表8　HBe抗原陽性のHBV単独感染患者における72週までの安全性データ（103試験）

	TDF-TDF (N=176)	ADV-TDF (N=90)
Study Drug-Related SAE (ALT Flare)	4% (3%)	7% (7%)
G3 Laboratory excluding ALT G3 ALT	18% 15%	10% 10%
G4 Laboratory	12%	11%
Confirmed ↓ phosphorus <2 mg/dL	1%	1%
Confirmed 0.5 mg/dL ↑ in creatinine	0	1%
Confirmed creatinine clearance <50 mL/min	0	0

含め，これまでHBV感染症に対するTDFの使用において腎機能の明らかな変動の報告はない。

TDF投与中に発現した腎障害の多くは可逆的であることが報告されているが，TDFを投与する場合には，血中クレアチニンや血中リン等の変動を注意深く観察し，異常が認められた場合には，投与を中止する等，適切な処置を行うことが必要である。最近，リスク因子の検討が進んでいるが，腎機能障害の既往がある患者や腎毒性のある薬剤が投与されている患者，あるいは糖尿病を合併している患者では注意すべきである。また，腎機能が低下している患者においては，テノホビルの血中濃度が上昇することによる副作用を発現する可能性があることから，添付文書通りの投与間隔の調節が必要である。なお，この他，TDFの主な副作用としては，鼓腸，下痢などの消化器系の症状が報告されている。

また，TDFの投与を中断する場合には，HBVが再増殖するおそれがあるので，十分注意が必要である。特に，非代償性の場合，重症化するおそれがあるので注意が望まれる。

おわりに

現在，本邦におけるB型慢性肝炎に対する抗ウイルス薬としてインターフェロンとLAM，ADVおよびETVの4剤が使用可能である。TDFは，既に，HIV/HBV合併例に対する多剤併用療法（HAART）として，海外の主要なガイドラインで選択されるべき薬剤として位置付けられており，本邦においても第一選択薬の一つと位置付けられている。しかしながら，TDFのHBV単独感染症に対する適応は，本邦では，まだ得られていない。

今回，5年間の観察が計画されているTDFの第Ⅲ相試験の中間成績を報告したが，これらの試験はさらに8年間の観察がされるようである。核酸アナログの耐性プロファイルや完全寛解に至る効果の強さは，薬剤によって異なっていることから，どのような使い方が，長期的に耐性の発現を最小にし，最大のウイルス抑制作用を持続させるのにベストであるのかを明らかにすることが課題である。今後，核酸アナログの長期使用成績が集積することで，「ベストな長期核酸アナログ療法」の評価が可能になると考えられる。

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Transient elastography for measurement of liver stiffness measurement can detect early significant hepatic fibrosis in Japanese patients with viral and nonviral liver diseases

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Background. Many studies have reported the efficiency of transient elastography, a noninvasive, reproducible, and reliable method for predicting liver fibrosis, in patients with chronic hepatitis C (CHC) and B (CHB), but there are few reports about nonviral chronic liver disease (CLD) such as primary biliary cirrhosis (PBC), nonalcoholic steatohepatitis (NAFLD), and autoimmune hepatitis (AIH). We therefore compared the efficiency of transient elastography between CHC and nonviral CLD. ***Methods.*** We assessed the accuracy of liver stiffness measurement (LSM) using Fibroscan, and compared these values with those of hyaluronic acid, type 4 collagen, platelet count, prothrombin index, and AST/platelet ratio index (APRI) as indices for the diagnosis of liver fibrosis in 114 patients with a variety of chronic liver diseases: CHC (n = 51), CHB (n = 11), NAFLD (n = 17), PBC (n = 20), and AIH (n = 15). The histology was assessed according to the METAVIR score by two pathologists. ***Results.*** The number of fibrosis stage (F0/1/2/3/4) with CHC was 9/15/12/6/10, and that with nonviral CLD was 10/21/11/4/6, respectively. The ability, assessed by area under receiver operating characteristic (AUROC) curve, to predict liver fibrosis $F \geq 2$ for LSM, HA, type 4 collagen, platelet count, prothrombin index, and APRI, was 0.92, 0.81, 0.87, 0.85, 0.85, and 0.92 in CHC patients, respectively; and 0.88, 0.72, 0.81, 0.67, 0.81, and 0.77 in nonviral CLD patients, respectively. ***Conclusions.*** In patients with nonviral CLD, LSM was most helpful in predicting significant fibrosis ($F \geq 2$). Transient elastography is a reliable method for predicting significant liver fibrosis, not only in CHC patients but also in nonviral CLD patients.

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Key words: transient elastography, fibrosis, chronic liver disease, NAFLD

Introduction

The prognosis and clinical management of chronic liver disease (CLD) are strongly influenced by the extent of liver fibrosis, because life-threatening complications mainly occur in patients with cirrhosis. The annual incidences of hepatocellular carcinoma, decompensation, and death are approximately 3%, 4%, and 3%, respectively.[1–5] underscoring the need for early identification of developing liver fibrosis to prevent complications. Although liver biopsy (LB) remains the gold standard for the assessment of the degree of fibrosis, this procedure is essentially invasive and with potentially fatal complications. Moreover, LB is an expensive procedure difficult for some patient to accept.[6,7] In addition, sampling error is common because only 1/50 000 of the organ is analyzed and thus up to 30% of results are false negatives.[8]

Liver stiffness measurement (LSM) by transient elastography is a rapid, noninvasive, and reproducible method[9–16] and has been compared with classical hematological markers, such as serum hyaluronic acid level,[17] type 4 collagen,[18] and the aspartate transaminase to platelet ratio index (APRI),[9,19] which have been reported to accurately represent the state of liver fibrosis.

However, a thorough comparison of the diagnostic ability of transient elastography to detect significant fibrosis between patients with chronic hepatitis C (CHC) and those with nonviral CLD, such as primary biliary cirrhosis (PBC), nonalcoholic steatohepatitis (NAFLD), and autoimmune hepatitis (AIH), has not been performed, especially in the Japanese population. The aim of the current study was to compare the diagnostic performance of transient elastography between CHC and nonviral CLD for the evaluation of liver fibrosis.

Material and methods

Patients

One hundred thirty-three consecutive patients of chronic liver disease (CLD) who underwent a liver biopsy (LB) and liver stiffness measurement (LSM) within a period of less than 6 months at the Division of Gastroenterology of Tohoku University Hospital from January 2006 to November 2007 were included in this study. Written informed consent was obtained from all patients before the procedures. Twelve patients had unsuitable biopsy specimens, and 7 patients had an unreliable LSM; thus, the analysis was performed in 114 patients with CHC (n = 51), chronic hepatitis B (CHB, n = 11), NAFLD (n = 17), PBC (n = 20), and AIH (n = 15).

CHC and CHB were diagnosed by the presence of serological hepatitis C virus (HCV)-RNA and hepatitis B virus (HBV)-DNA measured by polymerase chain reaction, respectively. The diagnosis of NAFLD was based on the following criteria: (1) elevated aminotransferases [aspartate aminotransferase (AST) or alanine aminotransferase (ALT)]; (2) liver biopsy showing steatosis in at least 10% of hepatocytes; and (3) exclusion of other etiological liver disease, including alcohol-induced or drug-induced liver disease, autoimmune or viral hepatitis, and cholestatic or metabolic/genetic liver disease. PBC was diagnosed by at least two of the following criteria: serum alkaline phosphatase (ALP) more than 1.5 times the upper limit of normal, a positive antimitochondrial antibody (>1:40), and compatible liver histology. The diagnosis of AIH was based on the revised descriptive criteria for the diagnosis of AIH reported by the International Autoimmune Hepatitis Group (IAHG) in 1999.[20] Criteria for exclusion from the study were the coexistence of hepatocellular carcinoma, a history of alcoholic abuse, receiving therapy of anticoagulants, obvious existence of ascites observed with sonography, and unsuitable LB and LSM.

Blood liver tests [i.e., serum bilirubin and albumin levels, gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), platelet count, prothrombin index, hyaluronic acid level, and type 4 collagen level] were performed before LB within a period of 1 week. Serum hyaluronic acid (HA) and type 4 collagen were evaluated using a Latex Agglutination-Turbidimetric Immunoassay.

Liver stiffness measurement (LSM)

Measurement of the liver stiffness by transient elastography was performed using a FibroScan (EchoSens, Paris, France), as reported previously.[14] In brief, vibrations of mild amplitude and low frequency are transmitted from the vibrator toward the tissues by the transducer itself, inducing an elastic shear wave that propagates through the tissue. All measures were performed on the right lobes of the liver through intercostal spaces while the patients were lying on their backs with the right arm in maximal abduction. An ultrasound guide was used to identify a target liver area, at least 6 cm thick without major vascular structures. The measurement depth was between 25 and 65 mm below the skin surface. The procedure was based on at least ten successful measurements, and a greater than 60% success rate (ratio between numbers of successful and total measurements) was obtained. Liver stiffness was recorded in kilopascals (kPa) as the median value of all measurements. The measurement of this liver stiffness was approved by the Institutional Review Board (IRB) (#2006-69).

Serum surrogate markers

The following parameters were determined using blood samples within 1 week before the LB: hyaluronic acid level,[17] type 4 collagen level,[18] platelet count, prothrombin index, and AST/platelet ratio index (APRI).[9,19] The APRI was calculated because it was previously reported to be associated with the severity of fibrosis in patients with CHC infection. The formula used was AST (per upper limit of normal; 35 IU/L) × 100/platelet count (10^9/l).[9,19]

Liver histology and staging

Liver biopsy was performed under sonography or laparoscopy. A Silverman needle (14-gauge) under laparoscopy or a Tru-cut needle (16-gauge) under sonography was used to obtain an adequate specimen of hepatic tissue. LB specimens were fixed in paraformaldehyde and embedded in paraffin. Sequentially, they were stained with hematoxylin and eosin or elastic Masson's trichrome staining. All specimens were analyzed by two independent, experienced liver pathologists blinded to the results of LSM and clinical data. LB specimens with a length of less than 10 mm, and those considered as unsuitable for fibrosis assessment by the pathologists, were excluded. Liver fibrosis was evaluated according to the METAVIR scoring system with some modifications.[21] Fibrosis was staged into a 0–4 scale as follows: F0 = no fibrosis; F1 = portal fibrosis, periportal fibrosis, or perisinusoidal fibrosis without septa; F2 = few septa; F3 = numerous septa without cirrhosis; and F4 = cirrhosis.

Statistical analysis

Patients were divided according to their consensus fibrosis stage. Differences between quantitative

Table 1. Characteristics of patients

	CHB (n = 11)	CHC (n = 51)	NAFLD (n = 17)	PBC (n = 20)	AIH (n = 15)	Total (n = 114)
Sex (male/female)	11/0	28/23	11/6	3/17	2/13	55/59
Age (years)	46 (24–64)	57.5 (27–74)	50 (25–71)	55.5 (38–75)	55 (18–63)	56 (18–75)
Height (cm)	171 (159–178)	161 (143–180)	160 (147–178)	155 (144–181)	155 (146–171)	160 (143–181)
Body weight (kg)	68 (58–82)	57 (40–128)	68 (44–107)	53 (41–71)	61 (38–67)	61 (38–128)
Stage of liver fibrosis F: 0/1/2/3/4	0/2/4/2/3	9/15/12/6/10	6/3/3/1/4	3/11/4/0/2	1/7/4/3/0	19/37/27/12/19
Total bilirubin (mg/dl)	1.1 (0.7–1.8)	0.9 (0.4–1.8)	1.2 (0.6–2.9)	0.9 (0.5–12.3)	0.9 (0.4–2.9)	1.0 (0.4–12.3)
AST (IU/l)	56 (20–420)	48 (13–291)	63 (27–160)	59 (16–241)	75 (31–351)	59 (13–420)
ALT (IU/l)	74 (26–800)	58 (9–433)	88 (18–245)	60 (14–175)	75 (33–972)	64 (9–972)
ALP (IU/l)	307 (214–726)	252 (85–782)	312 (158–727)	582 (151–3806)	386 (171–569)	4.0 (2.6–5.1)
GGT (IU/l)	63 (24–159)	48 (10–291)	77 (40–911)	189 (30–1861)	80 (13–406)	70 (10–1861)
Albumin (g/dl)	3.7 (3.1–4.9)	4.1 (2.7–5.0)	4.2 (3.0–5.1)	3.9 (2.6–4.3)	3.8 (2.8–4.7)	4.0 (2.6–5.1)
Platelet count (10^9/l)	166 (91–293)	159 (78–378)	224 (75–343)	221 (101–411)	205 (70–419)	184 (70–419)
Prothrombin index (%)	83 (63–120)	87 (63–120)	88 (40–118)	103 (63–120)	90 (57–109)	88 (40–120)
Hyaluronic acid (ng/ml)	90 (12–374)	110 (10–1307)	73 (10–8000)	75 (12–1053)	107 (10–885)	95 (10–8000)
Type 4 collagen (ng/ml)	216 (59–447)	152 (59–437)	136 (77–633)	150 (61–688)	151 (102–447)	150 (59–688)
Liver stiffness (kPa)	18.0 (5.0–27.4)	10.5 (3.3–36.3)	7.4 (4.8–69.1)	8.1 (4.2–75.0)	8.2 (4.8–35.3)	9.6 (3.3–75.0)

Median values are shown (ranges shown in parentheses)
CHC, chronic hepatitis C; CHB, chronic hepatitis B; NAFLD, nonalcoholic steatohepatitis; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase

variables were analyzed by a nonparametric test (Mann–Whitney U test). The trend between LSM and serum surrogate markers and ordinate fibrosis stages was estimated by the Spearman's ρ coefficient. The diagnostic performance of LSM and serum surrogate markers was determined in terms of sensitivity, specificity, positive predictive value (PPV), negative predictive values (NPV), and area under receiver operating characteristics (AUROC) curves. Optimal cutoff values between fibrosis categories were determined at the maximum sum of sensitivity plus specificity. The significance level was set at 0.05, and all P values were two-tailed. Statistical analyses were performed with Dr. SPSS II software (SPSS Japan, Tokyo, Japan).

Results

Patients

Among the 133 patients, 1 patient with CHB, 5 patients with CHC, 2 patients with NAFLD, 3 patients with PBC, and 1 patient with AIH were excluded because of unsuitable LB (n = 12). Similarly, 1 patient with CHB, 3 patients with CHC, and 3 patients with NAFLD were excluded because of defective LSM (n = 7). Data analysis was performed on the basis of the 114 remaining patients (CHB, n = 11; CHC, n = 51; NAFLD, n = 17; PBC, n = 20; AIH, n = 15). Their characteristics are provided in Table 1.

Liver biopsies

The median length of the liver biopsy samples was 17 mm, and the median number of fragments was one. The fibrosis grades of the 114 biopsy specimens of all patients were as follows: F0, n = 19; F1, n = 37; F2, n = 27; F3, n = 12; F4, n = 19. Those of the CHC patients were F0, n = 9; F1, n = 15; F2, n = 12; F3, n = 6; F4, n = 10. Those of the nonviral CLD patients were F0, n = 10; F1, n = 21; F2, n = 11; F3, n = 4; F4, n = 6 (see Table 1).

Relationship between the fibrosis stage and liver stiffness measurement

The liver stiffness values ranged from 3.3 to 75.0 kPa (median, 9.6 kPa). The median liver stiffness values in all patients with F0 (n = 19), F1 (n = 37), F2 (n = 27), F3 (n = 12), and F4 (n = 19) were 5.4, 6.8, 10.9, 19.7, and 27.4 kPa, respectively (Fig. 1A). Subgroup analysis demonstrated that the values in CHC patients with F0 (n = 9), F1 (n = 15), F2 (n = 12), F3 (n = 6), and F4 (n = 10) were 4.3, 6.7, 14.7 13.7, and 21.9 kPa, respectively; and in patients with nonviral CLD with F0

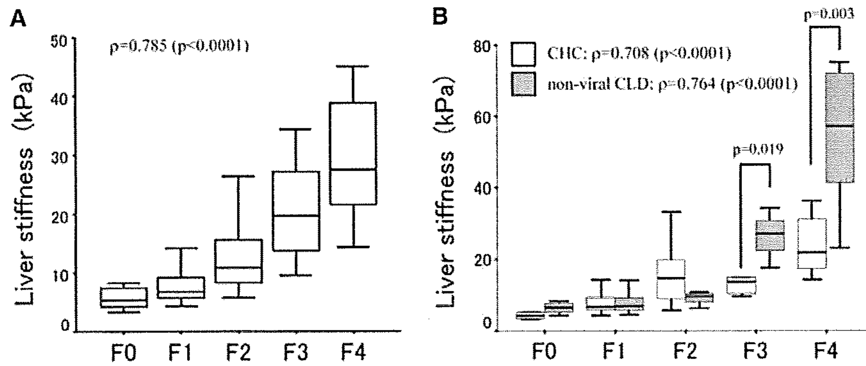

Fig. 1. Box plots of liver stiffness according to METAVIR fibrosis stage in all patients (**A**) and according to liver disease etiology (**B**). The lengths of the boxes represent the interquartile range within which 50% of the values were located. The *lines* in the boxes represent the median values. *Upper and lower error bars* are computed as upper quartile + 1.5* (interquartile range) and lower quartile + 1.5* (interquartile range), respectively. The ρ values are those obtained by the Spearman's ρ coefficient. There were statistically significant differences between chronic hepatitis C (*CHC*) and nonviral chronic liver disease (*CLD*) by the Mann–Whitney *U* test with F = 3 ($P = 0.019$) and F = 4 ($P = 0.003$)

Table 2. Correlation between liver fibrosis stage and surrogate markers

	LSM	Hyaluronic acid	Tyep 4 collagen	Platelet count	Prothrombin index	APRI
All patients	0.785	0.589	0.605	−0.579	−0.651	0.631
CHC patients	0.764	0.583	0.676	−0.662	−0.559	0.750
Nonviral CLD patients	0.708	0.528	0.550	−0.443	−0.619	0.492

Spearman's ρ coefficient
LSM, liver stiffness measurement; APRI, AST/platelet ratio index; CLD, chronic liver disease

Table 3. Performance of transient elastography for the determination of fibrosis stage in all patients, patients with chronic hepatitis C, and nonviral chronic liver disease

	All (n = 114)				CHC (n = 51)				Nonviral (n = 52)			
	F ≥ 1	F ≥ 2	F ≥ 3	F = 4	F ≥ 1	F ≥ 2	F ≥ 3	F = 4	F ≥ 1	F ≥ 2	F ≥ 3	F = 4
AUROC	0.84	0.94	0.94	0.94	0.87	0.92	0.85	0.90	0.76	0.88	0.99	0.99
Optimal cutoff (kPa)	8.4	9.5	14.2	17.2	5.6	9.5	10.3	17.2	8.4	8.1	15.8	21.8
Sensitivity	0.64	0.84	0.90	0.94	0.93	0.89	0.94	0.80	0.55	0.91	1.00	1.00
Specificity	0.95	0.83	0.84	0.85	0.78	0.83	0.69	0.88	1.00	0.76	0.95	0.91
Positive predictive value	0.64	0.84	0.71	0.55	0.95	0.86	0.96	0.95	1.00	0.73	0.83	0.60
Negative predictive value	0.95	0.91	0.96	0.98	0.70	0.86	0.58	0.62	0.31	0.92	1.00	1.00

AUROC, area under receiver operating characteristic

(n = 10), F1 (n = 21), F2 (n = 11), F3 (n = 4), and F4 (n = 6), they were 6.6, 6.9, 9.6, 27.2, and 57.1 kPa, respectively (Fig. 1B). There were significant differences between CHC and non-viral CLD with F = 3 (Mann–Whitney U test, $P = 0.019$) and F = 4 ($P = 0.003$), respectively.

There were significant correlations between the liver fibrosis and LSM by the Spearman's ρ coefficient in all patients ($\rho = 0.785$; $P < 0.0001$), in patients with CHC ($\rho = 0.764$; $P < 0.0001$), and in patients with nonviral CLD ($\rho = 0.708$; $P < 0.0001$) (Table 2). The optimal cutoff values are those giving the highest sum of sensitivity plus specificity. The AUROC curve in all patients for the diagnosis of fibrosis F ≥ 1, F ≥ 2, F ≥ 3, and F = 4 was 0.84, 0.94, 0.94, and 0.94, respectively; and the optimal cutoff values were 8.4, 9.5, 14.2, and 17.2 kPa for F ≥ 1, F ≥ 2, F ≥ 3, and F = 4, respectively (Fig. 2A, Table 3). The AUROC curve in patients with CHC for diagnosis of fibrosis F ≥ 1, F ≥ 2, F ≥ 3, and F = 4 was 0.87, 0.92, 0.85, and 0.90, respectively, and the optimal cutoff values were 5.6, 9.5, 10.3, and 17.2, respectively (Fig. 2B, Table 3). The AUROC curve in patients with nonviral CLD for the diagnosis of fibrosis F ≥ 1, F ≥ 2, F ≥ 3, and F = 4 was 0.76, 0.88, 0.99, and 0.99, respectively, and the optimal cutoff values were 8.4, 8.1, 15.8, and 21.8, respectively (Fig. 2C, Table 3).

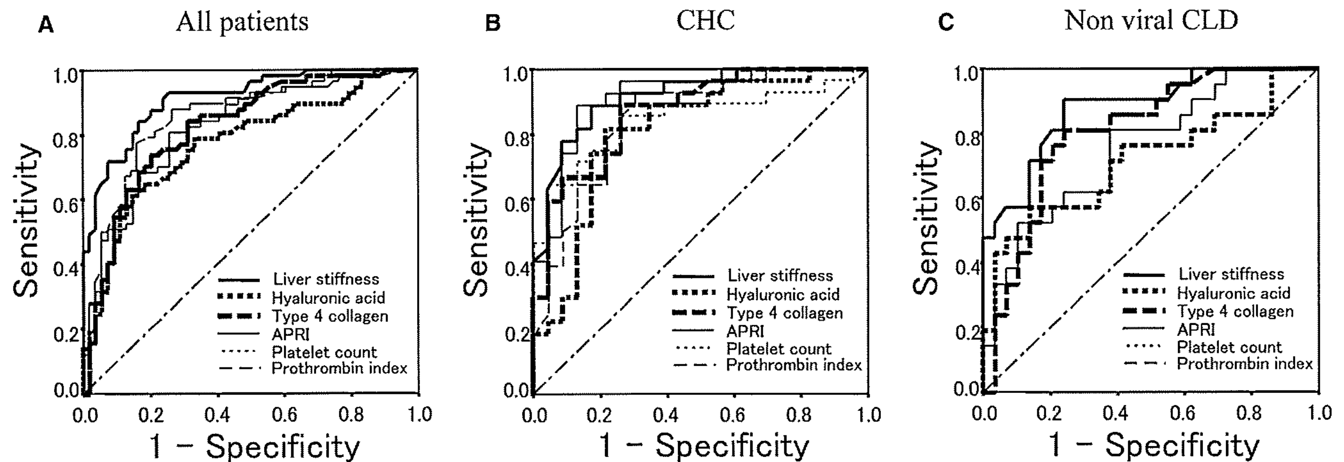

Fig. 2. Receiver operating characteristic curves for diagnosis of METAVIR fibrosis F ≥ 2 by transient elastography (*bold black line*), hyaluronic acid (*bold dotted line*), type 4 collagen (*bold dashed line*), AST/platelet ratio index (APRI) (*thin black line*), platelet count (*thin dotted line*), and prothrombin index (*thin dashed line*) in all patients (**A**), in patients with chronic hepatitis C (CHC) (**B**), and in nonviral chronic liver disease (CLD) (**C**)

Table 4. Comparisons of parameters associated with significant fibrosis (F ≥ 2) on liver biopsy

	F0–F1 (n = 56) median (range)	F2–F4 (n = 58) median (range)	P
Sex (male/female)	22/34	33/25	0.061
Age (years)	55.0 (25–75)	57 (18–74)	0.277
Height (cm)	158.5 (144–181)	164.0 (143–180)	0.340
Body weight (kg)	57.0 (38–107)	62.5 (41–128)	0.104
Total bilirubin (mg/dl)	0.85 (0.4–4.2)	1.1 (0.6–12.3)	<0.001
AST (IU/l)	40.0 (13–160)	71.5 (26–420)	<0.001
ALT (IU/l)	54.5 (9–245)	74.0 (18–972)	0.004
ALP (IU/l)	276.0 (85–1102)	367.0 (175–3806)	0.009
GGT (IU/l)	57.5 (10–932)	76.0 (19–1861)	0.046
Albumin (g/dl)	4.1 (3.0–5.1)	3.7 (2.6–4.9)	0.001
Platelet count (10^3/mm^3)	224.5 (101–378)	127.0 (70–419)	<0.001
Prothrombin index (%)	98.5 (63.7–120)	79.5 (40.4–120)	<0.001
Hyaluronic acid (ng/ml)	41.4 (10–427.5)	144.5 (11.5–8000)	<0.001
Type 4 collagen (ng/ml)	117.0 (59–688)	209.0 (84–652)	<0.001
APRI	0.52 (0.12–3.51)	1.59 (0.38–14.33)	<0.001
Liver stiffness (kPa)	6.45 (3.3–20.4)	17.2 (5.8–75.0)	<0.001

Mann-Whitney U-test

Relationship between the fibrosis stage and serum surrogate markers

To assess the predictive performance of the serum surrogate markers for significant fibrosis (F ≥ 2), we compared the minimal fibrosis group (F0–F1) and the significant fibrosis group (F2–F4). Significant differences were found in total bilirubin, AST, platelet count, prothrombin index, hyaluronic acid, type 4 collagen, and APRI between the minimal fibrosis group (F0–F1) and significant fibrosis group (Mann–Whitney U test, $P < 0.001$) (Table 4). We evaluated sequentially the correlations between the liver fibrosis by Spearman's ρ coefficient. Hyaluronic acid (ρ = 0.589; $P < 0.0001$), type 4 collagen (ρ = 0.605; $P < 0.0001$), and APRI (ρ = 0.631; $P < 0.0001$) were significantly positively correlated with the fibrotic stage, whereas the platelet count (ρ = −0.579; $P < 0.0001$) and prothrombin index (ρ = −0.651; $P < 0.0001$) had a significant negative correlation (see Table 2). Accordingly, we use those values to assess the predicting performance for significant fibrosis (F ≥ 2). The median hyaluronic acid level in all patients with F0, F1, F2, F3, and F4 was 27.4, 43.8, 93.6, 180.8, and 329.0 ng/ml, respectively. The median type 4 collagen level in all patients with F0, F1, F2, F3, and F4 was 104, 125, 165, 216, and 303 ng/ml, respectively. The median platelet

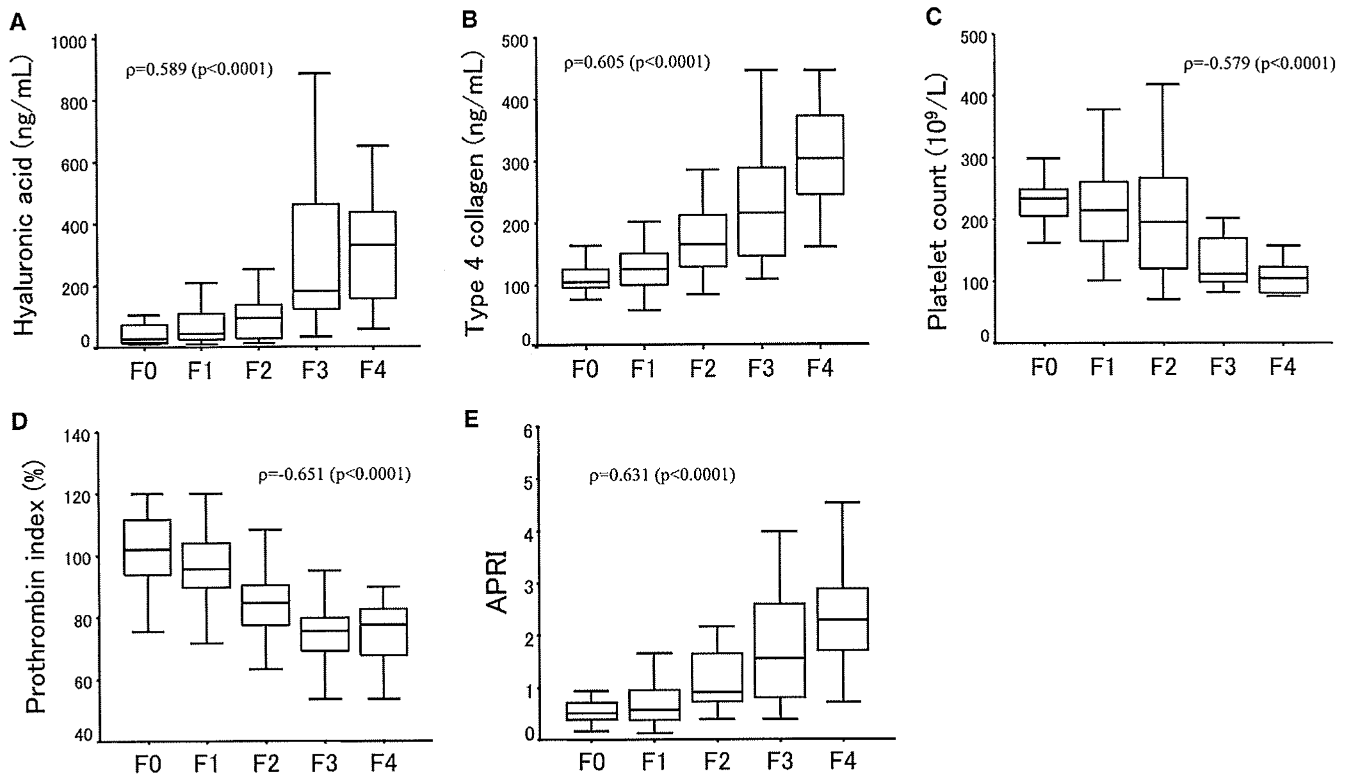

Fig. 3. Box plots of hyaluronic acid (**A**), type 4 collagen (**B**), platelet count (**C**), prothrombin index (**D**), and APRI (**E**) according to METAVIR fibrosis stage in all patients. The lengths of the boxes represent the interquartile range within which 50% of the values were located. The lines in the boxes represent the median values. *Upper and lower error bars* are computed as upper quartile + 1.5* (interquartile range) and lower quartile – 1.5* (interquartile range), respectively

count in all patients with F0, F1, F2, F3, and F4 was 233, 214, 195, 112, and 105 × 10^9/l, respectively. The median prothrombin index in all patients with F0, F1, F2, F3, and F4 was 101.9%, 95.5%, 84.7%, 75.4%, and 77.6%, respectively (Fig. 3A–E). The medial APRI in all patients with F0, F1, F2, F3, and F4 was 0.51, 0.56, 0.91, 1.55, and 2.29, respectively.

Comparison of transient elastography with the hyaluronic acid level, type 4 collagen level, platelet count, prothrombin index, and APRI for the diagnosis of significant fibrosis ($F \geq 2$)

ROC curve analysis for predicting significant fibrosis ($F \geq 2$) was performed on overall patients, CHC patients, and nonviral CLD patients, respectively (see Fig. 2). The AUROC curve of LSM, hyaluronic acid, type 4 collagen, platelet count, prothrombin index, and APRI was 0.94, 0.77, 0.82, 0.77, 0.85, and 0.83 for overall patients (n = 114), respectively. The highest AUROC curve was obtained by LSM. For patients with CHC (n = 51), the AUROC curve of LSM, hyaluronic acid, type 4 collagen, platelet count, prothrombin index, and APRI was 0.92, 0.81, 0.87, 0.85, 0.85, and 0.92, respectively; thus, LSM and APRI should be nearly the highest AUROC curve. In contrast, in patients with nonviral CLD (n = 52), the AUROC of LSM, hyaluronic acid, type 4 collagen, platelet count, prothrombin index, and APRI was 0.88, 0.72, 0.81, 0.67, 0.81, and 0.77, respectively. Finally, in comparison with the surrogate serum markers, LSM had the highest AUROC and correlated not only with the patients with CHC but also those with nonviral CLD (Fig. 2, Table 2, Table 5).

Discussion

In the present study we examined the ability of transient elastography to predict liver fibrosis in each stage. In nonviral CLD patients, the AUROC curves for the diagnosis of significant fibrosis ($F \geq 2$), severe fibrosis ($F \geq 3$), and cirrhosis were 0.88, 0.99, and 0.99, respectively (see Table 3). Although it seems to be more efficient to predict severe fibrosis and cirrhosis, it is more important to detect early-stage liver fibrosis from a clinical point of view for preventing developing cirrhosis and its complications. Thus, we compared the ability

Table 5. Diagnostic performance in predicting liver fibrosis (F ≥ 2)

	LSM	Hyaluronic acid	Type 4 collagen	Platelet count	Prothrombin index	APRI
All patients (n = 114)						
AUROC	0.94	0.77	0.82	0.77	0.85	0.83
Optimal cutoff	9.5	125.2	152.5	152.5	87.6	0.77
Sensitivity	0.84	0.61	0.74	0.59	0.78	0.81
Specificity	0.83	0.85	0.80	0.88	0.84	0.75
PPV	0.84	0.82	0.78	0.83	0.85	0.74
NPV	0.91	0.68	0.76	0.67	0.94	0.80
CHC patients (n = 51)						
AUROC	0.92	0.81	0.87	0.85	0.85	0.92
Optimal cutoff	9.5	96.6	128.0	122.0	90.7	0.70
Sensitivity	0.89	0.82	0.89	0.64	0.93	0.89
Specificity	0.83	0.78	0.74	0.96	0.70	0.87
PPV	0.86	0.82	0.80	0.95	0.79	0.89
NPV	0.86	0.78	0.81	0.69	0.89	0.87
Nonviral patients (n = 52)						
AUROC	0.88	0.72	0.81	0.67	0.81	0.77
Optimal cutoff	8.1	146.4	149.5	213.0	79.4	0.77
Sensitivity	0.91	0.48	0.81	0.71	0.62	0.81
Specificity	0.76	0.93	0.76	0.65	0.94	0.65
PPV	0.73	0.83	0.68	0.58	0.76	0.61
NPV	0.92	0.71	0.85	0.77	0.78	0.83

PPV, positive predictive value; NPV, negative predictive value

of transient elastography and surrogate serum markers to predict significant fibrosis (F ≥ 2) in patients with CHC and those with nonviral CLD. The AUROC curve predicting significant fibrosis in patients with CHC and nonviral CLD was 0.92 vs. 0.88 with LSM, 0.81 vs. 0.72 with hyaluronic acid, 0.87 vs. 0.81 with type 4 collagen, 0.85 vs. 0.67 with platelet count, 0.85 vs. 0.81 with prothrombin index, and 0.92 vs. 0.77 with APRI. Although all the indices had higher AUROC curves in patients with CHC than in those with nonviral CLD, LSM consistently had the highest AUROC curve value in comparison with the surrogate serum markers. Ganne-Carrié et al. had reported that alcoholic steatohepatitis and nonalcoholic steatohepatitis (NASH) exhibited higher cutoff values than CHC of 21.5 vs. 10.4 kPa for the diagnosis of cirrhosis.[13] Although our study grouped PBC, NAFLD, and AIH as nonviral CLD, a similar tendency was also observed; nonviral CLD had higher median LSM values than CHC in F = 3 with 27.2 vs. 13.7 kPa (P = 0.019) and F = 4 57.1 vs. 21.9 kPa (P = 0.003), respectively; and the cutoff values of predicting F ≥ 3 (15.8 vs. 10.3 kPa) and F = 4 (21.8 vs. 17.2) were also higher in patients with nonviral CLD than in those with CHC. In addition, the AUROC curve predicting F ≥ 3 (0.99 vs. 0.85) and F = 4 (0.99 vs. 0.90) was also higher in nonviral CLD than in CHC.

Although many surrogate serum markers have been developed to predict liver fibrosis (e.g., APRI, Forns' index, and Fibrotest), the majority of them were invented for patients with CHC infection, whereas few reliable surrogate markers were developed for nonviral CLD.[9,19,22–24] A strong association between liver stiffness and the degree of liver fibrosis has been demonstrated in patients with chronic viral hepatitis.[9,11,13]

However, in patients with nonviral CLD, few studies have been performed concerning the utility of transient elastography for the assessment of liver fibrosis in comparison with patients with viral hepatitis.[10,12,13,15] The diagnosis of CHC and CHB infection is commonly based on the presence of serological HCV-RNA or HBV-DNA. LB is performed to assess the extent of fibrosis (staging) and the activity. On the other hand, despite the invasiveness and expense of the procedure,[6,7] the diagnosis of NASH is essentially based on pathology,[25] and it is more dependent on LB than other types of CLD. In addition, PBC and AIH are difficult to be ruled out without histological diagnosis.[20,26,27] Furthermore, NAFLD has become the most common cause of CLD worldwide.[28,29] However, simple bland steatosis often remains stable for a number of years and will probably never progress in many patients,[30,31] whereas a subset of patients, particularly those with more advanced fibrosis, are at higher risk for progressing to decompensated cirrhosis, portal hypertension, and hepatocellular carcinoma (HCC).[32–34] Therefore, we need to perform LB for the patients with significant liver fibrosis who are at a high risk of progressing liver fibrosis and, eventually, end-stage liver disease. In spite of the lower morbidity of PBC and AIH compared with NAFLD, it is equally necessary for us to evaluate the staging of the progression of liver diseases. Especially, as liver transplantation is the only lifesaving therapy for those with

progressive liver disease, determining of disease stage is critical. In contrast, in patients with nonsignificant liver fibrosis, it may be better to observe them without performing LB.[35]

Transient elastography is a simple, safe, and reproducible method that measures a quantitative physical parameter directly on the liver with no possible interference from extrahepatic disorders.[14] It is able to determine the stiffness of a volume of the liver parenchyma that is approximately 100 times larger than that of a needle biopsy specimen and can be performed in about 95% of patients. Although it is difficult to assess LSM in patients with ascites, narrow intercostal spaces, or a body mass index above 28 kg/m^2,[36] LSM can be employed for most patients with a variety of liver diseases in daily practice.

Although differing from surrogate serum markers in that not all patients can be evaluated for liver stiffness by Fibroscan, our study indicated that it is more efficient for predicting liver fibrosis than the simple biomarkers and extracellular matrix markers used in routine laboratory tests. In the patients with nonviral CLD, advanced liver fibrosis ($F \geq 3$) tended to be accompanied by higher LSM values than CHC. We believe the heterogeneous components of the etiology of liver diseases are accountable for this observation. In patients with NAFLD, the pathological fibrosis initiates from zone 3 perisinusoidal fibrosis with possible additional portal/periportal fibrosis, leading to architectural remodeling.[37] A study comparing METAVIR portal-based scoring originally developed for CHC with a published system for nonalcoholic steatohepatitis demonstrated 47% agreement of fibrosis score; the differences were attributed to lack of detection of zone 3 perisinusoidal fibrosis by METAVIR.[38] Moreover, it is well known that patients with PBC sometimes develop esophageal varices before developing cirrhosis.[39] AIH has not been estimated for liver fibrosis quantitatively such as METAVIR score. Although more weight is given to histological activity than fibrosis,[20] the existence of significant liver fibrosis similarly influences prognosis. In either case, LSM could be different as a result of the etiology leading to hepatic fibrosis. Thus, it seems feasible to accumulate more data of LSM to perform better prediction of liver fibrosis in each etiology.

In conclusion, transient elastography demonstrated a better diagnostic accuracy and correlation with fibrosis stage than the existing serum surrogate markers, suggesting that transient elastography could be used as an alternative to LB for assessment of the liver fibrosis stage not in only patients with CHC but also those with nonviral CLD.

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Analysis of the Entire Nucleotide Sequence of Hepatitis B Causing Consecutive Cases of Fatal Fulminant Hepatitis in Miyagi Prefecture Japan

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We encountered five consecutive patients with fulminant hepatitis induced by acute hepatitis B virus (HBV) infection in 2000–2001 in Japan. They had not had previous contact each other, and were referred to us from different hospitals. Although a 69-year-old woman could be rescued by intensive internal treatment, the four patients died. We analyzed the partial (nt 278–646) and entire nucleotide sequences of the HBV obtained from them, and their divergences were 0–0.3% and 0–0.2%, respectively. The results suggested that they had been infected with the same HBV isolates. The isolates belonged to genotype B and subgenotype B2 on the phylogenetic tree analysis (AB302942–AB302946). As for the nucleotides sequences of them, previously reported mutations of G1896A, A1762T, and G1764A were present. Amino acid analysis revealed that previously reported Ile97-Leu and Pro130Non-Pro in the core region and Trp28Stop in the precore region were present. As for the entire nucleotide sequences among B2, AB302942 showed low divergences with AF121245 and AB073834 (1.7%), and X97850 from patients with fulminant hepatitis (3.2%). We compared the two consensus nucleotides derived from AB302942 and X97850 (fulminant hepatitis) versus AY121245 and AB073834 (non-fulminant hepatitis), which revealed a difference in nt 1,504 located in the P and X region. Nucleotide 1,504 was C for isolates from fulminant hepatitis and G for non-fulminant hepatitis, and it was recognized among most of the isolates belonging to B2 registered on GenBank. Further studies could disclose the mechanism of severe inflammation of liver that finally leads to fulminant hepatitis. ***J. Med. Virol. 80:967–973, 2008.*** © 2008 Wiley-Liss, Inc.

KEY WORDS: hepatitis B virus; genotype B; subgenotype B2; fulminant hepatitis

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INTRODUCTION

Hepatitis B virus (HBV) infection has a wide spectrum of clinical presentations, including self-limited acute hepatitis, fulminant hepatitis, liver cirrhosis and hepatocellular carcinoma [Lee, 1997]. The clinical manifestations of HBV infection are related to interactions between the virus and host immune responses to HBV antigens [Chisari and Ferrari, 1995].

HBV is each characterized according to the genotype based on the comparison of entire genomes, with intergroup divergences of more than 8% [Okamoto et al., 1988]. The distribution of genotypes throughout the world includes eight different genotypes of HBV, named A to H, that have been determined to date [Okamoto et al., 1988; Norder et al., 1992]. Recently, HBV strains have been shown to be composed of subgenotypes [Norder et al., 2004]. For example, genotype B (HBV/B) is divided into five subgroups, B1–B5, according to the countries where they are found [Norder et al., 2004; Nagasaki et al., 2006]. Thus, HBV needs to be examined with regards not only to its genotype but also to its subgenotype.

Clinically, there have been some reports that the outcomes vary according to the HBV genotype or subgenotype. For example, as for HBV/B and genotype C (HBV/C), which are commonly found in Asia, HBV/B has been found to cause HBe-seroconversion more frequently than HBV/C, and chronic infected patients with HBV/B appear to have better prognoses [Kikuchi

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et al., 2000]. Furthermore, as for HBV/B, B1 is known to show a good clinical prognosis compared with B2–B5.

Fulminant hepatitis is an often fatal complication of acute HBV infection or acute exacerbation of chronic HBV infection. The pathogenesis is not completely understood and it shows high mortality without liver transplantation. Virologically, the association between of the precore stop mutation (G1896A), the core promoter mutations (A1762T and G1764A) and fulminant hepatitis induced by HBV infection has been recognized [Carman et al., 1989; Aritomi et al., 1998; Friedt et al., 1999]. Similarly, an association between the amino acid Ile97Leu and Pro130Non-Pro in the core region and Trp28Stop in the precore region was previously reported [Kosaka et al., 1991; Aye et al., 1994].

We encountered five consecutive patients with fulminant hepatitis induced by acute HBV infection in 2000–2001. They were all residents of Miyagi prefecture in Japan. We investigated entire nucleotide sequences of the HBV detected in their serum samples and compared them with those of previous reports.

METHODS

Patients

The clinical characters of five patients are shown in Table I. The criteria for fulminant hepatitis induced by HBV infection were the development of hepatic encephalopathy, prolongation of the prothrombin time during the course of hepatitis, and immunoglobulin M antibody to hepatitis B core antigen [Perrillo and Aach, 1981]. They were all referred to our hospital from other clinics or hospitals. They were Japanese and had not had contact with known each other. They had never been abroad, had not met nor had with sexual contacts with foreigners, nor were they intravenous drug abusers. None of them had had prior hepatitis. The 66-year-old male patient (FH-3) had been administered medication for hyperlipidemia and hypertension, and the 71-year-old female patient (FH-4) had been administered medication for hypertension.

Thereafter, all but one patient (FH-4) died despite treatment in the intensive care unit due to multiple organ failure, including liver. We obtained serum samples from them and analyzed their HBV-DNA.

None of these five patients were positive for anti-hepatitis C virus antibody or for serum HCV-RNA (RT-PCR assay, Amplicor qualification assay, Roche Japan, Tokyo, Japan), the presence of which could worse the outcome of hepatitis B virus infection [Feray et al., 1993; Sagnelli et al., 2002; Liaw et al., 2004]. In addition, other serum markers related to acute infection of hepatitis A virus, EB virus, and Cytomegalovirus were all negative.

Assays of HBV Related Markers

HBeAg, anti-HBe, and anti-HBc were detected by immunoassays (Abbott Laboratories, N. Chicago, IL).

TABLE I. Clinical Characters and Data of Present Five Patients With Fulminant HBV Infection

Patients	Age	Sex	Onset	Symptom	Admission	Therapy	Outcome (hospital day)	T-Bil (mg/dl)	ALT (IU/L)	PT (%)	HBsAg	anti-HBs	HBeAg (COI)	anti-HBe (%)	anti-HBc (%)	IgM anti-HBc (COI)	HBV-DNA (LGE/ml)
FH-1	69	F	May 29, 2000	Fever, anorexia	June 2, 2000	—	Died (2)	10.2	3,945	8.0	−	−	− (0.57)	+ (98.5)	+ (98.5)	+ (2.81)	5.6
FH-2	71	M	July 26, 2000	Fever	August 3, 2000	PE	Died (44)	16.8	2,715	38.0	−	−	− (0.43)	+ (97.3)	+ (99.0)	+ (3.04)	7.5
FH-3	66	M	October 24, 2000	Fever	November 6, 2000	PE, CHDF, mPLS	Died (2)	13.9	6,950	15.0	−	+	− (0.62)	+ (97.1)	+ (98.9)	+ (2.50)	6.2
FH-4	71	F	September 1, 2000	Epigastralgia	January 4, 2001	—	rescued (50)	9.4	3,380	27.0	−	−	− (0.53)	+ (97.0)	+ (82.7)	+ (2.95)	4.9
FH-5	60	M	December 12, 2000	Fever, malaise	December 6, 2000	PE	Died (4)	4.4	7,884	24.0	+	−	− (0.71)	+ (70.0)	n.t.	+ (2.32)	6.2

HBeAg, anti-HBe, and anti-HBc were detected by immunoassays (positive range, HBeAg $\geq$1.0; anti-HBe and anti-HBc $\geq$70.0). IgM anti-HBc was detected by CORE-M-IMx (positive range, COI (cut off index) $\geq$1.0). Serum HBV-DNA levels were measured by transcription-mediated amplification-hybridization protection assay (TMA-HPA; Chugai Diagnostics, Ltd., Tokyo, Japan). Percent in each parenthesis meant for inhibition percent. For details, please see method section in the text.
F, female; M, male; PE, plasma exchange; CHDF, continuous hemodiafiltration; mPSL, methyl-prednisolone pulse administration; T-Bil, total bilirubin; ALT, alanine aminotransferase; PT, prothrombin time; HBsAg, hepatitis B s antigen; anti-HBs, antibody to hepatitis B s antigen; HBeAg, hepatitis B e antigen; anti-HBe, antibody to hepatitis e antigen; anti-HBc, antibody to hepatitis B c antigen; IgM anti-HBc, immunoglobulin M antibody to hepatitis c antigen; n.t., not tested; COI, cut off index; LGE, logarithm of the genome equivalent.

IgM anti-HBc was detected by CORE-M-IMx (Abbott Laboratories). Serum HBV-DNA levels were measured by transcription-mediated amplification-hybridization protection assay (TMA-HPA; Chugai Diagnostics, Ltd., Tokyo, Japan) with frozen stocked sera as described previously [Sakugawa et al., 2001; Kobayashi et al., 2007]. The results of this TMA-HPA assay were indicated as logarithm of the genome equivalent (LGE)/ml and its measurable range was 3.7–8.7 LGE/ml (equivalent to HBV-DNA $10^{3.7}$–$10^{8.7}$ copies/ml).

Amplification of HBV DNA by Polymerase Chain Reaction (PCR)

Nucleic acids were extracted from 200 μl of serum as described previously [Niitsuma et al., 1995]. For analysis of the entire nucleotide sequence, we divided the entire HBV genome into six overlapping segments and amplified each segment. Extracted DNA was subjected to the first round of PCR with each set of primers. PCR was performed with TaKaRa Ex Taq™ (TaKaRa Co. Ltd., Shiga, Japan) for 35 cycles (consisting of denaturation for 1 min at 93°C, annealing for 1 min at 55°C, and extension for 1 min at 74°C), followed by an extension cycle at 74°C for 8 min. The second round of PCR was carried out for 30 cycles consisting of the same protocol as in the first round.

The primers for the first and the second PCR rounds were as previously reported [Shan et al., 2002].

We used the standard numbering system method in this report, with the numbering of the bases commencing at the cleavage site for the restriction enzyme *Eco*RI in the preS2 region and counting of the full lengths of the 3,215 base pairs.

Nucleotide Sequences of HBV Isolates

We used each set of sequencing primers previously described [Shan et al., 2002]. Direct sequencing of the PCR products was carried out by a fluorescence autosequencer (model 377, PE Japan Applied Biosystems, Chiba, Japan) using a Big Dye Terminator Sequencing Kit (PE Japan Applied Biosystems) according to the manufacturer's instructions.

Phylogenetic Analysis of the Isolated HBV Clones

Six overlapping segments were joined and phylogenetic determination of the sequences of the HBV clone was performed by the neighbor-joining method with the aid of ClustalW (DNA Data Bank of Japan; DDBJ, http://www.ddbj.nig.ac.jp/search/clustalw-j.html).

We compared the present isolated clones with the eight reported HBV clones and confirmed their genotype by phylogenetic analysis. The accession numbers of the clones and genotypes of these HBV sequences used in the analysis were as follows: AB014370 (genotype A); X97850 (genotype B); X75665 (genotype C); J02203 (genotype D); X75664 (genotype E); X75663 (genotype F); AF160501 (genotype G); and AY090457 (genotype H).

RESULTS

Serum Test Findings

All of the patients were IgM class anti-HBc positive, as shown in Table I, and showed severe liver dysfunction, and coagulopathy compatible with fulminant hepatitis.

Entire Genome Sequences Detected From the Present Patients

We determined the entire nucleotide sequences except in one isolate (FH-5). The divergences among the four isolates were 0–0.2% (Table II). As for the partial nucleotide analysis in the HBs region (nt 278–646), all but one isolate were completely matched. Only one isolate (FH-4) demonstrated single different nucleotide.

Phylogenetic Analysis With HBV Entire Genome

We constructed a phylogenetic tree using the present four entire nucleotide sequences and other HBV isolates retrieved from the DNA database (DDBJ/GenBank) as representative genotypes (A, B, C, D, E, F, G, and H) (Fig. 1).

The genotype was B. The HBV/B isolates detected worldwide are divided into five subgenotypes: B1, B2, B3, B4, and B5; the present subgenotype was B2.

Comparison of the Nucleotides and Amino Acids of AB302942 With the Isolates From Previous Reports

Some nucleotide and amino acid mutations related to HBV fulminant hepatitis were previously reported [Carman et al., 1989; Aritomi et al., 1998; Sterneck et al., 1998; Friedt et al., 1999; Yuasa et al., 2000]. As for the nucleotides sequences of AB302942, the mutations of

TABLE II. Percentage Divergences of Entire HBV Nucleotide Sequences Among the Five Isolates From the Present Patients and Other HBV Isolates Registered on GenBank

		Accession number (country)						
Patient	Accession number	AB302942 (Japan)	AB302942 (Japan)	AB302942 (Japan)	AB302942 (Japan)	AF121245 (Vietnam)	AB073834 (Vietnam)	X97850 (China)
FH-1	AB302942	—	0.1	0	0.2	1.7	1.7	3.2
FH-2	AB302943		—	0.1	0.2	1.7	1.7	3.1
FH-3	AB302944			—	0.2	1.7	1.7	3.2
FH-4	AB302945				—	1.7	1.7	3.2

Fig. 1. Phylogenetic tree of the entire nucleotide sequences constructed by the neighbor-joining method using the present four isolates (*), HBV/B isolates and other HBV genotype (A, C, D, E, F, G, and H) isolates retrieved from DDBJ/GenBank.

G1896A in precore region, A1762T and G1764A in core promoter region were in accord with those of previous reports [Sato et al., 1995]. Similarly, amino acid analysis showed Ile97Leu and Pro130Non-Pro in the core region and Trp28Stop in the precore region as previously reported [Kosaka et al., 1991; Aye et al., 1994].

Divergences of the Entire Genome and Amino Acids Between the Present Isolates and HBV/B2 Isolates From GenBank

Divergences of the entire genome sequence of the present four isolates with other Asian HBV/B2 isolates registered on GenBank are shown in Table II. Among the sequences registered on GenBank, AY121245 and AB073834 from Vietnam showed low divergences with them, 1.7%. As for registered isolates from patients with fulminant hepatitis, X97850 showed divergences of 3.2%. We compared the two consensus sequences of the nucleotides and amino acids derived from AB302943 and X97850 (fulminant hepatitis) versus the isolates from AB121245 and AB073834 (non-fulminant hepatitis). Only one different nucleotide (nt 1,504) was revealed in the P and X region, but they were not located in either promoter or enhancer regions. Nevertheless, it was recognized that most of the 95 isolates (HBV genome length 3,215 nucleotides) belonged to B2 registered on Genbank showed that nt 1,504 was not C (83 isolates were G, just 2 isolates were C, and 10 were others). Similarly, as for the amino acids, aa 44 in the X region was Ala for isolates from fulminant hepatitis, but it was not common for non-fulminant hepatitis (66 isolates were Val, just 2 isolates were Ala, and 27 isolates were others). In contrast to it, aa 805 in the P region showed no mutation.

DISCUSSION

We encountered five cases of fulminant hepatitis induced by HBV acute infection that occurred during 8 months in 2000–2001. All had been referred to our hospital within a short period. They had had no sexual contact, had not abused illegal drugs, nor had had contact with foreigners. According to the retrospective investigations, it was revealed that they had not had previous contact with each other, and that the only thing they had in common was that they had seen the same physician as out-patients. It was thought to be very difficult to be determined their infectious routes, since not all of them had had chances such as intravenous injections in those days. Two of the patients had just been administered drugs for hyperlipidemia or hypertension. However, the later investigations revealed that they had be drawn blood tests with syringes which were not disposable, although it was impossible to prove their infectious source as the usage of this syringe at the time of investigation. However, the inadequate handling of autoclaving apparatus could possibly cause insufficient sterilization of glass syringes, which were still utilized at several clinics at that time. If the syringes, which were routinely used to draw blood, were contaminated with HBV, it is difficult to prevent fatal acute hepatitis, since the infected patients will never develop symptoms until large amount of infected hepatocytes will be collapsed. Thus, preventive medial intervention is extremely difficult in this setting once infection is established. They were all rather aged (average 67 years old), which might have worsened their clinical prognosis. As for the therapy, liver transplantation was not common in Japan in those days. Moreover, the worsening of the clinical course was so rapid that it would have been difficult to find suitable donors for them. We administered intensive therapy, including plasma exchange, continuous hemodiafiltration, and methyl-prednisolone administration (initially 1,000 mg/day and tapered). Four patients could not be rescued.

As for the clinical diagnosis, we confirmed that the patients suffered from fulminant hepatitis due to acute HBV infection as follows. First, none of them had a family history of hepatitis or prior liver diseases. Second, the serum tests of the present five patients showed anti IgM class anti-HBc positive, compatible with acute HBV infection.

Serologically, all of them were anti-HBe positive, similar to some previous reports from Japan [Kosaka et al., 1991; Omata et al., 1991; Sato et al., 1995; Inoue et al., 2006].

In this study, we at first determined the HBV partial nucleotide sequences in the S region (nt 278–646). Four sequences of them were in complete accord, and one isolate (FH-4) showed just one nucleotide difference. This result suggested the possibility of a common infectious route; this was based on just 11.5% of the entire HBV 3,215 nucleotides (369/3,125). Thereafter, we tried to determine the entire nucleotide sequences.

We determined the entire nucleotide sequences of the HBV from four patients, and their divergences showed 0–0.3%.

These results could imply that the patients were infected with the same HBV isolate. On the phylogenetic tree analysis, they belonged to HBV/B, subgroup B2. HBV/B is known to be widespread in Asian countries, but as we previously reported, HBV/B could be divided into five minor groups on the phylogenetic tree, whose geographical distances and genetic distances are well correlated. The HBV strain found in the present patients was not a typical strain in Japan. The typical HBV subgenotype in Japan is B1, and the B2 isolate is mainly prevalent in China, Vietnam, and Taiwan [Norder et al., 2004].

We could not determine the entire HBV nucleotide sequences in one patient (FH-5), possibly because of the presence of some mutations in the primer regions we used.

It was previously reported that patients with HBV/B2 have a rapidly progressive and severe clinical course [Sugauchi et al., 2002]. As for chronic infection, Sugauchi reported that Japanese patients with HBV/B2 showed HBe antigen more frequently than those with HBV/B1 [Orito et al., 2001].

As for acute HBV infection, it is not clear whether the genotypes of HBV show clinical differences. It is still controversial whether specific HBV genotypes are more often associated with fulminant hepatitis [Gandhe et al., 2003; Chen et al., 2004]. Further examination concerning the relation between the HBV subgenotypes and their clinical manifestations is needed.

The precore stop mutation (G1896A) and core promoter mutations (A1762T and G1764A) have been reported to have an association with fulminant hepatitis in Japan [Sato et al., 1995], but not common in other countries, such as the US and Germany [Laskus et al., 1993; Sterneck et al., 1998]. The association of G1896A, A1762T, and G1764A with fulminant hepatitis induced by HBV infection is controversial [Carman et al., 1989; Aritomi et al., 1998; Friedt et al., 1999; Yuasa et al., 2000; Chen et al., 2003; Kao et al., 2003]. A universal, specific genomic mutational pattern associated with fulminant hepatitis has not been found [Sterneck et al., 1996].

The prevalence of the above mutations is known to differ according to the HBV genotype. A1762T and G1764A are common in HBV/C, but not in HBV/B [Chan et al., 1999]. In contrast to it, G1896A is common in HBV/B and HBV/C, but rare in the HBV genotype A [Stuyver et al., 2000]. The above discrepancy might derive from the distribution of the HBV genotypes and their virological characteristics, but further studies including in vitro studies will be needed.

As for previously known nucleotide mutations in HBV/B, our present isolates had G1896A, A1762T, and G1764A. They were in accord with those previously reported for fulminant hepatitis induced by acute HBV/B infection [Ozasa et al., 2006].

Additionally, we analyzed for the subgenotype. We compared the entire two consensus nucleotide and amino acid sequences for fulminant hepatitis (AB302942 and X97851) versus non-fulminant hepatitis (AF121245 and AB073834) among the isolates belonging to HBV/B2. We chose two isolates for the following reason. We should compare the HBV genome from patients with fulminant hepatitis and acute hepatitis, but isolates from acute hepatitis belonging to B2 have not been confirmed on Genbank. Therefore, we at first compared two consensus nucleotides from isolates related to fulminant hepatitis and other isolates without G1896A related to fulminant hepatitis by HBV/B. Thereafter, the difference of nt 1,504 in the P and X region and the difference of aa 44 in the X region were revealed.

Nucleotide 1,504 in the P and X region was not located in either the promoter or the enhancer regions. It is difficult to determine the significance of this difference, it was outstanding among the 95 isolates (HBV genome length 3,215 nucleotides) of B2 registered on Genbank. These might have some relation to fulminant hepatitis by isolates belonging to HBV/B2. As for amino acids, aa 44 in the X region was different between them, but it was not involved in essential part of X protein [Runkel et al., 1993].

The previously reported nucleotide mutations, C1653T and T1753M, were not detected in the present isolates [Kaneko et al., 1995; Sato et al., 1995]. Our results might indicate the possibility of a relationship between fulminant hepatitis induced by HBV/B and A1762T and G1764A.

A similar analysis for amino acid mutations showed Ile97Leu and Pro130Non-Pro in the core region and Trp28Stop in the precore region as previously reported [Kosaka et al., 1991; Aye et al., 1994]. In contrast, Met1 non-Met and Ser183Pro were absent.

These results could imply the strong relationship between fulminant hepatitis induced by HBV infection and some nucleotide/amino acid mutations in pre-C/C region of HBV, and partially those in P/X region of HBV.

We analyzed the entire nucleotide sequences of HBV/B from five patients with fulminant hepatitis, all of whom presented during a short period of time and showed rapidly worsening clinical courses. Virologically, the present HBV isolates may have the potential to lead infected patients to a fatal outcome. We need further studies, including in vitro studies of isolates, and comparison with other isolates detected from fulminant hepatitis patients in the future.

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RAPID COMMUNICATION

Pegylated interferon plus ribavirin for genotype Ib chronic hepatitis C in Japan

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Abstract

AIM: To evaluate the efficacy of pegylated interferon α-2b (peg-IFNα-2b) plus ribavirin (RBV) therapy in Japanese patients with chronic hepatitis C (CHC) genotype Ib and a high viral load.

METHODS: One hundred and twenty CHC patients (58.3% male) who received peg-IFNα-2b plus RBV therapy for 48 wk were enrolled. Sustained virological response (SVR) and clinical parameters were evaluated.

RESULTS: One hundred (83.3%) of 120 patients completed 48 wk of treatment. 53 patients (44.3%) achieved SVR. Early virological response (EVR) and end of treatment response (ETR) rates were 50% and 73.3%, respectively. The clinical parameters (SVR *vs* non-SVR) associated with SVR, ALT (108.4 IU/L *vs* 74.5 IU/L, $P = 0.063$), EVR (76.4% *vs* 16.4%, $P < 0.0001$), adherence to peg-IFN (≥ 80% of planned dose) at week 12 (48.1% *vs* 13.6%, $P = 0.00036$), adherence to peg-IFN at week 48 (54.7% *vs* 16.2%, $P < 0.0001$) and adherence to RBV at week 48 (56.1% *vs* 32.1%, $P = 0.0102$) were determined using univariate analysis, and EVR and adherence to peg-IFN at week 48 were determined using multivariate analysis. In the older patient group (> 56 years), SVR in females was significantly lower than that in males (17% *vs* 50%, $P = 0.0262$). EVR and adherence to Peg-IFN were demonstrated to be the main factors associated with SVR.

CONCLUSION: Peg-IFNα-2b plus RBV combination therapy demonstrated good tolerability in Japanese patients with CHC and resulted in a SVR rate of 44.3%. Treatment of elderly female patients is still challenging and maintenance of adherence to peg-IFNα-2b is important in improving the SVR rate.

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Key words: Chronic hepatitis C; Pegylated interferon; Ribavirin

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INTRODUCTION

In Japan, annual mortality due to liver cancer exceeds 30 000 and 75% of liver cancer is associated with hepatitis C virus (HCV) infection[1]. The combination of pegylated interferon (peg-IFN) plus ribavirin (RBV) is one of the most effective therapies for chronic hepatitis C (CHC), and the effect of this combination is reported to be higher than conventional interferon[2,3]. However, the majority of Japanese CHC patients are infected with HCV genotype Ib and have a high viral load, and treatment with conventional interferon has its difficulties[4]. CHC patients in Japan tend to be older than CHC patients in other countries therefore, problems such as a higher incidence of liver cancer and lower tolerability to treatment have been observed[4,5]. The HCV strain and the efficacy of interferon treatment vary between races and countries[6,7]. Identification of the factors associated with treatment efficacy is extremely important, however, few studies involving large populations have reported on the treatment of Japanese CHC patients with pegylated interferon alpha-2b (peg-IFNα-2b) plus RBV[8,9]. In this study, we evaluated the efficacy and safety of peg-IFNα-2b plus RBV therapy in CHC genotype Ib patients with a high viral load. This treatment became available in Japan for health insurance approved treatment from December 2004. In addition, we attempted to identify predictive factors for treatment outcome.

MATERIALS AND METHODS

Study population

One hundred and thirty CHC genotype Ib patients with a high viral load, who received peg-IFNα-2b plus RBV therapy in our hospital or our affiliated institutions between December 2005 and November 2006 were enrolled in this study. The diagnosis of CHC was based on the following criteria; HCV antibody positive, HCV-RNA positive and elevation of serum alanine aminotransferase (ALT) activity (> 35 IU/L) within 6 mo of screening. Exclusion criteria were leucopenia [white blood cell (WBC) count < 3000/μL], neutropenia [neutrophil (ne) count <1500/μL], thrombocytopenia [platelet (PLT) count < 90 000/μL], anemia [hemoglobin (Hb) < 12 g/dL], cirrhosis, creatinine clearance < 50 mL/min, uncontrolled mental disorder, severe heart or lung disease, or autoimmune disease. The study was approved by the ethical committee of Tohoku University according to the Declaration of Helsinki. All patients gave written informed consent before enrollment.

Treatment regimen

The patients received peg-IFNα-2b (Pegintron®; Schering-Plough, Kenilworth, NJ, USA) at a dosage of 1.5 mg/kg every week subcutaneously for 48 wk. Daily RBV (Rebetol®, Schering-Plough) was given orally for 48 wk and the dosage was adjusted according to weight (600 mg for ≤ 60 kg, 800 mg for 60 to 80 kg, 1000 mg for > 80 kg). Blood samples were obtained every four weeks and were analyzed for biochemical parameters including ALT and HCV RNA levels. The HCV genotype was determined using a kit. HCV genotype was determined by PCR using a mixed primer set derived from the nucleotide sequences of the NS5 region. HCV RNA levels were measured by quantitative RT-PCR (Amplicor, Roche Diagnostic Systems, CA, USA). HCV RNA negativity was evaluated by qualitative RT-PCR (Amplicor, Roche), which has a higher sensitivity than the quantitative method. The lower limit of the assay in the quantitative method was 5 KIU/mL (equivalent to 5000 copies/mL) and was 50 IU/mL (equivalent to 50 copies/mL) in the qualitative method. Early virological response (EVR) was defined as undetectable HCV RNA after 12 wk. Sustained virological response (SVR) was defined as undetectable HCV RNA at 24 wk after completion of treatment.

Statistical analysis

Fisher's exact test and the Mann-Whitney *U* test were used to evaluate the parameters [age, sex, weight, body mass index (BMI), EVR, peg-IFN adherence, RBV adherence, HCV RNA, ALT, WBC, Hb, and PLT] to determine SVR. Quantitative data were divided into two groups using the median to examine the differences. We conducted multivariate analysis using binary logistic regression on the parameters which achieved statistical significance ($P < 0.05$) using univariate analysis. All analyses were performed using a statistical software package (StatView-J version 5.0, SAS Institute Inc. Cary, NC, USA).

RESULTS

Patient characteristics

The details of clinical background, blood biochemistry and virological data on the CHC patients who received peg-IFNα-2b plus RBV therapy are shown in Table 1. Seventy of 120 patients (58.3%) were male, and 50 patients (41.7%) were female. The mean age was 54.8 years, and the median age was 56 years. The mean age of males was 54.1 years, and the mean age of females was 55.8 years. The median BMI was 23.6. Seventy seven patients (64.2%) had no previous history of IFN treatment and 41 patients (34.2%) had been treated with IFN previously. Of these previously treated patients, 8 were null-responders (patients who did not achieve a virological or biochemical response during IFN treatment), 15 were relapsers, and 18 patients had no available virological response.

Treatment efficacy

One hundred of 120 patients (83.3%) completed 48 wk of treatment and 24 wk of follow up. Using intention to treat (ITT) analysis, 53 patients (44.3%) achieved SVR. The rate of EVR was 50%. Response rate at the end of treatment was 73.3%. The transition rate of HCV RNA negativity with time is shown in Figure 1. Patients discontinued treatment due to depression in 3, neutropenia in 3, retinopathy in 2, anemia in 1,

Table 1 Clinical characteristics of patients at baseline (mean ± SE)

No. of patients	120
Sex, *n* (%)	
Male	70 (58.3)
Female	50 (41.7)
Age (median, range, yr)	54.8 ± 0.98 (56, 27-75)
Male	54.1 ± 1.39 (55.5, 29-72)
Female	55.8 ± 1.33 (56, 27-75)
Weight	62.1 ± 1.09 (61.4, 35.0-99.8)
Body mass index (median, range, kg)	23.7 ± 0.32 (23.6, 14.6-34.1)
Viral load (kIU kirocopies/mL)	1510 (120->5000)
ALT (median, range, IU/L)	89.4 ± 7.39 (67, 18-636)
WBC (median, range, /μL)	5083 ± 136.6 (4900, 2400-9000)
Hemoglobin (median, range, g/dL)	14.4 ± 0.12 (14.1, 11.8-17.2)
Platelet (median, range, × 10^3/μL)	163.1 ± 4.71 (162.5, 8.1-33.2)
Interferon treatment history, *n* (%)	
Present	41 (34.2)
Null-responder/relapser/unknown	8/15/18
Absent	77 (64.2)
Unknown	2 (1.6)

ALT: Alanine aminotransferase; WBC: White blood cell.

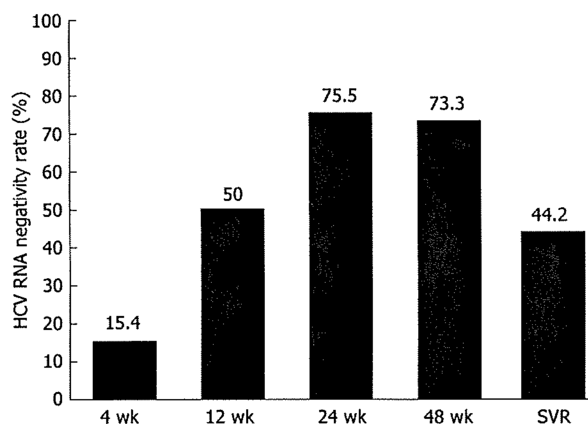

Figure 1 The transition rate of HCV RNA negativity with time.

cutaneous reaction in 1, palsy in 1, HSV infection in 1, and no response to treatment in 7.

Relationship between clinical parameters and SVR

The association between SVR rate and the baseline clinical parameters before treatment or treatment-related factors was examined using univariate analysis. The following baseline factors were analyzed: age, sex, BMI, HCV RNA level, ALT, WBC, Hb, and PLT. A summary of these results is shown in Table 2. The mean ALT level in patients who achieved SVR was 108.4 IU/L, which was significantly higher than the ALT level of 74.5 IU/L in the non-SVR group (P = 0.0478). The PLT level in patients in the SVR group was 1.73 ×10^5/μL, which was higher than the PLT level of 1.55 ×10^5/μL in the non-SVR group (P = 0.063). To determine the factors associated with treatment outcome, we examined the relationship between the SVR ratio and achievement of EVR or adherence to peg-IFN and RBV. A summary of these results is shown in Table 2 and Figure 2. As shown in Table 2, the ratio of patients who achieved EVR was

Table 2 Univariate analysis of association between sustained virological response (SVR) and influential factors (mean ± SE)

Factor	SVR patients (n = 53)	Non-SVR patients (n = 67)	P
Parameters before interferon treatment			
Age (yr)	52.5 ± 1.50	56.5 ± 1.26	0.0481
Sex (Male:Female)	35:18	35:32	0.1402
Body mass index	23.6 ± 0.48	23.8 ± 0.44	0.3611
Viral load (kirocopies/mL, median)	1500	1800	0.1963
ALT (IU/L)	108.4 ± 13.8	74.5 ± 7.04	0.0478
WBC (/μL)	5227 ± 201	4967 ± 186	0.2880
Hemoglobin (g/dL)	14.5 ± 0.18	14.3 ± 0.16	0.2352
Platelet (× 10^3/μL)	173 ± 7.7	155 ± 5.7	0.0630
Parameters associated with treatment			
EVR	42/51 (82.4%)	13/59 (22.0%)	< 0.0001
Cumulative exposure to peg-IFN			
12 wk (≥ 80%/< 80%)	38/41 (92.7%)	41/69 (68.3%)	0.0034
Overall (≥ 80%/< 80%)	35/41 (85.4%)	29/60 (48.3%)	0.0001
Cumulative exposure to RBV			
12 wk (≥ 80%/< 80%)	41/50 (82%)	44/63 (69.8%)	0.1882
Overall (≥ 80%/< 80%)	32/50 (64%)	25/63 (39.7%)	0.0138

ALT: Alanine aminotransferase; WBC: White blood cell; EVR: Early virological response; Peg-IFN: Pegylated interferon; RBV: Ribavirin.

Table 3 Multivariate analysis of association between sustained virological response and influential factors

Factor	Coefficient	χ^2	Odds Ratio (95% CI)	P
EVR (not achieved)	-2.725	19.325	0.066 (0.019-0.221)	< 0.0001
Cumulative exposure to peg-IFN				
Overall (≥ 80%)	2.392	6.600	10.934 (1.763-67.82)	0.0102
Constant	1.294			

EVR: Early virological response; Peg-IFN: Pegylated interferon.

significantly higher in the SVR group than in the non-SVR group (P < 0.0001). The SVR rate in patients who achieved EVR was 76.4%, and this was significantly higher than the SVR rate in the non-EVR group which was 16.4% (P < 0.0001). The ratio of patients who received 80% or more of the scheduled dose of peg-IFN or RBV was significantly higher in the SVR group than in the non-SVR group. The SVR rate in patients who received 80% or more of the scheduled dose of peg-IFN was 48.1% (12th wk) and 54.7% (overall). The SVR rate in patients who did not receive sufficient peg-IFN was 13.6% (12th wk) and 16.2% (overall), and these were significantly lower than the group who had good adherence. The group with adequate adherence to RBV (overall) showed an SVR rate of 56.1%, which was significantly higher than the SVR rate of 32.1% in the poor adherence group (P = 0.0102). For the factors which were determined as statistically significant by univariate analysis, we subsequently conducted multivariate analysis. The results of this analysis are shown in Table 3. Using binary logistic analysis, EVR and adherence to peg-IFN were determined to be independent predictive factors for SVR.

We examined a group of patients who were older than the median age (56 years). From the baseline factors obtained before treatment, sex was determined