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Foot notes

Table 1

Categorical variables are given as number. Continuous variables are given as median (range). Peg-IFN: pegylated interferon, RBV: ribavirin, BMI: Body mass index, AST: aspartate aminotransferase, ALT: alanine aminotransferase, γ-GTP: gamma-glutamyltransferase, LDL- cholesterol: low-density lipoprotein cholesterol, ISDR: interferon sensitivity-determining region, aa: amino acid, IL28B: interleukin 28B, ITPA: inosine triphosphatase, peg-IFN: pegylated-interferon, RBV: ribavirin

Table 2

Mild anemia was classified as hemoglobin levels <10 g/dl. Severe anemia was classified as hemoglobin levels <8.5 g/dl. Renal disorders were defined as serum creatinine concentration of 1.5 or more times above normal. Gastrointestinal disorders included nausea, diarrhea, and loss of appetite. Psychiatric disorders included insomnia and

depression. Increasing serum uric acid was defined as uric acid levels >8.5mg/dl. Skin rash included all grades.

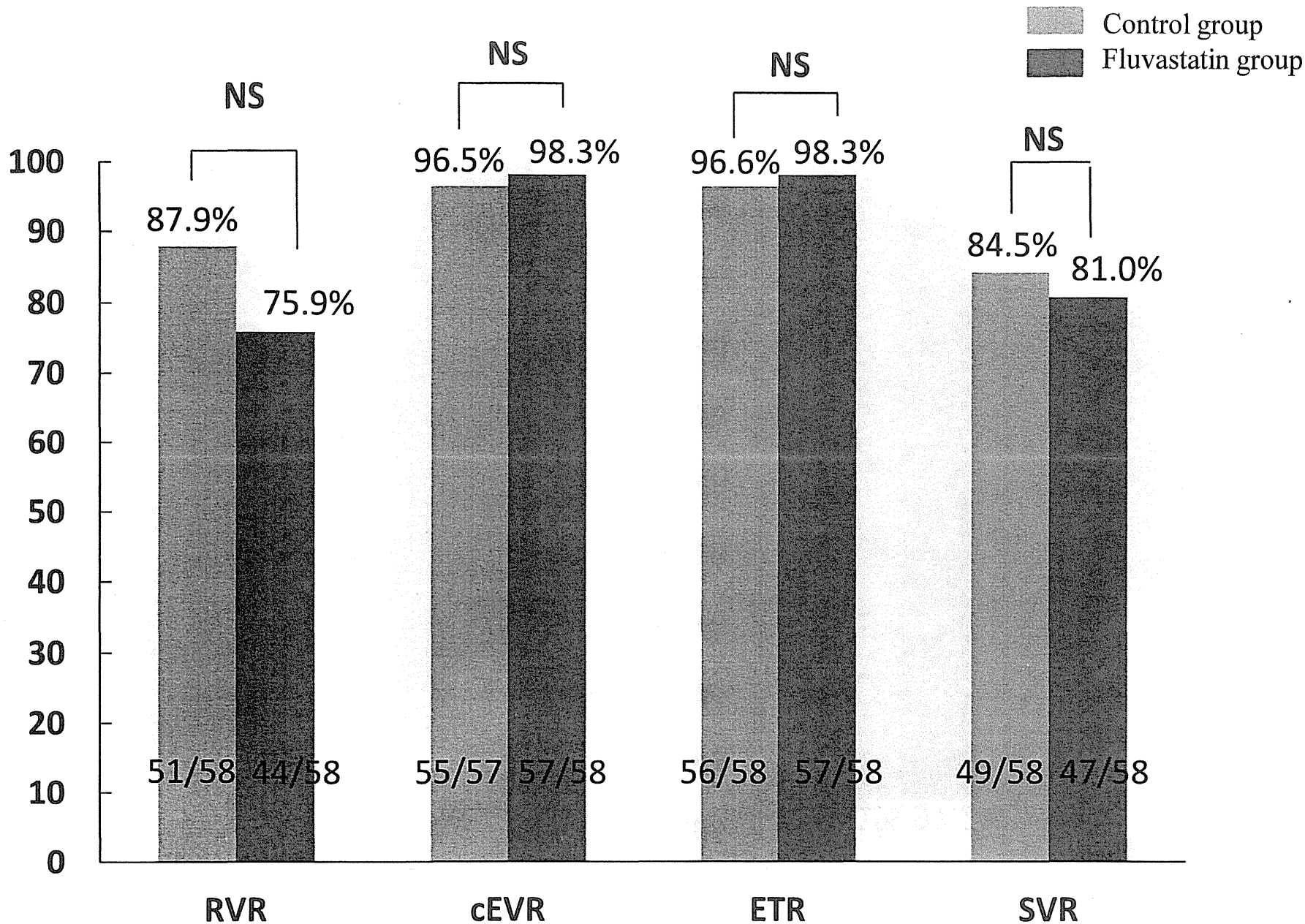
Figure 1. Comparison of the virological response rates between the fluvastatin group and the control group

RVR: rapid virological response, cEVR: complete early virological response, ETR : end of treatment response, SVR : sustained virological response, **NS: not significant.**

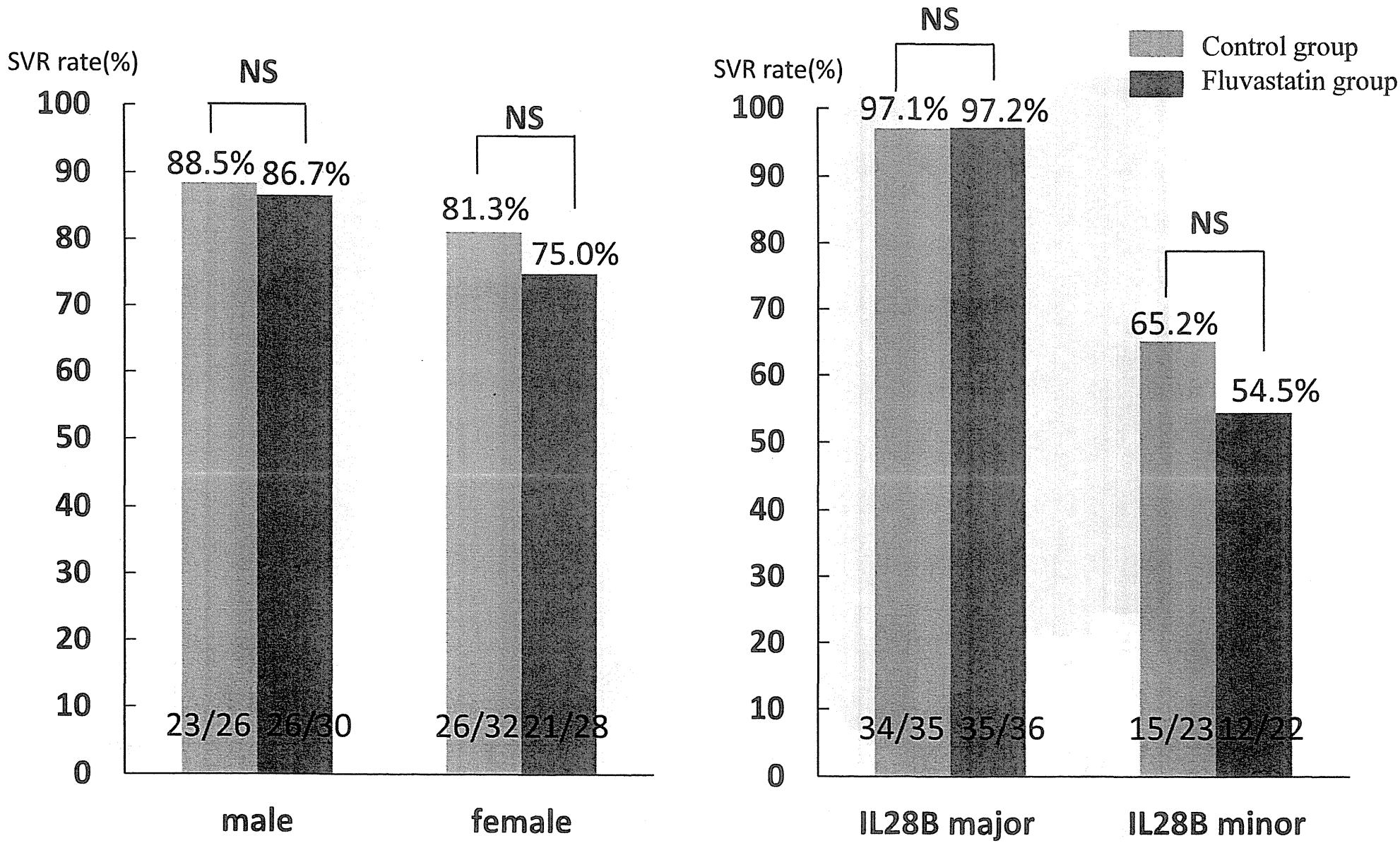
Figure 2. Comparison of the sustained virological response rates between the fluvastatin group and the control group according to gender or IL28B genotype

SVR : sustained virological response, **NS: not significant.**

Figure


Control group
Fluvastatin group
NS
NS
NS
NS
100
90
80
70
60
50
40
30
20
10
0
87.9%
75.9%
96.5%
98.3%
96.6%
98.3%
84.5%
81.0%
51/58
44/58
55/57
57/58
56/58
57/58
49/58
47/58
RVR
cEVR
ETR
SVR

Figure


SVR rate(%)
NS
NS
100
90
80
70
60
50
40
30
20
10
0
88.5%
86.7%
81.3%
75.0%
23/26
26/30
26/32
21/28
male
female
NS
SVR rate(%)
Control group
Fluvastatin group
97.1%
97.2%
NS
65.2%
54.5%
34/35
35/36
15/23
12/22
IL28B major
IL28B minor

Table

Table 1 Baseline characteristics and factors of the 116 patients who met study criteria

Factor	Telaprevir/peg-IFN/RBV with fluvastatin (n=58)	Telaprevir/peg-IFN/RBV without fluvastatin (n=58)	*P* value
Age (years)	58 (30-71)	61 (28-70)	0.536
Gender (males/females, number)	30/28	26/32	0.577
BMI (kg/m^2)	22.96 (15.94-33.31)	22.15 (17.93-34.29)	0.573
Previous treatment			
Naïve/relapse/NVR	34/18/6	23/22/13	0.131
White blood cells (/mm^3)	4880 (2820-8180)	4800 (2200-8200)	0.516
Hemoglobin (g/dl)	14.1 (10.9-17.2)	13.8 (10.7-16.8)	0.732
Platelets (×103 μl)	16.2 (6.9-33.6)	17.9 (7.0-16.8)	0.170
AST (IU/L)	43 (13-214)	40 (17-205)	0.657
ALT (IU/L)	50 (16-273)	41 (14-291)	0.577
γ-GTP (IU/L)	47 (12-296)	39 (11-339)	0.349
Total bilirubin (mg/dl)	0.6 (0.3-1.8)	0.8 (0.3-1.3)	0.072
Uric acid (mg/dl)	5.4 (2.8-7.9)	5.3 (2.4-9.4)	0.196
Serum creatinine (mg/dl)	0.67 (0.32-1.01)	0.64 (0.38-1.18)	0.208
Glucose (mg/dl)	105 (80-169)	95 (74-210)	0.196
LDL-cholesterol (mg/dl)	94 (21-155)	104 (39-189)	0.122
HCV RNA (logIU/ml)	6.7 (5.1-7.7)	6.6 (5.0-7.6)	0.344
Alpha-fetoprotein (ng/ml)	4.6 (1.1-625.7)	5.1 (2.0-90.1)	0.803
ISDR mutation 0,1/ ≥2	43/15	50/8	0.162
Core aa 70 wild/mutant	41/17	36/22	0.432
Core aa 91 wild/mutant	42/16	35/23	0.238
IL28B (rs8099917) TT/non-TT	36/22	35/23	1.000
ITPA (rs1127354) CC/non-CC	50/8	50/8	1.000

Categorical variables are given as number. Continuous variables are given as median (range).

Peg-IFN: pegylated interferon, RBV: ribavirin, BMI: Body mass index, AST: aspartate aminotransferase, ALT: alanine aminotransferase, γ-GTP: gamma-glutamyltransferase, LDL- cholesterol: low-density lipoprotein cholesterol, ISDR: interferon sensitivity-determining region, aa: amino acid, IL28B: interleukin 28B, ITPA: inosine triphosphatase, peg-IFN: pegylated-interferon, RBV: ribavirin

Table

Table 2 Incidence of adverse events according to treatment group

Adverse events	Fluvastatin group (n=58)	Control group (n=58)
	number (percent)	
Mild anemia	43 (74.1)	42 (72.4)
Severe anemia	26 (44.8)	20 (34.5)
Renal disorders	14 (24.1)	19 (32.8)
Skin rash and eruption	26 (44.8)	23 (40.0)
Gastrointestinal disorders	20 (34.5)	16 (27.6)
Psychiatric disorders	7 (12.1)	11 (19.0)
Serum uric acid increased	38 (65.5)	40 (69.0)
Discontinuation of treatment	2 (<0.1)	2 (<0.1)

Mild anemia was classified as hemoglobin levels <10 g/dl. Severe anemia was classified as hemoglobin levels <8.5 g/dl. Renal disorders were defined as serum creatinine concentration of 1.5 or more times above normal. Gastrointestinal disorders included nausea, diarrhea, and loss of appetite. Psychiatric disorders included insomnia and depression. Increasing serum uric acid was defined as uric acid levels >8.5mg/dl. Skin rash included all grades.

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Original Article

Serum 25-hydroxyvitamin D_3 levels affect treatment outcome in pegylated interferon/ribavirin combination therapy for compensated cirrhotic patients with hepatitis C virus genotype 1b and high viral load

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Aim: Much is unknown about the effect of 25-hydroxyvitamin D_3 levels on the outcome of pegylated interferon/ribavirin (PEG IFN/RBV) therapy for hepatitis C virus-related cirrhosis. The purpose of the present study was to analyze and elucidate factors, including 25-hydroxyvitamin D_3, that contribute to a sustained virological response (SVR) in patients with cirrhosis.

Methods: We analyzed whether 25-hydroxyvitamin D_3 contributes to the response to PEG IFN/RBV therapy among 134 cirrhotic patients.

Results: SVR was achieved in 43 patients. The median 25-hydroxyvitamin D_3 level was 20 ng/mL. Univariate analysis showed that the following factors contributed to SVR: low-density lipoprotein cholesterol, albumin, 25-hydroxyvitamin D_3, core a.a.70 (a.a.70) substitutions, the number of mutations at the interferon sensitivity-determining region and IL28B genotype. Multivariate analysis identified IL28B genotype and 25-hydroxyvitamin D_3 as independent factors contributing to SVR. Subsequently, SVR rate was examined by using 25-hydroxyvitamin D_3 and other important factors. The SVR rate was 51.8% in patients with core a.a.70 wild and ≥15 ng/mL of 25-hydroxyvitamin D_3, whereas the SVR rate was 7.1% in patients with core a.a.70 wild and <15 ng/mL of 25-hydroxyvitamin D_3. The SVR rate was 56.9% in patients with IL28B major genotype and ≥15 ng/mL of 25-hydroxyvitamin D_3. Surprisingly, the SVR rate was 0% in patients with IL28B minor genotype and <15 ng/mL of 25-hydroxyvitamin D_3.

Conclusion: IL28B genotype and 25-hydroxyvitamin D_3 were identified as independent factors contributing to SVR. Stratified analyses according to core a.a.70 substitution and IL28B genotype suggested that 25-hydroxyvitamin D_3 influences the outcome of PEG IFN/RBV therapy for cirrhosis.

Key words: cirrhosis, hepatitis C virus, 25-hydroxyvitamin D_3, pegylated interferon, ribavirin, sustained virological response

INTRODUCTION

WORLDWIDE, AN ESTIMATED 1.7 million people have chronic hepatitis C virus (HCV) infection.[1] If untreated, chronic hepatitis will progress to cirrhosis in many of these patients, some of whom will develop hepatocellular carcinoma.[2–5] Interferon (IFN) therapy for chronic hepatitis C inhibits progression to cirrhosis by achieving a sustained virological response (SVR).[6] The occurrence of hepatocellular carcinoma and liver disease-related death can be inhibited and decreased even after cirrhosis has already developed, by administrating IFN-based treatment and achieving SVR.[7–15]

Striking advances have been made in antiviral therapy for chronic hepatitis C. SVR is obtained in 40–50% of patients who receive pegylated (PEG) IFN/ribavirin (RBV) combination therapy for genotype 1 chronic hepatitis C, which is resistant to treatment.[16–18] Protease

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inhibitors, such as telaprevir and boceprevir, have also become available for use, and SVR is obtained in more than 70% of patients when protease inhibitors are combined with PEG IFN/RBV therapy.[19–21]

Nevertheless, the outcome of antiviral therapy for cirrhotic patients remains inadequate.[7,22,23] While PEG IFN/RBV in combination with a protease inhibitor has an outstanding antiviral effect on the infection, the combination therapy may possibly produce serious adverse events, specifically in patients with advanced fibrosis or cirrhosis.[24] Patients with chronic hepatitis including cirrhosis have a higher average age (~60 years old) in Japan as compared with patients in Western countries. Therefore, adherence to the treatment may be inadequate or treatment may be prematurely ceased in Japanese patients because of the decreased tolerability and increased incidence of more serious adverse events.[22,25]

Several factors that contribute to SVR to PEG IFN/RBV therapy in cirrhotic patients have been identified.[26–28] In recent years, a relationship between chronic hepatitis C and serum vitamin D levels has been suggested.[29–33] Vitamin D is produced in the skin or ingested from food. It exists in various forms in the body, including $1.25(OH)_2D_3$, which is an active form metabolized in the kidneys, and 25-hydroxyvitamin D_3, which is metabolized in the liver and determines the level of vitamin D in the body.[34] In chronic hepatitis C, forms of vitamin D, such as $1.25(OH)_2D_3$ and 25-hydroxyvitamin D_3, were reported to have important roles in host immune activation and inhibition of HCV replication.[35,36] The levels of serum 25-hydroxyvitamin D_3 were lower in chronic hepatitis C patients than in healthy patients and decreased with the progression of hepatic fibrosis.[29] The mechanism by which low serum 25-hydroxyvitamin D_3 levels in cirrhotic patients influence the disease condition and treatment response is unknown. Racial differences in serum 25-hydroxyvitamin D_3 levels have also been identified, but most studies have been performed in Caucasians.[30,32]

In the present study, we analyzed whether serum 25-hydroxyvitamin D_3 levels contribute to the response to PEG IFN/RBV therapy among genotype 1b, high viral load patients with compensated cirrhosis in Japan.

METHODS

Study design

THE INCLUSION CRITERIA were as follows: patient age between 20 and 85 years, high viral load (>5.0 log IU/mL) by quantitative analysis of HCV RNA with real-time polymerase chain reaction (PCR), infection with HCV genotype 1b, white blood cell count of more than 1500/mm^3, neutrophil count of more than 500/mm^3, platelet count of more than 50 000/mm^3 and hemoglobin level of more than 8.5 g/dL. Patients were also required to have a liver biopsy specimen indicative of cirrhosis (F4 based on the METAVIR classification system).[37] The exclusion criteria were as follows: other liver diseases, including autoimmune hepatitis, primary biliary cirrhosis and alcoholic disease; decompensated liver cirrhosis, such as poorly controlled ascites, hepatic encephalopathy or jaundice; liver failure; severe renal disorder; abnormal thyroid function; poorly controlled diabetes; poorly controlled hypertension; medication with Chinese herbal medicine; medical history of interstitial pneumonia; severe depression; and allergy to IFN, RBV and biological preparations such as vaccines.

There were 134 patients who visited Nippon Medical School Chiba Hokusoh Hospital and Shinmatsudo Central General Hospital between January 2006 and December 2011, and who met the inclusion criteria and agreed to receive PEG IFN/RBV therapy. The study protocol followed the ethical guidelines established in accordance with the 2004 Declaration of Helsinki and was approved by the ethics committee of Nippon Medical School Chiba Hokusoh Hospital and Shinmatsudo Central General Hospital. All patients provided written informed consent.

Treatment and definition of virological response

Patients received an s.c. injection of PEG IFN-α-2b (PegIntron; MSD, Tokyo, Japan) at a dose of 1.5 μg/kg per week and p.o. administration of RBV (Rebetol; MSD). The dose of RBV was determined on the basis of bodyweight (600, 800 and 1000 mg/day for <60, 60–80 and >80 kg, respectively) according to the manufacturer's instructions in Japan. The doses were reduced appropriately when a critical adverse event occurred during the treatment course, such as anemia. The treatment period was 48 weeks if the viral load was undetectable 12 weeks after the treatment initiation. However, the treatment course was prolonged to 72 weeks if the viral load became undetectable at 13 weeks or later. Patients who failed to achieve HCV RNA negativity by the end of treatment were considered to have a non-virological response (NVR). Patients in whom treatment was discontinued because of adverse effects or lack of effect were also considered to have an NVR. The patients were followed for 24 weeks after the completion of treatment. SVR was defined as virus-undetectable

status 24 weeks after treatment completion. Patients who exhibited end-of-treatment response but had detectable levels of virus 24 weeks after the completion of treatment were considered to have a relapse.

Laboratory tests

Peripheral blood examination and liver function tests were performed weekly until 8 weeks after treatment initiation and then monthly until 24 weeks after the completion of treatment. For biochemical tests before treatment initiation, data was obtained in the fasting state. Serum levels of 25-hydroxyvitamin D_3 were measured as that of vitamin D, because 25-hydroxyvitamin D_3 is stable in the blood circulation and comprises the major portion of vitamin D in the body. Serum 25-hydroxyvitamin D_3 levels were measured by double-antibody radioimmunoassay (RIA2 antibody assay) at a commercial laboratory (SRL Laboratory, Tokyo, Japan). HCV RNA levels were measured by real-time PCR (COBAS AmpliPrep; Roche Diagnostics, Tokyo, Japan). Gene mutations in the core and NS5A regions of the HCV genome were determined by the direct sequencing method. Core amino acid (a.a.)70 was defined as wild type (arginine) or mutant type (glutamine or histidine). Genomic DNA was extracted from whole blood by using a DNA Isolation kit on a MagNA Pure LC instrument (Roche Diagnostics, Basel, Switzerland). Single nucleotide polymorphisms (SNP) at rs8099917, which is located in the locus adjacent to the interleukin 28B (IL28B) gene on chromosome 19, were determined by real-time PCR with TaqMan SNP Genotyping Assays on a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The rs8099917 genotypes were classified into two categories: T/T (major genotype) and non-T/T (minor genotype: T/G or G/G).

Statistical analysis

Fisher's exact test was performed to compare the SVR rates according to IL28B genotype, core a.a.70 substitutions and serum 25-hydroxyvitamin D_3 levels. Logistic regression analysis for univariate comparison was performed to investigate whether each factor influenced SVR and decreased serum 25-hydroxyvitamin D_3 levels. Multiple logistic regression analyses were performed to identify significant independent factors that contributed to SVR and decreased serum 25-hydroxyvitamin D_3 levels. A receiver-operator curve (ROC) was generated to analyze the concentration of serum 25-hydroxyvitamin D_3 levels to most reasonably predict SVR. All statistical analyses were performed with IBM SPSS version 17.0 (IBM Japan, Tokyo, Japan). The level of statistical significance was set at $P < 0.05$.

RESULTS

Contributing factors to achieving SVR

PATIENT CHARACTERISTICS ARE shown in Table 1. The patient group consisted of 73 men and 61 women. The median patient age was 63 years (range, 41–82). Among the 134 patients, 43 (32.1%) achieved SVR, 39 (29.1%) relapsed into viremia and 52 (38.8%) showed NVR (38.8%).

Univariate analysis identified the following factors contributing to SVR: low-density lipoprotein ($P = 0.010$, odds ratio [OR] = 1.026, 95% confidence interval [CI] = 1.006–1.045), albumin level ($P = 0.006$, OR = 4.259, 95% CI = 1.521–11.928), 25-hydroxyvitamin D_3

Table 1 Baseline clinical and demographic characteristics of 134 patients with compensated cirrhosis

Factor	$n = 134$
Age (years), median (range)	63 (41–82)
Sex (men/women)	73/61
History of interferon therapy (naïve/relapse/non-response)	107/20/7
White blood cell count (/μL)	4355 (1800–10 400)
Hemoglobin (g/dL)	13.1 (9.7–17.2)
Platelet count (/mm^3) ×10^3	113 (53–373)
AST (IU/L)	71 (18–398)
ALT (IU/L)	64 (16–362)
γ-GT (IU/L)	57 (17–387)
Albumin (g/dL)	3.5 (2.5–5.0)
Total bilirubin (mg/dL)	0.8 (0.2–2.7)
LDL-C (mg/dL)	82 (34–137)
Triglycerides (mg/dL)	98 (34–235)
Cholinesterase (IU/mL)	208 (46–415)
25-hydroxyvitamin D_3 (ng/mL)	20 (7–45)
Alpha-fetoprotein (ng/mL)	12.1 (2.0–754.7)
Prothrombin time (%)	86.8 (36.5–141)
HCV RNA (log IU/mL)	6.5 (5.0–7.4)
ISDR (0–1/≥2)	99/29
Core a.a.70 (wild type/mutant type)	70/60
Core a.a.91 (wild type/mutant type)	77/53
IL28B (rs8099917) (TT/nonTT)	75/58

Categorical variables are given as number. Continuous variables are given as median (range).
γ-GT, γ-glutamyltransferase; a.a., amino acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; HCV, hepatitis C virus; IL28B, interleukin 28B; ISDR, interferon sensitivity-determining region; LDL-C, low-density lipoprotein cholesterol.

Table 2 Univariate logistic regression analyses of baseline factors associated with sustained virological response in patients with cirrhosis

		Univariate		
Factors	Category	OR	95% CI	*P*
LDL-C (mg/dL)	By 10 up	1.026	1.006–1.045	0.010
Albumin (mg/dL)	By 0.1	4.259	1.521–11.928	0.006
25-Hydroxyvitamin D3 (ng/mL)	By 1 up	1.064	1.001–1.132	0.049
ISDR mutation	Mutant type	2.750	1.143–6.620	0.024
Core a.a.70 substitution	Wild type	4.500	1.856–10.910	0.001
IL28B genotype	TT	8.041	3.067–21.081	0.00002

a.a., amino acid; CI, confidence interval; IL28B, interleukin 28B; ISDR, interferon sensitivity determining region; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio.

level ($P = 0.049$, OR = 1.064, 95% CI = 1.001–1.132), the number of mutations at the interferon sensitivity-determining region in NS5A ($P = 0.024$, OR = 2.750, 95% CI = 1.143–6.620), core a.a.70 wild type ($P = 0.001$, OR = 4.500, 95% CI = 1.856–10.910) and IL28B major genotype ($P = 0.00002$, OR = 8.041, 95% CI = 3.067–21.081) (Table 2).

Multivariate analysis identified 25-hydroxyvitamin D_3 level ($P = 0.048$, OR = 1.087, 95% CI = 1.001–1.181) and IL28B major genotype ($P = 0.001$, OR = 8.565, 95% CI = 2.491–29.450) as independent factors contributing to SVR (Table 3).

Relationship between serum 25-hydroxyvitamin D_3 levels and SVR rates

Serum 25-hydroxyvitamin D_3 levels were divided into three groups in accordance with previous reports: deficient (≤20 ng/mL), insufficient (21–29 ng/mL) and sufficient (≥30 ng/mL).[34] The deficient group contained 74 patients (55.2%), the insufficient group contained 50 patients (37.3%) and the sufficient group contained 10 patients (7.5%) (Fig. 1). The SVR rates were 29.7% (22/74) in the deficient group, 38.0% (19/50) in the insufficient group and 20% (2/10) in the sufficient group.

When the cut-off value for serum 25-hydroxyvitamin D_3 levels was 15 ng/mL (sensitivity = 0.91, specificity = 0.33, area under the curve = 0.564), the SVR rates were 11.8% (4/34) in patients with less than 15 ng/mL of serum 25-hydroxyvitamin D_3 and 39.0% (39/100) in patients with 15 ng/mL or more of serum 25-hydroxyvitamin D_3 ($P = 0.006$; Fig. 2).

Relationship between serum 25-hydroxyvitamin D_3 levels and core a.a.70 substitutions and SVR rates

Among patients with core a.a.70 wild type, the SVR rates were 51.8% (29/56) in those with 15 ng/mL or more of serum 25-hydroxyvitamin D_3 and 7.1% (1/14) in those with less than 15 ng/mL of serum 25-hydroxyvitamin D_3 ($P = 0.002$; Fig. 3). Among patients with core a.a.70 mutant type, the SVR rates were 19.5% (8/41) in those with 15 ng/mL or more of serum 25-hydroxyvitamin D_3 and 10.5% (2/19) in those with less than 15 ng/mL of

Table 3 Multivariate logistic regression analysis of baseline factors associated with sustained virological response in patients with cirrhosis

		Multivariate		
Factors	Category	OR	95% CI	*P*
25-Hydroxyvitamin D3 (ng/mL)	By 1 up	1.087	1.001–1.181	0.048
ISDR mutation	Mutant type	2.761	0.920–8.290	0.070
Core a.a.70 substitution	Wild type	3.052	0.994–9.374	0.051
IL28B genotype	TT	8.565	2.491–29.450	0.001

Multivariate analysis was performed with four selected factors significantly associated with sustained virological response by univariate analysis.
a.a., amino acid; CI, confidence interval; IL28B, interleukin 28B; ISDR, interferon sensitivity-determining region; OR, odds ratio.

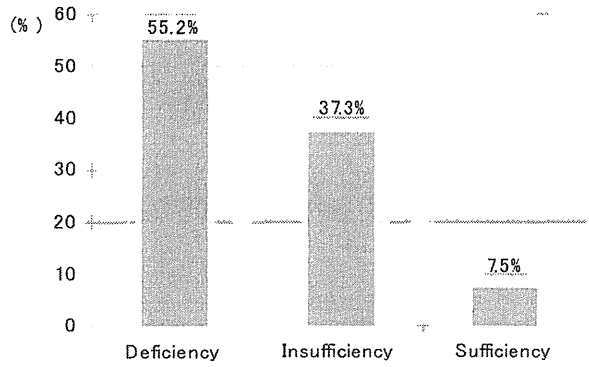

Figure 1 Categories of serum 25-hydroxyvitamin D_3 levels in the cirrhotic patient cohort. Serum 25-hydroxyvitamin D_3 levels were divided into three groups: deficient (≤20 ng/mL), insufficient (21–29 ng/mL) and sufficient (≥30 ng/mL).

serum 25-hydroxyvitamin D_3 ($P = 0.480$; Fig. 3). From the viewpoint of serum 25(OH)D_3 levels, a significant difference in the SVR rate was noted among patients with core a.a.70 wild type but not among patients with core a.a.70 mutant type.

Relationship between serum 25-hydroxyvitamin D_3 levels and IL28B genotype and SVR rates

Among patients with IL28B major genotype TT, the SVR rates were 56.9% (33/58) in those with 15 ng/mL

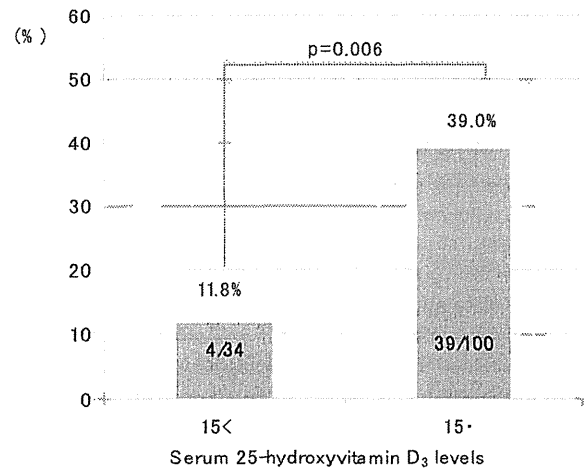

Figure 2 Comparison of sustained virological response (SVR) rates according to serum 25-hydroxyvitamin D_3 levels. The cut-off values of serum 25-hydroxyvitamin D_3 levels that predict SVR were determined from the receiver-operator curve analysis and divided into two groups: <15 ng/mL and ≥15 ng/mL.

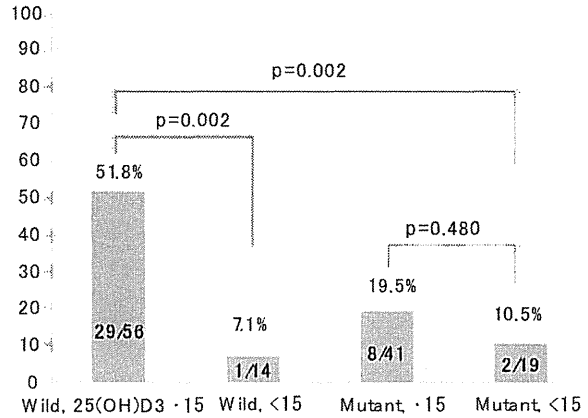

Figure 3 Comparison of sustained virological response (SVR) rates according to core amino acid (a.a.)70 substitutions and serum 25-hydroxyvitamin D_3 levels. Core a.a.70 substitutions were classified into two categories: wild type and mutant type.

or more of serum 25-hydroxyvitamin D_3 and 23.5% (4/17) in those with less than 15 ng/mL of serum 25-hydroxyvitamin D_3 ($P = 0.026$; Fig. 4). Among patients with IL28B minor genotype non-TT, the SVR rates were 14.6% (6/41) in those with 15 ng/mL or more of serum 25-hydroxyvitamin D_3 and 0% (0/17) in those with less than 15 ng/mL of serum 25-hydroxyvitamin D_3 ($P = 0.166$; Fig. 4). From the viewpoint of serum 25(OH)D_3 levels, a significant difference in the SVR rate was noted among IL28B TT patients,

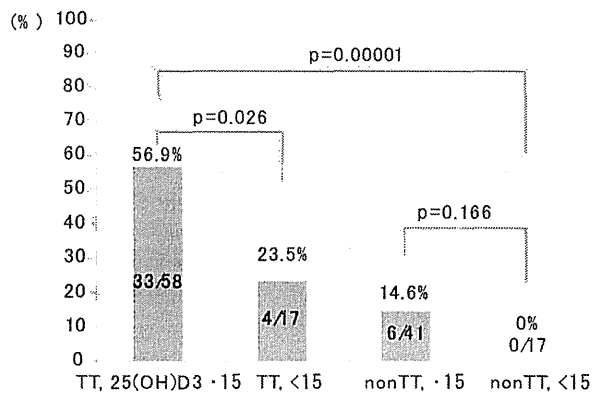

Figure 4 Comparison of sustained virological response (SVR) rates according to IL28B genotype (rs8099917) and serum 25-hydroxyvitamin D_3 levels. The IL28B (rs8099917) genotypes were classified into two categories: TT (major genotype) and non-TT (minor genotype: TG or GG).

Table 4 Univariate and multivariate logistic regression analyses of factors associated with decrease in serum 25-hydroxyvitamin D_3 levels in the 134 cirrhotic patients

		Univariate			Multivariate		
Factors	Category	OR	95% CI	*P*	OR	95% CI	*P*
Sex	Female	2.423	1.090–5.388	0.030			
Platelet count (/mm^3) $\times 10^3$	By 10 down	1.132	1.014–1.264	0.028			
Total bilirubin (mg/dL)	By 0.1 up	3.571	1.397–9.091	0.008			
Albumin (mg/dL)	By 0.1 down	7.781	2.582–23.443	0.0003	5.869	1.152–29.885	0.033
Cholinesterase (IU/mL)	By 10 down	1.001	1.001–1.013	0.020			
Creatinine (mg/dL)	By 0.1 up	12.560	1.167–134.958	0.037			
Prothrombin time (%)	By 10 down	1.031	1.002–1.062	0.036			

Multivariate analysis was performed with factors significantly associated with decreased 25-hydroxyvitamin D_3 levels by univariate analysis.
CI, confidence interval; OR, odds ratio.

whereas it was not observed among non-TT patients. In particular, the SVR rate of IL28B TT patients with 15 ng/mL or more of serum 25-hydroxyvitamin D_3 was significantly higher as compared to other patient subgroups (Fig. 4). By contrast, non-TT patients with less than 15 ng/mL of serum 25-hydroxyvitamin D_3 responded very poorly to the treatment.

Factors associated with low serum 25-hydroxyvitamin D_3 levels

The factors that influenced serum 25-hydroxyvitamin D_3 levels in cirrhotic patients were also investigated. Univariate analysis showed that the following factors were significantly associated with decreased levels of 25-hydroxyvitamin D_3: female sex ($P = 0.030$, OR = 2.423, 95% CI = 1.090–5.388), low platelet count ($P = 0.028$, OR = 1.132, 95% CI = 1.014–1.264), high total bilirubin level ($P = 0.008$, OR = 3.571, 95% CI = 1.397–9.091), low albumin level ($P = 0.0003$, OR = 7.781, 95% CI = 2.582–23.443), low cholinesterase level ($P = 0.020$, OR = 1.001, 95% CI = 1.001–1.013), high serum creatinine level ($P = 0.037$, OR = 12.560, 95% CI = 1.167–134.958) and low prothrombin time ($P = 0.036$, OR = 1.031, 95% CI = 1.002–1.062) (Table 4). Multivariate analysis identified albumin level ($P = 0.033$, OR = 5.869, 95% CI = 1.152–29.885) as the only independent factor associated with decreased levels of 25-hydroxyvitamin D_3 (Table 4).

DISCUSSION

THE CONTRIBUTION OF serum 25-hydroxyvitamin D_3 levels to the response to antiviral treatment or the association with liver fibrosis has been previously reported for patients with chronic hepatitis C infection.[30–33,38] However, these previous studies included exclusively Caucasian patients with various stages of liver fibrosis and different HCV genotypes. The present study was the first report to investigate serum 25-hydroxyvitamin D_3 status in HCV genotype 1b-mono-infected Asian patients with compensated cirrhosis and to clarify the influence of vitamin D on the outcome of PEG IFN/RBV combination therapy.

The association between the serum 25-hydroxyvitamin D_3 levels and SVR rates to PEG IFN/RBV combination therapy has been previously examined. In some studies, patients with higher serum 25-hydroxyvitamin D_3 levels were more likely to achieve SVR;[30,32] however, in another study, patients who had achieved SVR had lower serum 25-hydroxyvitamin D_3 levels.[38] The present study showed that it was difficult for cirrhotic patients with low serum 25-hydroxyvitamin D_3 levels to achieve SVR.

Advanced fibrosis or cirrhosis has a negative impact on the treatment outcome in PEG IFN/RBV therapy and even in protease inhibitor-based combination therapy.[7,20,22,23] Indeed, the SVR rate was only 32.1% in this cirrhotic patient cohort. The refractory reason is that cirrhotic patients are commonly characterized by cytopenia resulting from hypersplenism and/or impaired thrombopoiesis. Cytopenia results in reduced adherence to the treatment and/or premature treatment cessation. The mean doses of PEG IFN-α-2b and RBV in this study were 1.42 μg/kg per week (range, 0.74–1.67) and 9.05 mg/kg per day (range, 1.1–14.0), respectively, which were less than the generally recommended doses of 1.5 μg/kg per week and 13 mg/kg per day, respectively.[39] Another possible reason is that patients with

HCV-related cirrhosis are commonly elderly and thus less tolerant of the treatment, resulting in an inadequate treatment regimen.[40] Additionally, low vitamin D activity in cirrhotic patients may negatively influence the efficacy of treatment. Vitamin D supplementation may improve the poor response to treatment in cirrhotic patients.

Multivariable regression analysis in the present study identified IL28B genotype and 25-hydroxyvitamin D_3 levels as significant independent factors associated with SVR. Despite the presence of cirrhosis, IL28B major genotype patients had the SVR rate of 49.3%. In contrast, the SVR rate was very low (11.1%) in patients with IL28B minor genotype. Moreover, the present study found that serum vitamin D levels were also important for cirrhotic patients to achieve SVR.

Serum 25-hydroxyvitamin D_3 levels are often divided into categories based on several proposed criteria. According to the criteria proposed by Holick *et al.*,[34] 92.5% of the cirrhotic patients in the present study were either deficient or insufficient in serum 25-hydroxyvitamin D_3 concentration. According to previous reports, the serum 25-hydroxyvitamin D_3 levels of 46% of chronic hepatitis C patients were less than 20 ng/mL (deficient)[30,32] and those of healthy individuals were an average of 28.4 ng/mL (range, 9.5–54.8) with only 14.4% of them having deficient levels.[32] In the present study, 55.2% of the cirrhotic patients had less than 20 ng/mL of serum 25-hydroxyvitamin D_3. In patients with compensated cirrhosis, it is unclear which factors influence low serum 25-hydroxyvitamin D_3 levels. The present study identified several factors that were associated with decreased serum 25-hydroxyvitamin D_3 levels, especially as multivariate analysis identified serum albumin level as the only independent factor associated with decreased serum 25-hydroxyvitamin D_3 levels. Serum albumin level reduction may reflect the severity of liver fibrosis. Previously reported causes of vitamin D deficiency are aging, obesity, liver failure, nephrotic syndrome, chronic kidney disease and lack of sunlight.[34]

In a comparison of the SVR rate with serum 25-hydroxyvitamin D_3 levels of 15 ng/mL as the cut-off value obtained from the ROC, the SVR rate was lower in cirrhosis patients with less than 15 ng/mL of serum 25-hydroxyvitamin D_3. Based on these findings, it may be possible to designate 15 ng/mL as the cut-off value for deficient/insufficient serum 25-hydroxyvitamin D_3 levels in cirrhotic patients who receive PEG IFN/RBV combination therapy. Surprisingly, the SVR rate in patients with IL28B minor genotype and less than 5 ng/mL of serum 25-hydroxyvitamin D_3 was extremely poor (0%, 0/17). Improving the treatment outcomes of cirrhotic patients with such decreased serum 25-hydroxyvitamin D_3 levels will be crucial. These findings suggest that the SVR rate may be predicted by using the combination of serum 25-hydroxyvitamin D_3 levels and IL28B genotype,[41–43] which is currently the most influential factor in the therapeutic outcome of IFN-based treatment of chronic hepatitis C.

The addition of vitamin D to PEG IFN/RBV combination therapy was reported to improve the treatment outcome for chronic hepatitis C patients.[44] *In vitro*, vitamin D appears to play an important role in immune activation such as enhancement of the antigen-presenting capacity of dendritic cells and the cytotoxic activity of natural killer cells.[45,46] Moreover, it was reported that $1.25(OH)_2D_3$ and 25-hydroxyvitamin D_3 demonstrated a direct anti-HCV effect.[35,36] However, there are no reports of vitamin D administration to patients with compensated cirrhosis. The results of the present study encourage us to investigate the efficacy and roles of vitamin D in HCV-related cirrhosis. It is possible that supplementary vitamin D may improve treatment efficacy, especially in patients with compensated cirrhosis who have low 25-hydroxyvitamin D_3 levels.

As shown in the present investigation, it is thought that treatment should be aggressively introduced in patients with core a.a.70 wild type and/or IL28B major genotype with 15 ng/mL or more of serum 25-hydroxyvitamin D_3. On the other hand, more effective drug therapy is needed for patients in whom the response is poor, such as patients with core a.a.70 mutant type and/or IL28B minor genotype with very low serum 25-hydroxyvitamin D_3 levels.

The limitations of the present study include its small sample size and the fact that the serum 25-hydroxyvitamin D_3 levels were measured in various seasons. The serum 25-hydroxyvitamin D_3 levels are higher in summer and autumn than in winter and spring.[38] Additionally, although genes related to vitamin D levels, CYP2R1 (rs1993116, rs10741657), GC (rs2282679) and DHCR7 (rs7944926, rs12785878), have been reported,[47,48] we did not investigate these factors. Also, although an association between the serum vitamin D levels and insulin resistance has been reported,[34] it was not evaluated as a background factor in our study.

In conclusion, serum 25-hydroxyvitamin D_3 levels were decreased overall in patients with compensated cirrhosis. 25-Hydroxyvitamin D_3 levels were identified

as independent factors contributing to SVR. Stratified analyses according to core a.a.70 substitutions and IL28B genotype suggested that serum 25-hydroxyvitamin D_3 levels critically influence the outcome of PEG IFN/RBV combination therapy for HCV genotype 1b patients with compensated cirrhosis.

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Published online 2013 December 23. **Research Article**

Efficacy of Alfacalcidol on PEG-IFN/ Ribavirin Combination Therapy for Elderly Patients With Chronic Hepatitis C: A Pilot Study

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Background: Serum vitamin D concentration is reported to show a decrease in older age. Patients with chronic hepatitis C (CHC) in Japan are older on average than those in Western countries. Moreover, the outcome of pegylated-interferon (PEG-IFN)/ ribavirin therapy combined with vitamin D in elderly patients is unclear.
Objectives: This pilot study explored the efficacy and safety of alfacalcidol as vitamin D source in PEG-IFN/ ribavirin combination therapy for elderly CHC patients infected with hepatitis C virus genotype 1b.
Patients and Methods: Consecutive twenty CHC patients aged ≥ 65 years were enrolled in this pilot study. Fifteen patients met the inclusion criteria and received PEG-IFN/ ribavirin therapy combined with alfacalcidol. Four-week lead-in of oral alfacalcidol was conducted, and it was subsequently and concurrently administered in PEG-IFN/ ribavirin combination therapy (vitamin D group). Age, gender, and IL28B genotype-matched patients, who received PEG-IFN/ ribavirin alone, were saved as control group (n = 15) to compare the treatment outcome with the vitamin D group.
Results: Subjects consisted of 14 males and 16 females, with a median age of 70 years (65-78). The serum 25 (OH) D3 concentration in females (20 ng/ml, 11-37) was significantly lower than males (27 ng/mL, 13-49) (P = 0.004). Sustained virological response (SVR) rates were 33.3% (5/15) in the control group and 80.0% (12/15) in the vitamin D group, respectively (P = 0.025). While no significant difference was shown in the SVR rate between the two groups among males (P = 0.592), in females the SVR rate was significantly higher in the vitamin D group (87.5%, 7/8) than the control group (25.0%, 2/8) (P = 0.041). The relapse rates in the groups with and without alfacalcidol were 7.7% (1/13) and 61.5% (8/13), respectively (P = 0.011). Interestingly, in females, the relapse in the control group was shown in 5 of 7 (71.4%), whereas in the vitamin D group the relapse rate was decreased (1/8, 12.5%) (P = 0.041). No specific adverse events were observed in the vitamin D group.
Conclusions: PEG-IFN/ ribavirin combined with alfacalcidol may be effective and safe in elderly CHC patients. In particular, concomitant administration of alfacalcidol may lead to a reduced relapse rate, and consequently improving the SVR rate in elderly females.

Keywords: 1-hydroxycholecalciferol; Vitamin D; Ribavirin; Aged; Hepatitis C, Chronic

1. Background

Therapy for patients with chronic hepatitis C infected with high-viral-load hepatitis C virus (HCV) genotype 1b, which is difficult to treat, has progressed rapidly in the recent years (1, 2). The current standard therapy is pegylated-interferon (PEG-IFN)/ribavirin-based therapy in combination with first-generation protease inhibitors, such as telaprevir and boceprevir. The triple combination treatment increases the rate of sustained virological response (SVR) from 45%–50% to approximately 70% (3-7). However, some patients remain uncured, and adverse effects may occur more frequently and severely using protease inhibitors, prompting further improvement in the therapeutic modalities. Particularly in the elderly patients, the use of such protease inhibitors must be carefully evaluated, because adverse events may become more serious.

Next-generation protease inhibitors that cause adverse events less frequently (8, 9) and therapeutic modalities using a combination of direct antiviral agents alone without IFN (10) are expected to be developed. Young patients

Implication for health policy/practice/research/medical education:
Our study suggested that it is very important to clarify the association between vitamin D and outcome of PEG-IFN/ribavirin therapy in elderly patients with chronic hepatitis C. We hope that our study will contribute to development of treatment for patients with chronic hepatitis C, especially elderly ones.

at low risk of developing hepatocellular carcinoma can wait until the approval of the next-generation therapeutic modalities. In contrast, elderly patients at high risk of developing hepatocellular carcinoma urgently require new antiviral modalities (11, 12).

The activation of innate and acquired immunity is important for the elimination of the hepatitis C virus. Vitamin D is closely associated with host immunity. Vitamin D enhances the antigen-presenting capacity of dendritic cells, promotes their phagocytic activity toward exogenous antigens (13), and increases the cytotoxic activity of natural killer (NK) cells (14). In vitamin D-deficient mice, abnormal macrophage differentiation is observed (15). Macrophages express vitamin D receptors in the innate immune system (16). Furthermore, mature T-cells and B-cells, which are components of the acquired immune system, also express vitamin D receptors (16), and T-cell receptor signaling is controlled by 1alpha, 25 (OH) 2D3 via receptors (17).

A clinical study reported that the combination of vitamin D supplementation with PEG-IFN/ ribavirin therapy improved the SVR rate in patients with chronic hepatitis C (18). Another study showed that patients with low serum vitamin D levels had a decreased likelihood of achieving SVR in response to PEG-IFN/ ribavirin therapy (19). Furthermore, it has been reported that treatment outcomes could be significantly influenced by serum vitamin D levels and single nucleotide polymorphisms (SNPs) near the interleukin 28B (IL28B) gene (20), which is the strongest predictor of response to PEG-IFN/ ribavirin therapy for patients with chronic hepatitis C of genotype 1 (21-23).

It is conceivable that serum vitamin D levels are lower in the elderly compared to the young patients. Serum vitamin D concentration is reported to show a decrease in older age, particularly in elderly women (19). Patients with chronic hepatitis C in Japan are older on average than those in Western countries. Moreover, the outcome of PEG-IFN/ ribavirin therapy combined with vitamin D in elderly patients is unclear. The present study therefore focused on elderly patients with chronic hepatitis C aged 65 years and older in analyzing the association between serum vitamin D levels and successful therapy.

Alfacalcidol [1alpha (OH) D3] as a vitamin D supplement is directly metabolized into its active form 1alpha, 25 (OH) 2D3, and is metabolized in the liver [25(OH) ase].

2. Objectives

We administered alfacalcidol as vitamin D source in combination with PEG-IFN/ ribavirin therapy for elderly patients with chronic hepatitis C infection of high-viral-load HCV genotype 1b, and compared therapeutic outcome and safety with patients matched for age, gender and IL28B genotype, who received PEG-IFN/ ribavirin alone without alfacalcidol.

3. Patients and Methods

3.1. Study Design

Pilot study explored the efficacy and safety of PEG-IFN/ ribavirin combination therapy with alfacalcidol for elderly patients with chronic hepatitis C infection of genotype 1b. The inclusion criteria were as follows: age of 65-80 years, high viral load (> 5.0 log IU per mL) by quantitative analysis of HCV-RNA with real-time polymerase chain reaction (PCR), white blood cell count > 2500 per mm3, platelet count > 80000 per mm3, and hemoglobin level > 12 g per dL in laboratory tests. The exclusion criteria were as follows: positive results for hepatitis B surface antigen and antibody to human immunodeficiency virus type-1 (HIV-1), other liver diseases, including autoimmune hepatitis, primary biliary cirrhosis and alcoholic liver disease, liver cirrhosis, current development of hepatocellular carcinoma, severe renal dysfunction, abnormal thyroid function, abnormal parathyroid function, hypercalcemia, poorly controlled diabetes and hypertension, medication with Chinese herbal medicine, history of interstitial pneumonia, severe depression, and allergy to PEG-IFN, ribavirin, alfacalcidol.

Consecutive twenty patients with chronic hepatitis C of genotype 1b and high-viral-load aged > 65 years visited Nippon Medical School Chiba Hokusoh Hospital between January 2011 and December 2011. Five patients were excluded; 2 cirrhosis, one current development of hepatocellular carcinoma, and two rejected receiving alfacalcidol. Thus, 15 patients met the inclusion criteria and agreed to receive PEG-IFN/ ribavirin therapy combined with alfacalcidol (Table 1 and Figure 1).

Age, gender and IL28B genotype-matched patients, who received PEG-IFN/ ribavirin alone without alfacalcidol, were recruited as the control group to compare the treatment outcome with the vitamin D group. The control group was composed of 15 patients who received PEG-IFN/ ribavirin combination therapy between January 2009 and December 2010 at Nippon Medical School Chiba Hokusoh Hospital and Shinmatsudo Central General Hospital (Table 1). The study protocol was prepared following the ethical guidelines established in accordance with the 2008 Declaration of Helsinki, and was approved by the Ethics Committee of Nippon Medical School Chiba Hokusoh Hospital (Approval numbers: 523010). All patients provided written informed consent.

Table 1. Baseline Characteristics of Patients in Each Group [a]

Factors	Vitamin D Group [b]	Control Group [b]	P value
Prior IFN [b] mono therapy response naive/relapse/non-virological response	11/2/2	13/1/1	0.686
White blood cell count, /mm3	4760 (2650-6530)	4750 (2820-7170)	0.767
Hemoglobin, g/dL	13.4 (12.4-14.5)	14.1 (12.3-15.3)	0.128
Platelet count, $\times 10^3/\mu L$	143 (92-223)	149 (82-227)	0.814
AST [b], IU/L	59 (20-159)	41 (19-95)	0.280
ALT [b], IU/L	48 (19-260)	39 (20-149)	0.846
γGTP , IU/L[b], IU/L	33 (13-102)	33 (14-165)	0.927
T-BIL [b], mg/dL	0.5 (0.3-0.8)	0.6 (0.3-1.6)	0.237
LDL-C [b], mg/dL	93 (49-153)	103 (53-158)	0.361
Serum Albumin, g/dL	4.1 (3.1-4.6)	4.2 (3.5-5.0)	0.260
AFP [b], ng/mL	4.6 (2.0-130.3)	4.9 (1.6-130.3)	> 0.999
Prothrombin Time, %	89.4 (68.8-108.4)	96.7 (68.8-120.3)	0.134
25 (OH) D3 ng/mL	22 (11-37)	25 (11-40)	0.751
1alpha, 25 (OH) 2D3, pg/mL	63 (36-135)	63 (23-97)	0.480
HCV-RNA, Log IU/mL	6.7 (5.1-7.7)	6.6 (5.3-7.3)	0.863
Fibrosis, F1-2/F3	12/3	11/4	> 0.999
ISDR [b] mutation 0 or 1/ ≥ 2	11/4	7/6	0.433
Core aa 70 Wild/Mutant[b]	11/4	10/3	> 0.999
Core aa 91 Wild/ Mutant	11/4	8/5	0.410
IL28B (rs8099917) TT/ nonTT[b]	12/3	13/2	> 0.999

[a] Categorical values are represented as the number of patients. Continuous variables are represented as median (range). Relapse was defined as achievement of the end of treatment response and reappearance of HCV RNA after IFN mono-therapy. Patients who failed to achieve HCV-RNA negativity by the end of prior IFN mono-therapy were considered as non-virological response.

[b] Abbreviations: PEG-IFN, pegylated-interferon; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDL-C, low-density lipoprotein cholesterol; γ-GTP, gamma-glutamyltransferase; T-Bil, total bilirubin; ISDR, interferon sensitivity determining region; aa, amino acid; IL28B SNP, Interleukin 28B single nucleotide polymorphism; AFP, alpha-fetoprotein; Vitamin D group, PEG-IFN/ ribavirin/ alfacalcidol; Control group, PEG-IFN/ ribavirin.

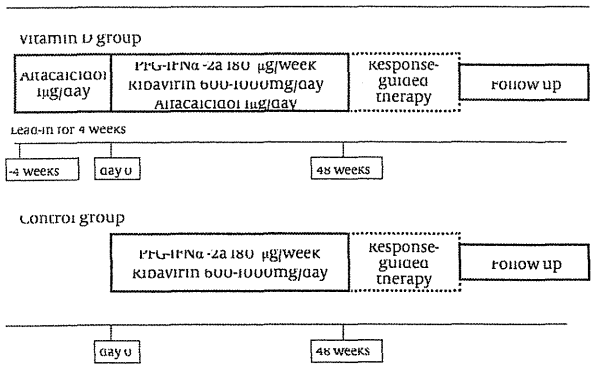

Figure 1. Treatment Protocol

3.2. Treatment and Definition of Virological Response

PEG-IFNα-2a (PEGASYS; Chugai, Tokyo, Japan) was injected subcutaneously 180 μg per week and oral administration of ribavirin (COPEGUS; Chugai, Tokyo, Japan). Ribavirin dose was adjusted by body weight (600 mg, 800 mg, and 1000 mg per day for < 60kg, 60kg-80kg, and > 80 kg, respectively) based on the guidelines of the Ministry of Health, Labor and Welfare of Japan. During the treatment course, the doses were reduced appropriately when a potentially fatal adverse event such as anemia occurred. For these 15 patients, alfacalcidol (ALFAROL; Chugai, Tokyo, Japan) was administered orally 1 μg/ day as vitamin D source. In the vitamin D group, alfacalcidol was administered for 4 weeks in advance of the combination therapy (Figure 1). The treatment period was 48 weeks if HCV-RNA was undetectable at 12 weeks after the initiation of treatment and was prolonged to 72 weeks if the HCV-RNA became undetectable at 13 weeks or later. Patients who had undetectable levels of the HCV-RNA at the completion of treatment were defined as having an end-of-treatment response (ETR). SVR was defined as HCV-RNA-undetectable status 24 weeks after the completion of treatment. Patients who exhibited an ETR but had detectable levels of the HCV-RNA, 24 weeks after the completion were consid-

ered as relapse. Patients who failed to achieve HCV-RNA negativity by the ETR were considered as non-virological response (NVR).The SVR and relapse rates were compared between the vitamin D and control groups.

3.3. Laboratory Tests

Peripheral blood examination, liver function tests and renal function tests were performed weekly until 8 weeks after the initiation of treatment, and then monthly until 24 weeks after the completion of treatment. Both serum 25 (OH) D3, which stably circulates in the body, and 1alpha, 25 (OH) 2D3, an activated form of vitamin D, were measured as serum vitamin D evaluation. The serum 25 (OH) D3 and 1alpha, 25 (OH) 2D3 were measured two times in vitamin D group: at the start of oral alfacalcidol and PEG-IFN/ ribavirin administration in the vitamin D group, and after the administration of alfacalcidol for 4 weeks. Serum 1alpha, 25 (OH) 2D3 and 25 (OH) D3 concentrations were measured by Double-antibody Radioimmunoassay (RIA2 antibody assay) at a commercial laboratory (SRL Laboratory, Tokyo, Japan). HCV-RNA levels were measured using real-time PCR (COBAS AmpliPrep; Roche Diagnostics, Tokyo, Japan). Amino acid substitutions in the core 70 and 91and NS5A regions (interferon-sensitivity determining region; ISDR) of the HCV genome were determined using the direct sequencing method. Core amino acid at position 70 was defined as wild type (arginine) or mutant type (glutamine or histidine), and core amino acid at position 91 was defined as wild type (leucine) or mutant type (methionine). Amino acid mutations in ISDR were defined as wild type (0, 1) or mutant type ($\geq$ 2). Genomic DNA was extracted from whole blood using a DNA Isolation Kit on a MagNA Pure LC instrument (Roche Diagnostics, Basel, Switzerland). SNPs at rs8099917, which is located in the locus adjacent to the IL28B gene on chromosome 19, were determined by real-time PCR using TaqMan® SNP Genotyping Assays on a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, The USA). The rs8099917 genotypes were classified into two categories: T/T (major genotype), and non-T/T (minor genotype: T/G or G/G).

3.4. Statistical Analysis

Fisher's exact test and the Mann-Whitney U-test were performed to compare the baseline characteristics. Fisher's exact test was performed to compare the SVR and relapse rates between the vitamin D and control groups. To confirm the reliability of results regarding the limited number of cases, SVR and relapse rates were analyzed, adopting the bootstrap method. Wilcoxon Signed Ranks Test was performed to compare variables such as 1alpha, 25 (OH) 2D3 between the baseline values and that after 4 weeks of alfacalcidol. The planned sample size was based on the assumption that the SVR rate would be 30% in the control group, and 60% in the vitamin D group, resulting in a necessary sample size of 38 patients in each group with a two-sided significance level of 5%, and statistical power of 80%. However, we were not able to enroll all the 38 patients in each group required by the statistical calculations. The study was stopped at 20 enrolled patients in the vitamin D group due to the slowness of the enrolment procedure. Statistical analyses were performed using IBM SPSS version 17.0 (IBM Japan, Tokyo, Japan). Bootstrap method was performed using SAS Version 9.2 (SAS Institute Japan). The level of significance was set at $P < 0.05$.

4. Results

A total of 30 patients with a median age of 70 years (range; 65-78), including 14 males and 16 females, were analyzed. Prior treatment response with IFN mono-therapy was relapse and non-virological response in 3 and 3 patients, respectively. There were no significant differences between the 15 patients received PEG-IFN/ ribavirin with alfacalcidol (vitamin D group), and the 15 patients matched for age, gender and IL28B genotype (rs8099917) received PEG-IFN/ ribavirin alone without alfacalcidol (control group) regarding background factors (Table 1). In addition, there were no significant differences between the control and vitamin D groups after 4weeks of alfacalcidol regarding background factors (Tables 2 and 3).

Table 2. Change in Variable Profile After 4 Weeks of Alfacalcidol 1μg Daily [a]

Variable (Vitamin D Group)	Pre [b]	Post [b]	P value [c]	Difference
AST [b], IU/L	59 (20-159)	42 (17-199)	0.301	-16 (± 37)
ALT [b], IU/L	48 (19-260)	37 (19-171)	0.240	-26 (± 51)
HCV-RNA, Log IU/mL	6.7 (5.1-7.7)	6.4 (4.3-7.7)	0.078	-1.1(± 2.3)
25 (OH) D3, ng/mL	22 (11-37)	27 (7-36)	0.352	2 (± 6)
1alpha, 25 (OH) 2D3, pg/mL	63 (36-135)	67 (30-136)	> 0.999	-11(± 43)

[a] Continuous variables are represented as median (range). Differences are represented as mean (± SD).
[b] Pre, baseline; post, after 4weeks of alfacalcidol; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
[c] Wilcoxon Signed Ranks Test was performed to compare variables between baseline value and that after 4 weeks of alfacalcidol.