ± 3.5 years.

Bone Fracture

Thirty-one out of 420 patients sustained bone fracture. Seventeen patients had vertebral fracture alone

TABLE I. Characteristics of Subjects Enrolled

Characteristic	
N	420
Age (years)	64.1 ± 3.5
BMI	22.1 ± 3.6
HCV-genotype (1b/2a/2b/others)	237/110/46/27
HCV RNA level (KIU/ml)	1,193 ± 1,151
Staging (chronic hepatitis/liver cirrhosis)	310/110
AST (IU/L)	80 ± 56
ALT (IU/L)	101 ± 70
Albumin (g/dl)	4.0 ± 0.3
Total cholesterol (mg/dl)	165 ± 30
Platelet count ($\times 10^4$/mm^3)	13.6 ± 4.8
IFN-alpha[a]/IFN-beta[a]	300/120
Total dose of IFN (Megaunit)	582 ± 204
Efficacy of treatment (virus clearance/non-virus clearance)	111/309
Follow-up period (year)	7.2 ± 4.4

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; IFN, interferon.
Data are number of patients, median (range), or mean ± standard deviation.
[a]Outbreak of IFN monotherapy: recombinant IFN alpha 2a, 35 cases; recombinant IFN alpha 2b, 23 cases; natural IFN alpha, 242 cases; natural IFN beta, 120 cases.

and seven patients had hip fracture alone. One patient had both vertebral and hip fracture. Remaining six patients had bone fractures except in vertebral or hip. The cumulative appearance rate of bone fracture was 3.6% at 5th year, 9.2% at 10th year, and 17.4% at the 15th year in all the patients (Fig. 1).

Determinants of Bone Fracture

Table II shows the factors associated with bone fracture after cessation of IFN therapy in all the 420 women with postmenopausal osteoporosis and chronic liver disease caused by HCV. Univariate analysis identified the following four factors that influenced incidence of bone fracture: liver staging ($P = 0.002$), serum albumin level ($P = 0.016$), efficacy of IFN ($P = 0.039$), and kind of IFN ($P = 0.095$). These four parameters were entered into multivariate Cox proportional hazard analysis. Multivariate Cox proportional hazards analysis showed that bone fracture occurred when patient had liver cirrhosis (hazard ratio (HR): 2.54; 95% confidence interval (CI) = 1.21–5.31; $P = 0.013$), serum albumin level of <3.5 mg/dl (HR: 2.25; 95% CI = 1.10–4.59; $P = 0.026$), and non-virus clearance (HR: 3.65; 95% CI = 1.11–12.05; $P = 0.033$).

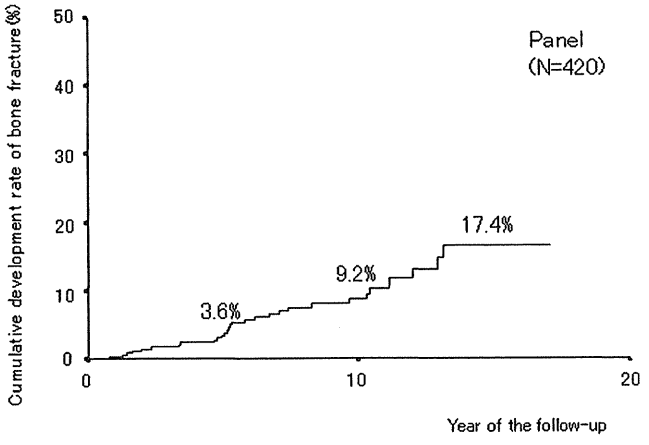

Fig. 1. Cumulative development rate of the bone fracture after the termination of IFN treatment in a total of women with osteoporosis and type C chronic liver disease.

Causes of Death After Bone Fracture

During the observation period after an episode of bone fracture, 10 of the 31 patients died. Four patients died of liver-related disease (HCC, decompensated liver cirrhosis, rupture of esophageal varices). On the other hand, six patients died of infection and deterioration of general condition. In a total of 10 patients died after the development of bone fracture, liver-related death corresponded to 40% (4/10) of all deaths.

DISCUSSION

Bone fracture after cessation of IFN monotherapy in postmenopausal women with osteoporosis and chronic liver disease caused by HCV is described in the present study. The study was limited because a retrospective cohort trial. Postmenopausal women aged 55–75 years and diagnosed as having osteoporosis were selected. The reason for the selection of women aged 55–75 years as follows; (1) Onset of bone fracture based on osteoporosis is rare in young females with <55 years and/or males, (2) patients over the age of 75 years have a tendency to avoid IFN therapy due to some IFN-related side effects. Other limitations are the followings: (1) serum levels of vitamin D were not measured, (2) bone density measurement was not measured.

However, several findings were obtained with regard to bone fracture after cessation of IFN in postmenopausal women with osteoporosis and chronic liver disease caused by HCV. First, the annual rate of bone fracture among female patients with osteoporosis and chronic liver disease caused by HCV was about 1%. Second, the development rate of bone fracture in patients with virus clearance was low with statistical significance compared to that without virus clearance. In a previous study, it was reported that virus clearance reduce the onset of malignant lymphoma and/or type 2 diabetes in HCV patients treated with IFN [Kawamura et al., 2007; Arase et al., 2009a]. The present study shows that virus clearance reduces the development of bone fracture in HCV patients. The reasons for the reduction of bone fracture in patients with virus clearance are unclear. Possible reasons are that improvement of nutrition and physical activity after virus clearance might reduce the development of bone fracture. Third, in addition to virus clearance, slight fibrosis of the liver and serum albumin level of ≥3.5 g/dl reduced the onset of bone fracture in HCV patients treated with IFN. These results suggest that maintaining a serum albumin level of ≥3.5 g/dl is important for

TABLE II. Predictive Factors for Appearance of Bone Fracture*

Variables	Univariate analysis		Cox-regression	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Age (years) (≥65/<65)	1.90 (0.93–3.89)	0.078		
BMI (≥25/<25)	1.46 (0.65–3.21)	0.362		
HCV load (KIU/ml) (≥1,000/<1,000)	1.12 (0.96–1.32)	0.155		
Genotype (1/2)	0.74 (0.35–1.57)	0.431		
ALT (IU/L) (<50/≥50)	0.50 (0.14–1.77)	0.289		
Platelet count ($\times 10^4/mm^3$) (<15/≥15)	1.52 (0.64–3.61)	0.345		
Albumin (g/dl) (<3.5/≥3.5)	2.37 (1.17–4.80)	0.016	2.25 (1.10–4.59)	0.026
Cholesterol (mg/dl, ≥180/<180)	0.33 (0.04–2.72)	0.305		
Staging (liver cirrhosis/chronic hepatitis)	2.85 (1.38–5.90)	0.005	2.54 (1.21–5.31)	0.013
Kind of IFN (beta/alpha)	0.44 (0.17–1.15)	0.095	0.40 (0.15–1.05)	0.062
Total dose of IFN (MU) (≥500/<500)	0.91 (0.59–1.40)	0.672		
Efficacy (non-virus clearance/virus clearance)	3.50 (1.06–11.53)	0.039	3.65 (1.11–12.05)	0.033

ALT, alanine aminotransferase; BMI, body mass index; HR, hazards ratio; IFN, interferon.
*Data are number of patients or mean ± standard deviation.

protecting the bone fracture in HCV patients. Definitive treatment of maintaining a serum albumin level of ≥3.5 g/dl is unclear. However, the use of branched-chain amino acid granules has been shown to improve hypoalbuminemia in decompensated patients with cirrhosis and hypoalbuminemia despite adequate dietary intake [Yoshida et al., 1989; Muto et al., 2005].

Bone fracture in patients treated with IFN-beta was slightly lower compared with that in patients treated with IFN-alpha as shown in Figure 2 in spite of $P > 0.05$. Takayanagi et al. [2002] have reported that administration of IFN-beta into the site of inflammation resulted in marked inhibition of osteoclast formation and bone resorption. Although the role of IFN-beta in the bone

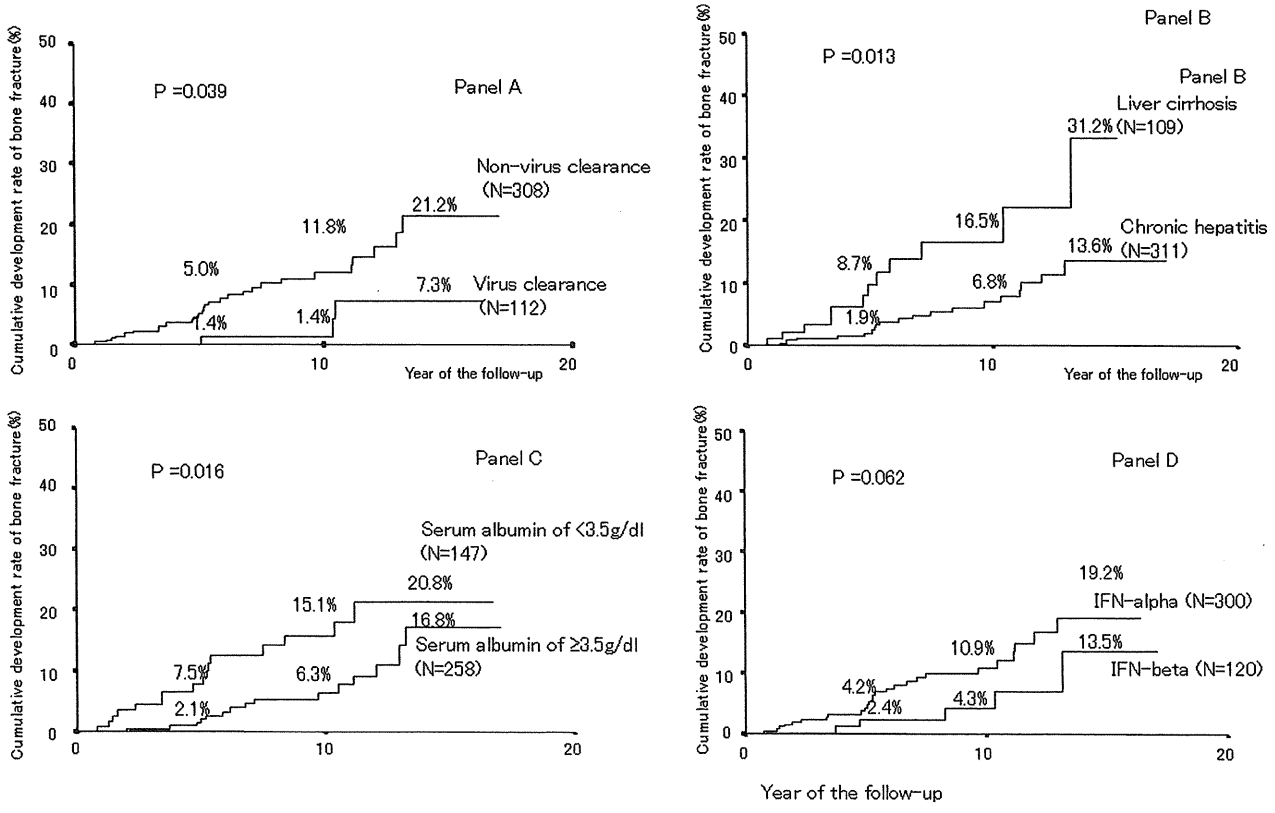

Fig. 2. **Panel A**: Cumulative development rate of the bone fracture based to difference of treatment efficacy. **Panel B**: Cumulative development rate of the bone fracture based to difference of histological staging of the liver. **Panel C**: Cumulative development rate of the bone fracture based to difference of serum albumin level. **Panel D**: Cumulative development rate of the bone fracture based to difference of kind of IFN.

metabolism remains speculative, the following possible mechanism have been considered [Abraham et al., 2009], (1) binding of IFN-beta to its biological receptor of nuclear factor-kappaB (RANK) ligand (RANKL) initiates a signal transduction cascade through the classic JAK/STAT pathway, causing an inhibition of osteoclast proliferation and differentiation; (2) another mechanism pertinent to the anti-resorptive effect of IFN-beta is the induction of nitric oxide which has been shown to inhibit osteoclast formation. On the other hand, IFN-alpha did not inhibit osteoclast formation and bone resorption. This result may indicate that the role of IFN-beta in bone metabolism way warrant systematic evaluation as a potential adjunct to therapeutic regimens of osteolytic diseases

IFN-beta should be given intravenously. Intravenous injection is not convenient for treatment compared to intramuscular or subcutaneous injection. However, IFN-beta-related side effects are mild and few compared to combination therapy with IFN-alpha. IFN-beta-induced mental disorders are milder than those induced by IFN-alpha [Katamura et al., 2008]. IFN-beta could also be given in elderly patients of 70 or older years because of mild side effects [Arase et al., 2009b]. Thus, about 10% of HCV patients are given IFN-beta in Japan.

Recent studied have reported that osteodystrophy occurs not only in patients with alcoholic cirrhosis, but also in those with cirrhosis caused by hepatitis C or B virus. Due to improvement of treatment, patients with cirrhosis live longer; an increasing proportion of such patients are found to have bone disease [Tsuneoka et al., 1996]. Thus, physicians undertaking the daily management of patients with hepatitis virus should check the bone condition of the patients in addition to the liver.

In conclusion, the present retrospective study shows that the annual incidence of bone fracture after cessation of IFN therapy among postmenopausal women with osteoporosis and chronic liver disease caused by HCV was about 1%. Virus clearance causes a two-thirds reduction in the risk of bone fracture after cessation of IFN in postmenopausal women with osteoporosis and HCV.

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Original Article

Correlation of YMDD mutation and breakthrough hepatitis with hepatitis B virus DNA and serum ALT during lamivudine treatment

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Aim: Continuous lamivudine treatment is associated with high frequency of drug resistance. We analyzed the incidence of tyrosine-methionine-aspartate-aspartate (YMDD) motif mutant and breakthrough hepatitis (BTH) in hepatitis B virus (HBV) DNA positive patients receiving lamivudine for > 1 year and correlated it with HBV DNA and alanine aminotransferase (ALT) levels to evaluate if these measurements can provide a practical option for monitoring patients in clinical practice and define early switch from lamivudine therapy.

Methods: Of the 929 patients receiving lamivudine for > 1 year, 359 patients who maintained an ALT level of ≤ 40 IU/L during the course of lamivudine treatment were stratified into two groups based on the duration of lamivudine treatment – one receiving lamivudine for < 3 years and the other for ≥ 3 years.

Results: The incidence of YMDD motif in patients receiving lamivudine for < 3 years was 27% in patients with ALT ≤ 20 IU/L, 58% with ALT ≤ 30 IU/L, and 63% with ALT ≤ 40 IU/L, ($P = 0.002$). The corresponding incidence of BTH was 2%, 7%, and 48% ($P < 0.001$). The incidence of YMDD motif and BTH in these patients was 7% and 2% with HBV DNA < 2.6 (log copies/mL) and ALT ≤ 20 IU/L, while with ALT at 21–30, the YMDD motif mutant was 16% and BTH was 0%.

Conclusion: Correlation of ALT and HBV DNA levels with YMDD motif mutant and BTH indicates that these measurements can be used in clinical practice for deciding early switch from lamivudine to other suitable antiviral therapies.

Key words: alanine transaminase, breakthrough hepatitis, hepatitis B virus, lamivudine, mutation, viral DNA

INTRODUCTION

LAMIVUDINE HAS GAINED increasing popularity since its approval in 1998 for the treatment of chronic hepatitis B virus (CHBV).[1–4] Lamivudine blocks HBV replication, reduces HBV DNA levels, normalizes alanine aminotransferase (ALT) levels, thereby resulting in histological improvement of the liver.[5] It is a reverse transcriptase inhibitor that acts by competing with the natural polymerase substrate deoxycytidine triphosphate (dCTP) and thus inhibits the elongation of HBV DNA minus strand. It incorporates into the nascent DNA strand and thereby acts as a chain terminator. Although lamivudine is very effective in inhibiting viral replication, the incidence of resistance is high, with an estimated 14–32% of patients developing resistance after 1 year of treatment, 38% after 2 years of treatment, and 53–76% after 3 years of treatment.

Resistance to lamivudine, which increases over years is due to development of mutations in the tyrosine-methionine-aspartate-aspartate (YMDD) motif in the DNA polymerase/reverse transcriptase, which is the main target of lamivudine.[4,6–9] This amino acid sequence in YMDD motif is predominantly involved in deoxynucleoside triphosphate (dNTP) binding in the catalytic site of the HBV DNA polymerase.

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Table 1 2007 Ministry of Health, Labour and Welfare of Japan guidelines for hepatits B virus (HBV)-positive patients for nucleoside analogue treatment for patients with chronic HBV receiving lamivudine therapy

Lamivudine therapy HBV DNA		< 3 years	≥ 3 years
Keep < 2.6 log copies/mL		Switch to entecavir 0.5 mg/day	Continue lamivudine 100 mg/day
≥ 2.6 log copies/mL	No BTH†	Switch to entecavir 0.5 mg/day	
	With BTH	Adefovir 10mg/day (duo therapy with lamivudine)	Adefovir 10 mg/day (duo therapy with lamivudine)

†After checking for absence of tyrosine-methionine-aspartate-aspartate (YMDD) motif mutation. BTH, breakthrough hepatitis.

Long-term lamivudine therapy is associated with amino acid substitutions mainly in the YMDD motif and also in the proximal FLLAQ (phenylalanine, leucine, alanine, glutamine) motif.[10] Common mutation may occur in the YMDD motif where the methionine residue is replaced either by valine (rtM204V) or isoleucine (rtM204I).[11] These amino acid substitutions form the basis of emergence of lamivudine-resistant strains of HBV and when these occur, the clinical condition may worsen, which is usually accompanied by increase in viral load and serum aminotransferase levels. YMDD mutants cause breakthrough hepatitis (BTH) and, therefore, require withdrawal or switch-over from lamivudine treatment. The American Association for the Study of Liver Diseases (AASLD) and the United States Algorithm for Management of Patients with Drug Resistance recommend either switching over to entecavir or adding adefovir in the event of lamivudine resistance.[12] The 2007 Japanese guidelines of the study group (Ministry of Health, Labour and Welfare of Japan)[13] on standardization of treatment for HBV positive patients for nucleoside analogue treatment for patients with CHBV receiving lamivudine therapy are explained below and also summarized in Table 1.

According to the 2007 guidelines for patients on lamivudine therapy, switching over criteria from lamivudine therapy has been changed from BTH to HBeAg status in patients maintaining HBV DNA copies ≥ 2.6 log copies/mL. Patients on lamivudine for < 3 years and maintaining HBV DNA copies ≥ 2.6 log copies/mL can be switched over to entecavir 0.5 mg/day if they are also HBeAg negative, whereas HBeAg-positive patients can be co-administered adefovir 10 mg/day in both the treatment duration groups (> 3 years or < 3 years).

Unfortunately, the cost of measuring HBV resistance to lamivudine by molecular methods is high and is not presently covered by Japanese reimbursement system in clinical practice. Development of HBV resistance to lamivudine is typically indicated by an increase in HBV DNA followed by an increase in serum ALT levels. Increase in HBV DNA represents active viral replication whereas serum ALT levels provide an indirect assessment of the degree of liver injury.[14]

Hence, in this study, we analyzed the correlation of the incidence of YMDD motif mutant and BTH with HBV DNA and serum ALT levels, either separately or together, in HBV DNA-positive patients who are treated with lamivudine for ≥ 1 year and who had maintained an ALT level of ≤ 40 IU/L until the development of BTH during the course of lamivudine treatment.

METHODS

Patients

THIS WAS A retrospective, nonrandomized study that enrolled 929 HBV DNA-positive-patients receiving 100 mg of lamivudine daily and followed up for a period of 1 year or longer between 1995 and 2006. Since long-term treatment with lamivudine was associated with a high frequency of YMDD motif mutant and BTH (BTH can be defined as abnormal variations in serum transaminase level due to YMDD motif mutant), we analyzed patients who had a possibility to switch to other nucleoside analogues. Patients (n = 395) with ALT ≤ 40 IU/L during follow-up (for 48 patients who developed BTH, data was used until 1 month before the patient developed BTH). Patients were not treated with either adefovir or entecavir during follow-up (for patients who used adefovir or entecavir because of BTH development, data was used until the point before the patient started adefovir or entecavir treatment). Patients were negative for anti-hepatitis C virus (HCV) (third-generation enzyme immunoassay; Chiron, Emerville, CA) and negative for HCV RNA with PCR (Amplicor; Roche Diagnostic Systems, Pleasanton, CA), did not have hepatocellular carcinoma, nore other forms of liver injury such as hemochromatosis, Wilson's disease,

primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease.

Informed consent was obtained from each patient included in the study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Patients were stratified into 2 groups based on the duration of lamivudine treatment – one receiving lamivudine for < 3 years ($n = 125$) and the other for ≥ 3 years ($n = 234$). In addition, we also analyzed patients based on their ALT level (IU/L) grouped into ≤20, 21–30, and 31–40, and HBV DNA (log copies/mL) divided into < 2.6, 2.6–5.0, and ≥ 5.1.

During treatment, patients were followed up each month for liver function and serum markers of HBV infection. The serum sample of the patients were collected and preserved at –80 °C. All the collected samples up to this time period were analyzed for HBV DNA in June 2001. From July 2001, the serum samples were collected and analyzed once a month at the clinical treatment facility.

YMDD motif mutants were determined at the baseline and monitored at 6 months and during the study as well as at the development of breakthrough hepatitis. YMDD motif mutants were analyzed in the serum preserved at –80 °C altogether.

Markers of HBV infection

The HBeAg was estimated by enzyme-linked immunosorbent assay (ELISA) (F-HBe; Sysmex, Kobe). HBV DNA was determined by PCR followed by hybridization (Amplicor HBV Monitor: Roche Molecular Systems, Branchburg, NJ), and the results were expressed in log copy per milliliter over a range of 2.6–7.6. The 6 major genotypes of HBV (A–F) were determined serologically by ELISA (HBV GENOTYPE EIA; Institute of Immunology) and the PCR-invader method with genotype-specific probes.[15] YMDD motif mutants were determined by PCR followed by restriction fragment length polymorphism (RFLP)[8] or enzyme-linked minisequence assay with commercial assay kits (PCR-ELMA; Genome Science).

Statistical analyses

Frequencies were compared between groups by the χ^2-test, Fisher's exact test, and HBV DNA values by Mann–Whitney *U*-test. Emergence of YMDD motif mutants and BTH were compared in the Kaplan–Meier life table by using the production limit method. A *P*-value < 0.05 was considered significant. Analyses of all data were performed with SAS 9.1.3.

RESULTS

DURING THE PERIOD of 12 years from 1995 to 2006, 929 HBV DNA-positive patients received 100 mg of lamivudine daily. From the total of 929 patients who received lamivudine for 1 year or more, 359 patients who maintained an ALT level of ≤ 40 IU/L were stratified based on the duration of lamivudine treatment and divided into 2 groups – one receiving lamivudine for < 3 years ($n = 125$) and the other for ≥ 3 years ($n = 234$). Demographic features and clinical background of the two study groups were uniformly matched with no significant differences in age, sex, serum transaminase levels, HBV DNA, hepatitis B e-antigen (HBeAg), and HBV genotype (Table 2). The median ALT values were 112 IU/L and 145 IU/L in both the groups, respectively, and the median HBV DNA level was identical at 6.1 log copies/mL in both the groups.

Incidence of YMDD motif mutant and BTH after lamivudine treatment for < 3 years

The incidence of YMDD motif mutant within 3 years of treatment with lamivudine by ALT (IU/L) level was 27% in 53 patients maintaining an ALT level of ≤ 20 (group A), 58% in 46 patients maintaining an ALT level of ≤ 30 (group B); and 63% in 26 patients maintaining an ALT level of ≤ 40 (group C), with statistical differences among the 3 groups ($P = 0.002$). The incidence of BTH was 2% in group A, 7% in group B, and 48% in group C ($P < 0.001$). The lowest incidence of YMDD motif mutant and BTH was noted in patients with ALT level of ≤ 20 (IU/L) (Fig. 1a,b). Follow-up for patients who developed BTH was discontinued upon the detection of YMDD motif mutant.

The incidence of YMDD motif mutant within 3 years of treatment with lamivudine based on the HBV DNA (log copies/mL) level was 28% in patients maintaining an HBV DNA level of < 2.6; 83% in patients maintaining an HBV DNA level of 2.6–5.0; and 100% in patients maintaining an HBV DNA level of ≥ 5.1, with significant differences among the 3 groups ($P < 0.001$). The incidence of BTH was 4%, 30%, and 40%, respectively, in patients with HBV DNA level of < 2.6, 2.6–5.0, and ≥ 5.1 log copies/mL ($P = 0.004$) (Fig. 2a,b). The lowest incidence of YMDD motif mutant and BTH was seen in patients maintaining an HBV DNA level of < 2.6 log

Table 2 Background of 359 patients using lamivudine treatment for ≥ 1 year at the start of lamivudine therapy

Factors	Duration of lamivudine therapy		
	< 3 years *n* = 125	≥ 3 years *n* = 234	Differences (*P*-value)
Age (years)	23–75 (43)†	18–76 (43)†	NS‡
Male	93 (73%)	182 (77.1%)	NS‡
HBV infection in mother	47 (37%)	82 (35%)	NS‡
Chronic hepatitis	109 (85%)	212 (90%)	NS‡
AST (IU/L)	15–866 (80)†	19–2593 (83)†	NS‡
ALT (IU/L)	11–2092 (112)†	14–2142 (145)†	NS‡
Total bilirubin (mg/dL)	0.2–3.8 (0.7)†	0.2–10.6 (0.7)†	NS‡
γ-GTP (IU/L)	16–440 (54)†	13–468 (65)†	NS‡
HBV DNA (log copy/mL)	<2.6–>7.6 (6.1)†	<2.6–>7.6 (6.1)†	NS‡
HBeAg	66(52%)	107 (45%)	NS‡
HBV genotype (A, B, C, ND)	4:15:98:8	5:21:207:1	NS‡

†Median value where indicated. ‡Not significant. ALT, alanine transaminase; AST, aspartate aminotransferase; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; γ-GTP, gamma glutamyl transferase.

copies/mL. The BTH incidence was particularly high in patients with an HBV DNA level of ≥ 5.1, which was 40% within 1 year.

The incidence of YMDD motif mutant within 3 years of treatment with lamivudine in patients based on both the ALT (IU/L) and HBV DNA (log copies/mL) level during the course of lamivudine treatment was evaluated (Table 3).

In patients maintaining HBV DNA < 2.6 and ALT ≤ 20, the incidence of YMDD motif mutant and BTH was 7% and 2%, respectively. Whereas in patients with HBV DNA level of < 2.6 and ALT 21–30, the incidence of YMDD motif mutant was higher at 16% and BTH was 0%, and in patients with ALT 31–40, YMDD motif mutant and BTH was further higher at 42% and 17%, respectively.

In patients with HBV DNA level at 2.6–5.0 and ALT ≤ 20, the incidence of YMDD motif mutant was 33% in patients with 0% incidence of BTH. Nevertheless, in patients maintaining HBV DNA at 2.6–5.0 but with ALT 21–30, the incidence of YMDD motif mutant was 73% and BTH was 18%; whereas in patients with ALT 31–40, the incidence of YMDD motif mutant was 50% and BTH was 42%.

In patients maintaining HBV DNA ≥ 5.1 and ALT 31–40, both YMDD motif mutant and BTH was 100%.

Incidence of YMDD motif mutant and BTH after lamivudine treatment for ≥ 3 years

In patients treated with lamivudine for 3 years or more, the incidence of YMDD motif mutant by ALT (IU/L) level was 58% in 113 patients in group A, 60% in 84

Table 3 Incidences of tyrosine-methionine-aspartate-aspartate (YMDD) mutant and breakthrough hepatitis (BTH) by hepatits B virus (HBV) DNA and alanine transaminase (ALT) level in patients during lamivudine treatment for < 3 years (125 patients)

HBV DNA† (Amplicor: log copies/mL)	ALT level (IU/L)†					
	≤ 20		21–30		31–40	
	YMDD	BTH	YMDD	BTH	YMDD	BTH
< 2.6	3/41 (7%)	1/41 (2%)	5/32 (16%)	0/32 (0%)	5/12 (42%)	2/12 (17%)
2.6–5.0	4/12 (33%)	0/12 (0%)	8/11 (73%)	2/11 (18%)	6/12 (50%)	5/12 (42%)
≥ 5.1	0	0	3/3 (100%)	0/3 (0%)	2/2 (100%)	2/2 (100%)

†The HBV DNA and ALT levels are shown based on the treatment duration of lamivudine.

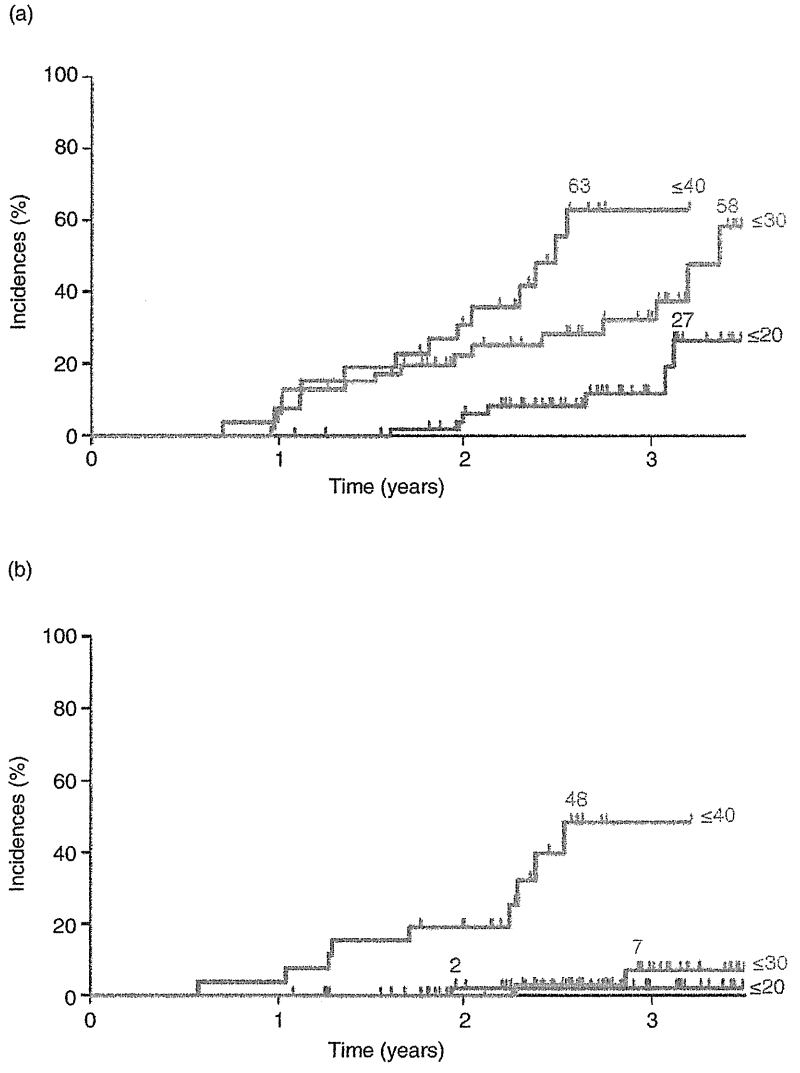

Figure 1 The incidence of tyrosine-methionine-aspartate-aspartate (YMDD) motif mutant and breakthrough hepatits was noted in patients with alanine aminotransferase level of ≤ 20 (IU/L) (a) Incidence of YMDD mutants over time ($P = 0.0017$). (b) Incidence of break throughhepatitis over time ($P < 0.0001$).

patients in group B, and 80% in 37 patients in group C ($P = 0.002$), and that of BTH in the corresponding groups was 7%, 14%, and 57% ($P < 0.001$) (Fig. 3a,b).

In patients treated with lamivudine for ≥ 3 years, the increased incidence of YMDD motif mutant by HBV DNA (log copies/mL) level was 65% in patients maintaining an HBV DNA level of < 2.6, 78% in patients maintaining an HBV DNA level of 2.6–5.0, and 92% in patients maintaining an HBV DNA level of ≥ 5.1, and that of BTH in the corresponding groups was 10%, 18%, and 77% ($P < 0.001$) (Fig. 4a,b).

The incidence of YMDD motif mutant in ≥ 3 years treatment with lamivudine in patients by both ALT (IU/L) and HBV DNA (log copies/mL) levels during the course of lamivudine treatment was also analyzed (Table 4).

In patients maintaining HBV DNA < 2.6 and ALT ≤ 20, the incidence of YMDD motif mutant and BTH was 38% and 7%, respectively. At the same HBV DNA level of < 2.6 and ALT 21–30, the incidence of YMDD motif mutant was 48% and BTH was 8%; whereas at ALT 31–40, YMDD motif mutant was 36% and BTH was 9%.

In patients maintaining HBV DNA 2.6–5.0 and ALT ≤ 20, the incidence of YMDD motif mutant and BTH was 60% and 4%, respectively. At the same HBV DNA

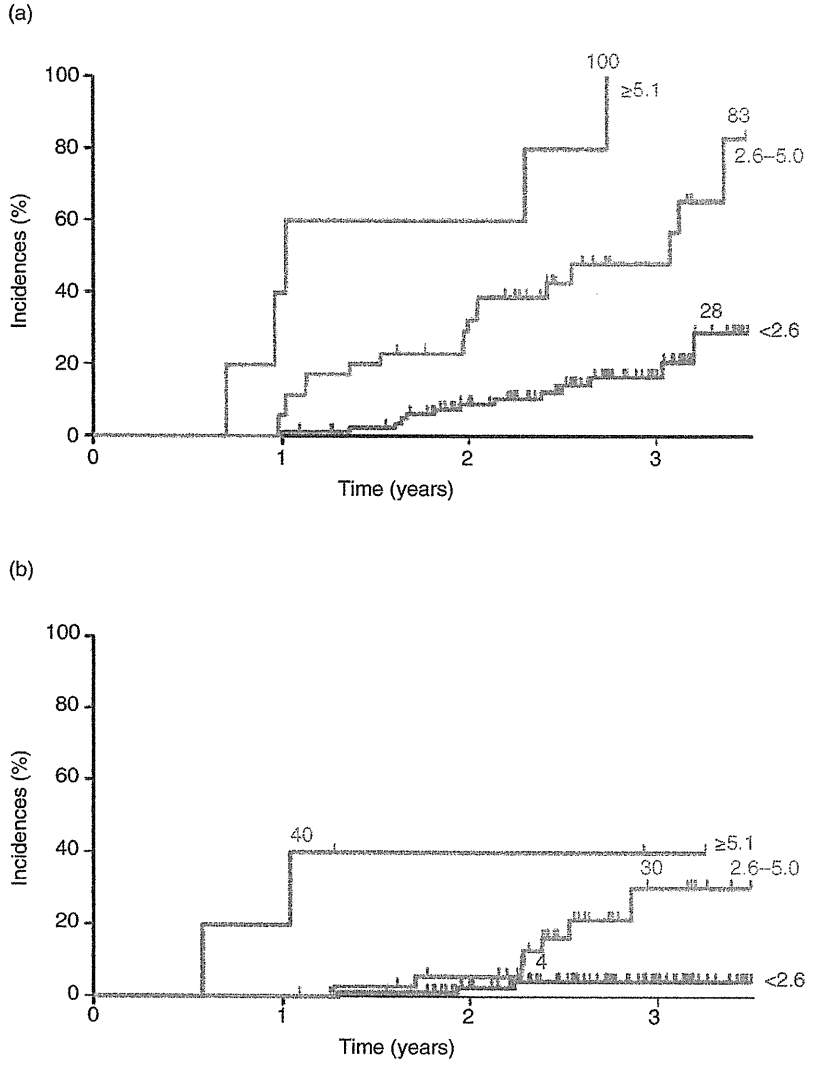

Figure 2 incidence of BTH was 4%, 30%, and 40%, respectively, in patients with HBV DNA level of < 2.6, 2.6–5.0, and ≥ 5.1 log copies/mL ($P = 0.004$). (a) Incidence of YMDD mutants over time ($P = 0.0001$). (b) Incidence of breakthrough hepatitis over time ($P < 0.0037$).

level, 2.6–5.0 and ALT 21–30, the incidence of YMDD motif mutant was 86% and BTH was 18%; whereas at ALT 31–40, YMDD motif mutant was 92% and BTH was 42%.

In patients maintaining HBV DNA ≥ 5.1 and ALT 31–40, YMDD motif mutant was 93% and BTH was 86%.

DISCUSSION

LONG-TERM THERAPY for CHBV can lead to the development of HBV drug-resistant mutants. Early detection of the YMDD motif mutants in lamivudine-treated patients and timely switch to other nucleoside analogues with low viral resistance is crucial to prevent viral and biochemical flares and ineffective therapeutic response. Although development of YMDD mutants results in decreased viral susceptibility to lamivudine, viral replication rate is lower in mutant strains than in wild type.[6]

Among the 359 patients who received lamivudine for > 1 year and maintained an ALT level of ≤ 40 IU/L, the rate of YMDD motif mutant was 11% (1 year), 29% (2 year), 42% (3 year), 49% (4 year) and 61% (5 year). BTH occurrences were 3% (1 year), 8% (2year), 13% (3 year), 15% (4 year) and 19% (5 year). The rate of

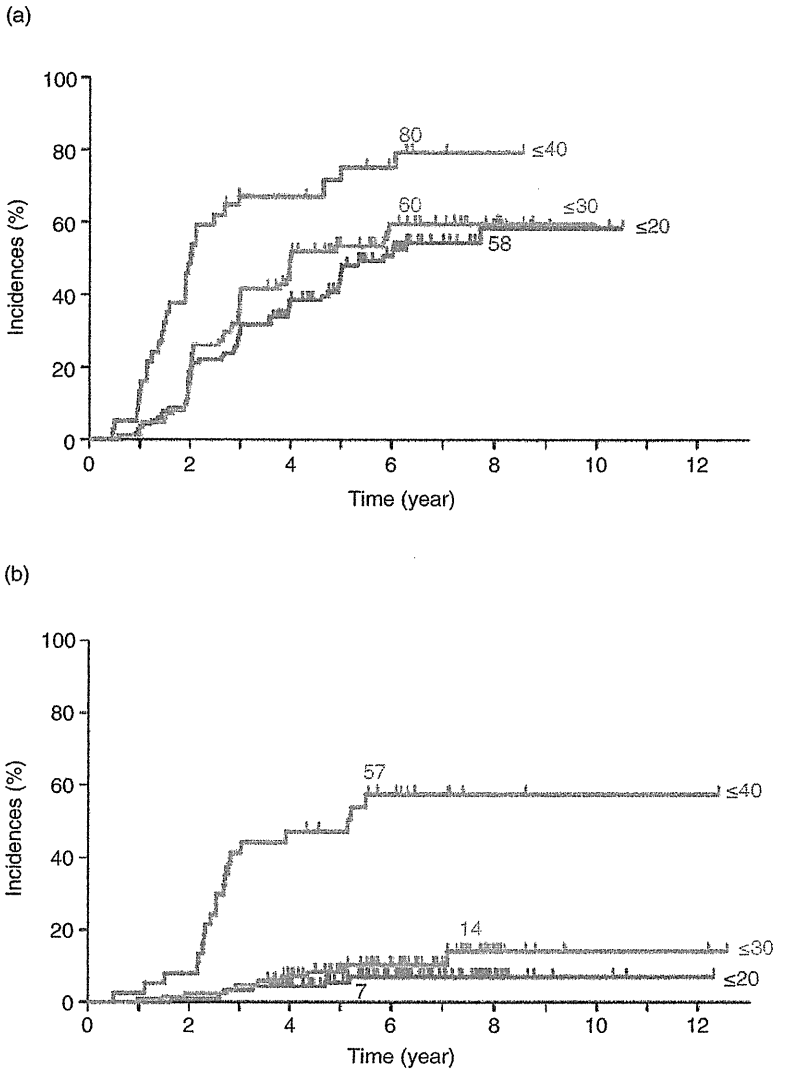

Figure 3 In patients treated with lamivudine for 3 years or more, the incidence of tyrosine-methionine-aspartate-aspartate (YMDD) motif mutant by alanine aminotransferase (IU/L) level was 58% in 113 patients in group A, 60% in 84 patients in group B, and 80% in 37 patients in group C ($P = 0.002$), and that of BTH in the corresponding groups was 7%, 14%, and 57% ($P < 0.001$). (a) Incidence of YMDD mutants over time ($P = 0.0015$). (b) Incidence of breakthrough hepatitis over time ($P < 0.0001$).

YMDD motif mutant and BTH were low after 3 or more years of treatment with lamivudine. Therefore, the year of switching treatment from lamivudine to other nucleic acid analogue will be at 3 years. Accordingly, in this study, we examined patients treated with lamivudine for < 3 and ≥ 3 years.

Among the patients treated with lamivudine for < 3 years, the lowest incidence of YMDD motif mutant and BTH was seen in patients with ALT < 20 IU/L maintaining HBV DNA level of 2.6–5.0. The other category for lowest incidence was in patients with ALT 21–30 IU/L and HBV DNA level of < 2.6 log copies/mL. In this study, within 3 years of treatment with lamivudine, the group of patients with the recommended HBV DNA (< 2.6 log copies/mL) and ALT maintained at 21–30 IU/L may be considered eligible to be switched to entecavir therapy as per Japanese guidelines. We, however, believe it is important to consider the prognosis for patients who are switched from lamivudine to entecavir. Similarly, in patients maintaining HBV DNA level in the range of 2.6–5.0 log copies/mL and ALT < 20 IU/L, switching to dual therapy with adefovir in combination with lamivudine depends on the related viral breakthrough. In a study by Li Zhou *et al.*,[16] some patients with YMDD motif mutants had significantly lower HBV DNA and ALT levels compared with baseline

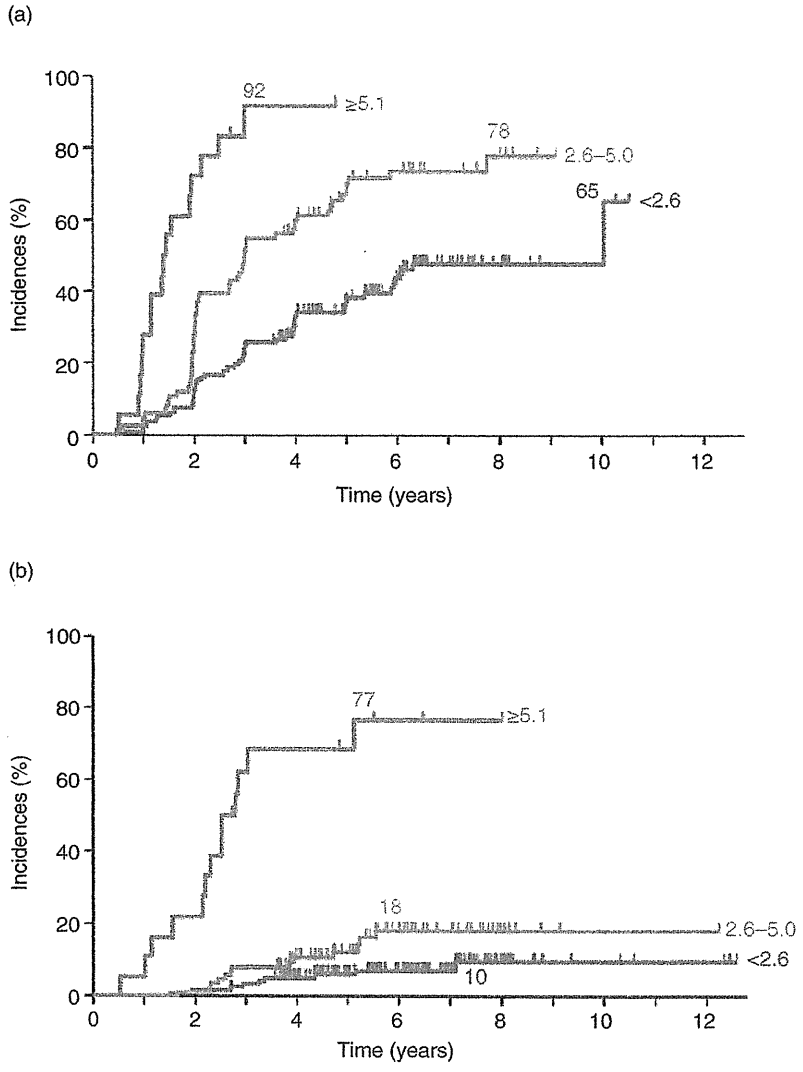

Figure 4 In patients treated with lamivudine for ≥ 3 years, the increased incidence of tyrosine-methionine-aspartate-aspartate (YMDD) motif mutant by hepatitis B virus (HBV) DNA (log copies/mL) level was 65% in patients maintaining an HBV DNA level of < 2.6, 78% in patients maintaining an HBV DNA level of 2.6–5.0, and 92% in patients maintaining an HBV DNA level of ≥ 5.1, and that of BTH in the corresponding groups was 10%, 18%, and 77% ($P < 0.001$). (a) Incidence of YMDD mutants over time ($P = 0.0001$). (b) Incidence of breakthrough hepatitis over time ($P < 0.0001$).

values, which might be due to decreased replication efficiency of the HBV mutants.

HBeAg, severe liver disease, high HBV DNA, and low ALT levels at the baseline were factors accelerating the development of BTH. This was in confirmation of previous results.[17–19] Development of BTH, however, was not influenced by HBV genotypes. This is probably due to the response in HBeAg-positive patients, which was comparable among those with different genotypes though it differed among HBeAg-negative patients.[20]

In a study of Japanese adult patients treated with lamivudine for > 12 months, the YMDD motif mutation was detected in 26% patients, with 23, 16, and 21 patients correspondingly positive for YIDD, YVDD, and YIDD + YVDD mutants. The occurrence of mutations steadily increased and two, five, and 52 patients with genotypes A, B, and C, respectively developed resistance.[21] Lamivudine retreatment could induce rapid re-emergence of YMDD motif mutants with associated viral and hepatic flares[22] and should be avoided. Next, we were interested to know if any difference in sensitivity existed in detecting YMDD mutants by the two different methods used in this study, PCR-RFLP and PCR-ELMA. We studied the rate of detection of YMDD motif mutant by both methods in 20 patients who received lamivudine for more than two years. The detection rate

Table 4 Incidences of tyrosine-methionine-aspartate-aspartate (YMDD) mutant and breakthrough hepatitis (BTH) by hepatitis B virus (HBV) DNA and alanine transaminase (ALT) level in patients during lamivudine treatment for ≥ 3 years (234 patients)

HBV DNA† (Amplicor: log copies/mL)	ALT level (IU/L)†					
	≤ 20		21–30		31–40	
	YMDD	BTH	YMDD	BTH	YMDD	BTH
< 2.6	23/60 (38%)	4/60 (7%)	29/61 (48%)	5/61 (8%)	4/11 (36%)	1/11 (9%)
2.6–5.0	30/50 (60%)	2/50 (4%)	19/22 (86%)	4/22 (18%)	11/12 (92%)	5/12 (42%)
≥ 5.1	3/3 (100%)	1/3 (33%)	0/1 (0%)	0/1 (0%)	13/14 (93%)	12/14 (86%)

†The HBV DNA and ALT levels are shown based on the treatment duration of lamivudine.

between PCR-RFLP and PCR-ELMA was similar; eight patients (40%) and nine patients (45%), respectively.[23]

CONCLUSION

CORRELATION OF ALT and HBV DNA levels with YMDD motif mutant and viral breakthrough can be used as an indirect method of estimating susceptibility to develop lamivudine resistance. The low incidence of YMDD motif mutant and BTH associated with an HBV DNA level of < 2.6 log copies/mL and ALT level of ≤ 30 IU/L and an HBV DNA level of 2.6–5.0 log copies/mL and ALT level of ≤ 20 IU/L during only less than 3 year-treatments can be utilized as a clinically relevant tool to monitor patients' criteria in switching to other nucleoside analogue drugs. Using these simple methods, which can be easily pursued in clinical practice, it may be feasible in the future to switch from lamivudine to other nucleoside analogue drugs with low rates of inducing resistant mutants in CHBV patients. This is important considering the risk of continuous lamivudine treatment causing YMDD motif mutant and BTH.

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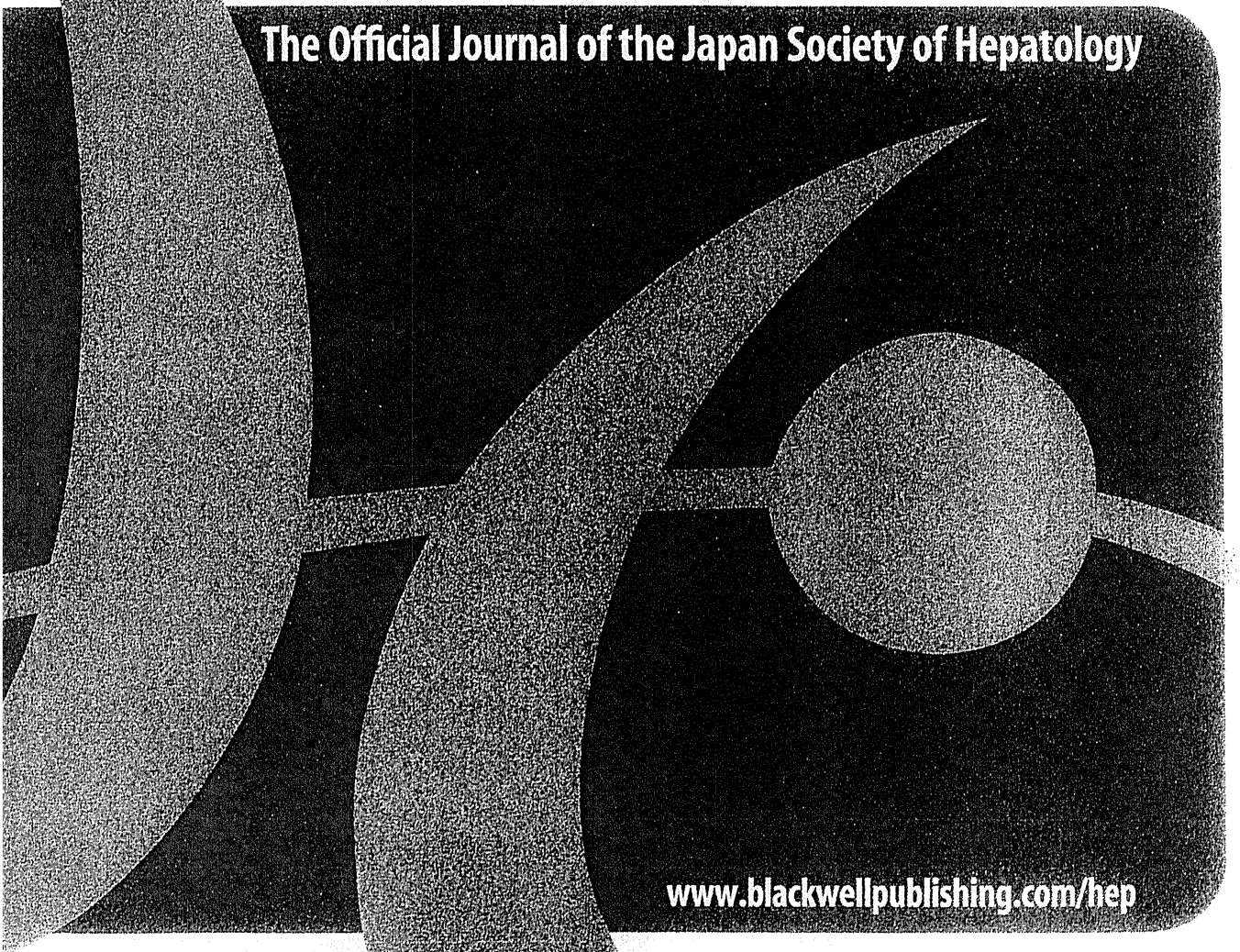

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