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Hepatitis C Virus-infected Patients with Persistently Normal Alanine Aminotransferase Levels whose Platelet Count less than 150,000 /μL and whose Age over 55 Years Old should be Recommended Antiviral Therapy

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Abstract：Background and aim: There have been still controversies whether hepatitis C virus（HCV）-infected patients with normal alanine aminotransferase（ALT）levels should require antiviral therapy. The aim of this study is clarify antiviral therapeutic adaptation according to histological findings and evaluated antiviral therapy retrospectively for HCV carriers with normal ALT levels（<40 IU/L）.

Material and methods: Sixty HCV carriers with normal ALT levels were performed liver biopsy and evaluated by New Inuyama's classification, then 43 received peginterferon alpha-2b（PEG-IFN）plus ribavirin combination therapy. We assessed the relationships between each factors using statistical analyses.

Results: Of the 60 subjects studied, 21（35%）had moderate to severe fibrosis（over F2 stage）. Compared the F0-F1 group and the F2-F3 group, Age; over 55 years old（p=0.0191, odds ratio=5.26, 95% confidence interval（CI）=1.40-23）and platelet count; less than 150,000 /μL（p=0.0152, odds ratio=5.05, 95% CI=1.41-20）were the strong predictable factors in the multivariate logistic regression analysis. Whereas PEG-IFN plus ribavirin combination therapy was performed for 43 patients, sustained virological response（SVR）was achieved in 33.6%（12/33）with genotype 1b, and in 70%（7/10）with genotype 2a/2b. The most important factor to achieve SVR was HCV RNA undetectable within 12 weeks after beginning of the combination therapy（early virological response; EVR: p=0.02, odds ratio=12.1, 95% CI=2.1-126）in the multivariate logistic regression analysis.

Conclusions: According to this current study, HCV carriers with normal ALT levels whose age is over 55 years old and platelet count is less than 150,000 /μL should be recommended with antiviral therapy.

Key words：**Chronic hepatitis C, Normal serum ALT levels, Peginterferon plus ribavirin combination therapy, Early virological response**

Introduction

Chronic hepatitis C virus（HCV）infection has been an important global health problem since HCV was identified in 1989 [1,2]. According to the World Health Organization（WHO）, more than 170 million people are infected worldwide [3], and most of the mortality from HCV is caused by the complications of decompensated cirrhosis and hepatocellular carcinoma（HCC）[4]. Following serum alanine aminotransferase（ALT）levels is an insensitive means of as creating the presence of ongoing hepatic inflammation and injury. One study has reported that as many as 30% of patients with chronic HCV

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infection have normal serum ALT levels [5]. Although HCV-infected patients with non-elevated ALT seem to have significantly milder disease activity and slower progression to hepatic cirrhosis [6,7], articles have discussed the therapeutic necessity for such individuals and have provided treatment recommendations, and efficacy results for several of these proposed treatment regimens [8,9]. Recently, the need for antiviral therapy for HCV-infected patients with normal ALT levels has also been presented [10]. Here, we describe the clinical course and biochemical and histological characteristics resulting from peginterferon alpha-2b (PEG-IFN) plus ribavirin combination therapy in HCV carriers with normal ALT levels. The aim of the present study is to explore factors that may emphasize the usefulness of antiviral therapeutic adaptation for HCV-infected patients without a discernable increase in ALT in Japan.

Patients and methods

1. Eligibility and definition

The current study was conducted from January 2005 to July 2007. Among 214 patients with positive for serum HCV RNA who underwent liver biopsy in order to detect the progression of hepatitis state before antiviral therapy in Fukuoka university hospital, we enrolled 60 patients who had normal serum ALT levels (< 40 IU/L). Patients positive for hepatitis B surface antigen (HBsAg), antinuclear antibody (ANA), or anti-mitochondrial antibody (AMA), or those with a history of heavy alcohol abuse or thyroid disease were excluded from this study. All patients were negative for HCC, as assessed by abdominal ultrasonography as well as serum tumor markers. Serum ALT was evaluated twice - first at the time of liver biopsy and then just before treatment; the evaluation term took place within six months. Of 60 patients, 43 patients were treated with PEG-IFN plus ribavirin combination therapy after liver biopsy.

2. Liver biopsy

60 patients gave written informed consent before enrolling in this trial, and underwent liver biopsies by automatic biopsy needle (diameter 1.5 mm) using ultrasound guidance prior to entry. All liver tissue was fixed in formalin and stained with hematoxylin-eosin for routine morphological evaluation and with Masson's trichrome stain for assessment of fibrosis. Tissues were evaluated by pathologists belonging to the Fukuoka University Hospital, and the histological findings were interpreted and scored according to the New Inuyama classification. Staging criteria of fibrosis is classified as F0 to F4: F0; no fibrosis, F1; mild fibrosis; fibrous portal expansion, F2; moderate fibrosis; bridging fibrous (portal-portal or portal-central linkage), F3; severe fibrosis; bridging fibrosis with lobular distortion (disorganization), and F4; cirrhosis. Grading criteria of inflammation is classified as A0 to A3: A0; no necro-inflammatory reaction, A1; mild necro-inflammatory reaction, A2; moderate necro-inflammatory reaction, A3; severe necro-inflammatory reaction [11].

3. Evaluation of liver tissue

We first categorized these 60 patients into histological groups based on the degree of progression of fibrosis, and compared selected characteristics of these subjects. The groups were designated as the mild fibrosis group (F0 plus F1 stage patients) and progressive fibrosis group (F2 plus F3 stage patients). Subjects were evaluated for medical history, gender, age, body mass index (BMI), past history of IFN therapy, complete blood count, serum ALT levels (6-40 IU/L), total cholesterol (T-cho; 150-219 mg/dL), triglyceride (TG; 50-149 mg/dL), immunoglobulin G (Ig-G; 870-1700 mg/dL), and markers for HCV. Qualitative analysis of HCV RNA was performed using polymerase chain reaction (PCR) assay (Cobas Amplicor Hepatitis C Virus Test, version 2.0; Roche Diagnostics, Branchburg, NJ; detection limit: 5 KIU/mL). HCV genotype was determined by the method described by Ohno et al [12].

4. Evaluation of antiviral therapy

Among 60 HCV carriers with normal ALT levels, 43 were treated with PEG-IFN (peginterferon alpha-2b; Pegintron®, Schering-Plough Corp, Kenilworth, NJ, USA) plus ribavirin (Rebetol®, Schering-Plough Corp) combination therapy after liver biopsy. Among 17 patients who were not received combination therapy, 6 patients were treated with peginterferon alpha-2a (Pegasys®, Roche, Basle, Switzerland) mono-therapy, 5 patients were observed continuously in our hospital without receiving antiviral therapy. We couldn't follow up 6 patients because the patients changed the institution after introducing combination therapy. Combination therapy was performed 48 weeks among patients of the HCV genotype 1b, and for 24 weeks among those of genotype 2a/2b. Subjects had weekly patient visits during the first months

Table 1. Backgrounds of HCV-infected patients with normal ALT levels (n=60)

Gender ;		
Male : Female	20 : 40	
HCV genotype;		
1b : 2a/2b : Unknown	42 : 17 : 1	
Past IFN therapy		
Yes : No	15 : 45	
	Mean ± SD	(Range)
Age (years)	54.6 ± 13.5	(22 - 79)
Body mass index (kg/m^2)	22.6 ± 2.8	(18.0 - 34.2)
ALT (IU/L)	25.1 ± 7.4	(6 - 40)
Hemoglobin (g/dL)	13.3 ± 1.3	(10.7 - 17.1)
Platelet count (x10^4/μL)	17.8 ± 4.9	(9.0 - 29.6)
Total cholesterol (mg/dL)	176 ± 32.3	(127 - 279)
Triglyceride (mg/dL)	87.1 ± 35.5	(36 - 170)
Immunoglobulin G (mg/dL)	1642 ± 409	(963 - 2740)
Zinc turbidity test (Kunkel･U)	14.4 ± 6.5	(3.2 - 26.9)
HCV RNA (KIU/mL)	2132 ± 1508	(5 - 5000<)

and monthly visits for the remainder of the treatment period. Serum HCV RNA levels were determined at base line and monthly until the end 24-week follow-up period. Sustained virological response (SVR) was defined as patients who were negative for serum HCV RNA six months after completion of the combination therapy regimen. Combination therapy was discontinued if adverse events occurred, such as pancytopenia, severe anemia, depression, and erythema caused by peginterferon or ribavirin.

5. Statistical analyses

For comparison between the two groups distinguished by liver histology and duration of PEG-IFN plus ribavirin combination therapy, we used Fisher's exact test for gender, HCV genotype, history of past IFN therapy, evaluation liver histology (fibrosis) and early virological response (EVR). Comparisons of the means ± standard deviations were performed using Student's t-test for age, BMI, platelet count, hemoglobin, serum biochemical markers and serum HCV-RNA levels. A p value of less than 0.05 was considered statistically significant. For multivariate analysis, stepwise logistical regression was used to analyze the subjects considered to be statistically significant by univariate analysis (software; JMP®, version 5.1, SAS Institute, Cary, NC, USA).

	F 0	F 1	F 2	F 3	A
A 0	2	0	0	0	2
A 1	2	24	6	0	32
A 2	1	10	11	3	25
A 3	0	0	0	1	1
F	5	34	17	4	60

Figure 1. Result of histology. Liver fibrosis was existed in 55 subjects (91.6%) among the patients with normal ALT (n=60). Furthermore, mild to severe fibrosis (F2 puls F3) were shown in 21 subjects (35%) evaluated by New Inuyama classification (F0; no, F1; mild, F2; moderate, F3; severe fibrosis). Liver cirrhosis was not detected.

Results

1. Background and clinical features of liver histology

The demographic and clinical features of the patients who underwent liver biopsies are summarized in table 1. Of the 60 patients, 20 were male (33%) and 40 were female (67%). 42 were HCV genotype 1b (70%), 17 were genotype 2a/2b (28%), and only one patient was genotype unknown. The 45 patients had never been treated with interferon therapy. 15 patients had been previously treated with interferon for a diagnosis of chronic hepatitis

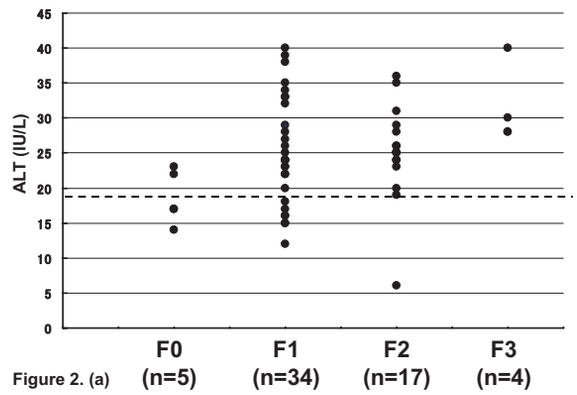

Figure 2. (a)

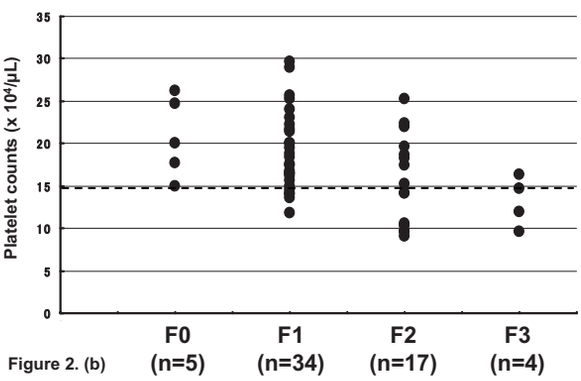

Figure 2. (b)

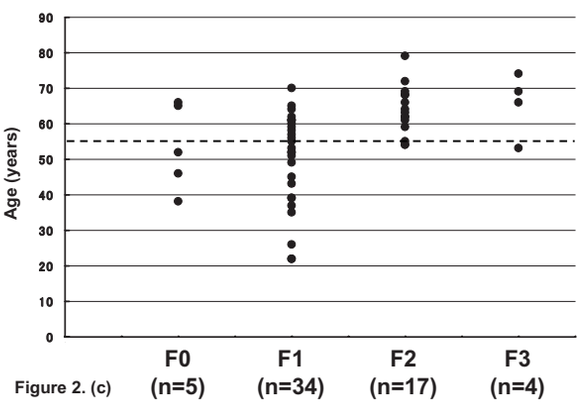

Figure 2. (c)

Figure 2. Relationships between each factor and the fibrosis stage are shown in the distribution maps (n=60).
(a) Among the most cases of F2 and F3 stage, ALT levels were more than 18 IU/L (A dividing line is shown).
(b) Among the most cases of F2 and F3 stage, platelets counts were more than 15 x 10^4 /μL (A dividing line is shown).
(c) Among the most cases of F2 and F3 stage, ages were more than 55 years old (A dividing line is shown).

type C, but IFN therapy had been discontinued at least 1 year prior to enrollment in this current study. Overall, the mean age was 54.6 ± 13.5 years. The mean serum ALT levels were 25.1 ± 7.4 IU/L. The mean platelet count was 17.8 ± 4.9 x 10^4/μL. These values were all within normal ranges. The mean HCV RNA levels were 2132 ± 1508 KIU/mL, and only one patient had a level less than 5 KIU/mL.

2. Evaluation of liver histology among HCV carriers

Histological findings in the liver biopsies (n=60) are described in Figure 1. Although only two subjects showed almost normal liver histology (A0/F0), most of the biopsies showed fibrosis greater than the F1 stage and activity greater than A1 grade of inflammation, features which define chronic hepatitis. The incidence of damage exceeding the F1/A1 stage was 91.6% (55/60). Mild fibrosis (F1 stage) was seen in 34 (56.7%) cases; 17 (28.3%) had moderate fibrosis (F2 stage); and 4 (6.7%) cases had severe fibrosis (F3 stage). None were defined as cirrhotic (F4 stage).

The relationships between age, BMI, laboratory data, and histological stage are shown in Table 2. We divided these 60 subjects into the F0 plus F1 group (n=39) and the F2 plus F3 group (n=21), and statistically compared them with respect to factors contributing to the development of fibrosis. Age (50.3 ± 12.3 vs. 63.4 ± 7.1 years; p<0.0001), Ig-G (1467 ± 245 vs. 2061 ± 1560 mg/dL; p<0.0001) of the F2 plus F3 group were significantly higher, and platelet counts (19.3 ± 4.3 vs. 14.9 ± 4.7 x 10^4/μL; p=0.0002) of the F2 plus F3 group were significantly lower than those of the F0 plus F1 group by univariate analysis. The scatter chart with relationships between selected factors (serum ALT level, platelet count, age) suspected of having a strong correlation with fibrosis are shown in figure 2. We fixed the boundary line according to the scatter chart in each three factors. ALT levels more than 18 IU/L (p=0.0438), platelet count less than 15 x 10^4/μL (p=0.0026), age more than 55 years (p=0.0087) showed strong correlations within each fibrosis group (Table 2).

Parameters differing significantly according to the univariate analysis of subjects with advanced liver fibrosis were included in a multivariate logistic regression analysis. These palameters- age older than 55 years (p=0.0191, odds ratio=5.26, 95% confidence interval; CI=1.40-23) and platelet count less than 15 x10^4 /μL (p=0.0152, odds ratio=5.05, 95% CI=1.41-20) -

Table 2. Relationships with HCV carriers who received liver biopsy between F0+F1 group and F2+F3 group（n=60）

	F0+F1 (n=39)	F2+F3 (n=21)	*p*
Gender			
Male : Female	12 : 27	8 : 13	0.806
Past IFN therapy			
Yes : No	31 : 8	13 : 8	0.1231
Age (years)	50.3 ± 12.3	63.4 ± 7.1	<0.0001
Body mass index (kg/m^2)	22.3 ± 2.4	23 ± 3.4	0.1796
ALT (IU/L)	24.5 ± 7.7	26.2 ± 6.8	0.1924
Hemoglobin (g/dL)	13.4 ± 1.4	13.1 ± 1.1	0.1588
Platelet count (10^4/μL)	19.3 ± 4.3	14.9 ± 4.7	0.0002
Total cholesterol (mg/dL)	180 ± 31.1	170 ± 32.8	0.1201
Triglyceride (mg/dL)	88.1 ± 41.6	85.3 ± 21.4	0.3856
Immunoglobulin G (mg/dl)	1467 ± 245	1965 ± 458	<0.0001
Zinc turbidity test (Kunkel･U)	11.7 ± 4.3	19.5 ± 6.4	<0.0001
HCV RNA (KIU/mL)	2169 ± 1499	2061 ± 1560	0.3968
ALT (IU/L)			
<18 : 18≦	10 : 29	1 : 20	0.0438
Platelet count (x 10^4/μL)			
<15 : 15≦	7 : 32	12 : 9	0.0026
Age (years)			
<55 : 55≦	21 : 18	4 : 17	0.0087

Table 3. Multivariate logistic regression analysis of the subjects associated with advanced liver fibrosis according to the significant differences in univariate analysis.

Subjects	F0 +F1 (%) (n=39)	F2 + F3 (%) (n=21)	Odds ratio (95%CI)	*p* value
Age (years)				
<55	21 (53)	4 (19)	1	
55≦	18 (47)	17 (81)	5.26 (1.40 - 23)	0.0191
Platelet count (x 10^4 /μL)				
<15	7 (18)	12 (57)	5.05 (1.41 - 20)	0.0152
15≦	32 (82)	9 (43)	1	

Only variables that achieved statistical significance (*p*<0.05) on multivariate logistic regression are shown.

CI: Confidence Interval

were statistically important factors in the evolution and progression of fibrosis（Table 3）.

3. Background and clinical features of patients treated by PEG-IFN plus ribavirin combination therapy

Among 60 patients from whom the liver biopsies were obtained, 43 patients with persistently normal ALT levels were treated by PEG-IFN plus ribavirin combination therapy. The background of the patients who were treated PEG-IFN plus ribavirin combination therapy is shown in Table 4. Among 43 patients, 15 were male, 33 were HCV genotype 1b, and 13 had prior IFN therapy but had no treatment for the past one year or more. The results of PEG-IFN plus ribavirin combination therapy are shown in Figure 3. The rate of sustained virological response（SVR）was 44.2%（19/43）, non virological response（NR）was 48.8%（21/43）, and drop out was 7%（3/43）in the intention-to-treat analysis. In the group of HCV genotype 1b patients（n=33）, SVR was achieved in 36.3%（12/33）, NR was 54.5%（18/33）, and drop out was 9%（3/33）. In the group of HCV genotype 2a/2b patients（n=10）, SVR was achieved in 70%（7/10）patients, NR was 30%（3/10）, and drop out was 0%.

Table 4. Background of HCV carriers who received PEF-IFN plus ribavirin combination therapy (n=43)

Gender ;		
Male : Female	**15 : 28**	
HCV genotype;		
1b : 2a/2b	**33 : 10**	
Past IFN therapy		
Yes : No	**13 : 30**	
	Mean ± SD	**(Range)**
Age (years)	**55.2 ±11.7**	**(26 - 79)**
Body mass index (kg/m^2)	**22.7 ±2.8**	**(18.1 - 34.2)**
ALT (IU/L)	**26.6 ± 7.1**	**(15 - 39)**
Hemoglobin (g/dL)	**13.3 ± 1.1**	**(10.7 - 15.4)**
Platelet count (x10^4/uL)	**17.4 ± 4.5**	**(9.0 - 28.9)**
Total cholesterol (mg/dL)	**180 ± 33.8**	**(127 - 261)**
Triglyceride (mg/dL)	**84.5 ± 34.3**	**(36 - 154)**
Immunoglobulin G (mg/dL)	**1693 ± 419**	**(963 - 2740)**
Zinc turbidity test (Kunkel･U)	**15.0 ± 6.6**	**(3.2 - 26.9)**
HCV RNA (KIU/mL)	**2124 ± 1582**	**(5 - 5000<)**

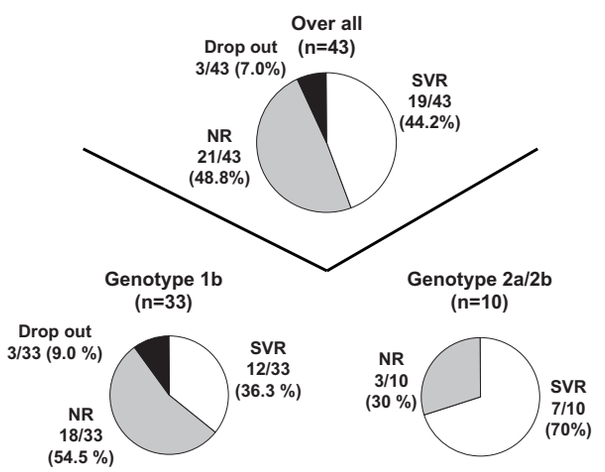

Figure 3. The outcome of the PEG-IFN plus ribavirin combination therapy is shown.
(a) The study included total of 60 patients. SVR was achieved in 19 cases (44.2), while NR was observed in 21 cases (48.8%).
(b) Among 33 patients with genotype 1b, SVR was achieved in 12 cases (36.3%).
(c) Among 10 patients with genotype 2a/2b, SVR was achieved in 7 cases (70%). (Intention-to-treat analysis)
SVR; Sustained virological response.

4. Factors associated with SVR by PEG-IFN plus ribavirin combination therapy among HCV carriers

Among 43 patients who were treated PEG-IFN plus ribavirin combination therapy, the relationship between SVR (n=19) and NR groups (n=21) is shown in Table 5. By univariate analysis, the rate of EVR (p=0.002), and BMI (21.8 ± 2.2 vs. 23.5 ± 3.1 kg/m^2; p=0.0253) were the significant predictable factors associated with SVR. In the genotype 1b patients (n=30), the rate of EVR (p=0.0080), BMI (21.5 ± 2.2 vs. 23.5 ± 3.3 kg/m^2; p=0.0319), and total cholesterol levels (185 ± 30.6 vs. 162 ± 20.8 mg/dL; p=0.0119) were the significant predictable factors associated with SVR (Table 6). In the genotype 2a/2b patients (n=10), age (50.8 ± 7.8 vs. 67.3 ± 10.1 years; p=0.0226) was the significant predictable factor with SVR (Table 7). Using the significant differences found by univariate analysis pertaining to subjects with SVR, multivariate logistic regression analysis demonstrated that EVR (p=0.02, odds ratio=12.1, 95% CI=2.1-126) was the most statistically important factor contributing SVR (Table 8).

Discussion

In Japan, the number of HCV carriers is 1.5-2 million [13], and 600,000 - 700,000 of them have chronic hepatitis or liver cirrhosis that should be treated. Importantly, chronic HCV infection has become the leading cause of HCC among the types of viral hepatitis [14]. The rate of HCC occurrence in HCV-infected patients with liver cirrhosis is 7% to 8% per year; HCC will develop in 70% to 80% of these patients within a decade. Earlier antiviral treatment of these patients may improve their prognosis by limiting progression to decompensated cirrhosis [15].

Table 5. Univariate analysis of PEG-IFN plus ribavirin combination therapy（n=40）

	SVR (n=19)	NR (n=21)	*p*
Gender			
Male : Female	8 : 11	6 : 15	0.5096
HCV genotype			
1b : 2a/2b	12 : 7	18 : 3	0.1482
Past IFN therapy			
Yes : No	6 : 13	7 : 14	1.0000
Stage of fibrosis			
F0+F1 : F2+F3	12 : 7	10 : 11	0.3596
EVR			
Yes : No	16 : 3	8 : 13	0.0041
Age (years)	52.9 ± 10.9	58.6 ± 11.8	0.1589
Body mass index (kg/m^2)	21.8 ± 2.2	23.5 ± 3.1	0.0253
ALT (IU/L)	26.2 ± 8.5	26.1 ± 5.3	0.4879
Hemoglobin (g/dL)	13.5 ± 1.1	13.1 ± 1.2	0.1589
Platelet count (10^4/μL)	17.8 ± 5.3	16.8 ± 4.1	0.2392
Total cholesterol (mg/dL)	187 ± 34.6	170 ± 32.3	0.0616
Trigliceride (mg/dL)	86.2 ± 37.4	79.5 ± 25.0	0.2564
Immunoglobulin G (mg/dL)	1677 ± 394	1699 ± 469	0.4852
Zinc turbidity test (Kunkel･U)	14.9 ± 6.7	14.7 ± 7.0	0.4746
HCV RNA (KIU/mL)	1969 ± 1747	2263 ± 1447	0.2821

Table 6. Univariate analysis of PEG-IFN plus ribavirin combination therapy among HCV carriers with genotype 1b (n=30)

	SVR (n=12)	NR (n=18)	*p*
Gender			
Male : Female	8 : 11	6 : 15	0.2663
Past IFN therapy			
Yes : No	6 : 6	7 : 11	0.7106
Stage of fibrosis			
F0+F1 : F2+F3	7 : 5	10 : 8	1.0000
EVR			
Yes : No	9 : 3	4 : 14	0.0080
Age (years)	54.1 ± 12.5	57.2 ± 11.7	0.2511
Body mass index (kg/m^2)	21.5 ± 2.2	23.5 ± 3.3	0.0319
ALT (IU/L)	26.5 ± 7.6	27.0 ± 5.2	0.4301
Hemoglobin (g/dL)	13.6 ± 1.3	12.9 ± 1.2	0.0862
Platelet count (10^4/μL)	17.4 ± 3.7	16.6 ± 3.6	0.2749
Total cholesterol (mg/dL)	185 ± 30.6	162 ± 20.8	0.0119
Triglyceride (mg/dL)	77.0 ± 33.6	78.2 ± 26.7	0.4553
Immunoglobulin G (mg/dL)	1668 ± 365	1685 ± 485	0.4583
Zinc turbidity test (Kunkel･U)	15.5 ± 6.2	14.3 ± 7.4	0.3308
HCV RNA (KIU/mL)	1853 ± 1773	2117 ± 1463	0.3299

Table 7. Univariate analysis of PEG-IFN plus ribavirin combination therapy among HCV carriers with genotype 2a/2b (n=10)

	SVR (n=7)	NR (n=3)	*p*
Gender			
Male : Female	2 : 5	1 : 2	1.0000
Past IFN therapy			
Yes : No	0 : 7	0 : 3	-
Stage of fibrosis			
F0+F1 : F2+F3	5 : 2	0 : 3	0.1667
EVR			
Yes : No	7 : 0	3 : 0	-
Age (yrs)	50.8 ± 7.8	67.3 ± 10.1	0.0226
BMI (kg/m^2)	22.4 ± 2.2	23.3 ± 1.2	0.2671
ALT (IU/L)	25.5 ± 10.5	21.0 ± 2.6	0.2457
Hemoglobin (g/dL)	13.2 ± 0.7	13.9 ± 0.8	0.1115
Platelet count (10^4/μL)	18.6 ± 7.5	17.9 ± 7.2	0.4517
Total cholesterol (mg/dL)	189 ± 42.5	216 ± 55.1	0.2244
Triglyceride (mg/dL)	100 ± 40.9	87.3 ± 9.07	0.3013
Immunoglobulin G (mg/dL)	1680 ± 470	1773 ± 448	0.2144
Zinc turbidity test (Kunkel･U)	15.4 ± 7.6	17.3 ± 4.4	0.3521
HCV RNA (KIU/mL)	2168 ± 1821	3140 ± 1183	0.2139

Table 8. Multivariate logistic regression analysis of the subjects associated with sustained virological response according to the significant differences in univariate analysis.

Subjects	SVR (%) (n=19)	NR (%) (n=21)	Odds ratio (95% CI)	*p*
EVR				
Yes	16 (84.2)	8 (38.1)	12.1 (2.1-126)	0.02
No	3 (15.8)	13 (61.9)	1	

Only variables that achieved statistical significance ($p<0.05$) on multivariate logistic regression are shown.

CI: Confidence Interval

The primary treatment goal is permanent eradication of HCV, and if HCV RNA is undetectable (using an assay with a sensitivity of ≤ 5 IU/mL) six months after completion of therapy, this is regarded as SVR [16]. Interferon therapy has been proven useful for eliminating HCV RNA in human serum and hepatic tissue and the gold standard for HCV treatment has recently become combination therapy with pegylated interferon and ribavirin [17]. It had been accepted thought that patients with moderate-to-severe fibrosis should receive interferon therapy, but that patients with normal ALT levels need not be treated except in experimental protocols [18]. In contrast, Okanoue and colleagues reported that the rate of histological progression of fibrosis is high among HCV carriers with persistently normal ALT levels, that fibrosis progresses more rapidly in cases with greater than F2 stage fibrosis, and that such cases need to be considered more often for HCV treatment [19]. In the United States, the National Institutes of Health (NIH) consensus panel in 2002 recommended that treatment for HCV carriers should be approached considering the patient's HCV RNA genotype, histological findings, clinical history, and age. It has been shown that SVR rates for patients with normal ALT levels were no different from those of patients with elevated ALT levels [16].

In this study, 91.6% of the 60 HCV carriers with normal ALT levels had mild fibrosis (over the F1 stage), 35% had moderate to severe fibrosis (over the F2 stage), and fibrosis progressed in accordance with age and decreased platelet count. Those results suggested that even if a patient had normal ALT levels, fibrosis continued to worsen. Consequently, such patients are at definitive risk of developing liver cirrhosis and HCC over the long term, even if ALT levels are normal. Chee-Kin and colleagues have reported that histological and clinically progressive diseases do occur in these patients [20]. Therefore, we advocate the use of interferon therapy for such patients in an effort to slow fibrosis progression to cirrhosis and decrease chances of HCC development. However, even among HCV carriers with normal ALT levels, a few patients have no histological changes. This promoted us to investigate other potential predictors of evolving liver fibrosis. In this study, among HCV carriers with normal ALT levels, patients who had progression of F2 stage fibrosis were over 55 years old and had platelet counts of less than $15 \times 10^4/\mu L$ by the multivariate logistic regression analysis; thus, we considered that one indication for interferon therapy in these patients could be a platelet counts less than $15 \times 10^4/\mu L$. Along those lines, the guidelines regarding treatment for HCV carriers were announced in Japan in April 2006, in which interferon therapy was deemed necessary for cases in which the platelet count is below $15 \times 10^4/\mu L$ [21]. Whereas almost patients with ALT levels less than 18 IU/L were not shown progression of fibrosis by the univariate analysis, such patients might be observed carefully unless receiving antiviral therapy. But it needs more consideration about the safety range of ALT levels with further observation period among these patients. Therefore, we suggested the first criteria in this current study that antiviral therapy was required for the patients with normal ALT levels whose age over 55 years old and platelet count less than $15 \times 10^4/\mu L$. This is the first report that the indications of antiviral therapy for the patients with chronic hepatitis C correlated with age and platelet count. Recently, we reported that interferon mono-therapy is recommended for young patients with early-stage liver fibrosis, and that the SVR rate of interferon mono-therapy in patients who are old or have progression of fibrosis in histology was low [22]. It is not efficacious to wait for an elevation in ALT (over 40 IU/L) before initiating interferon therapy in HCV carriers with persistently normal ALT levels. This is due to the fact that in HCV-infected patients treated with interferon, the SVR rate decreases as they age and fibrosis progresses. Consequently, it is important to adopt more applicable treatment criteria based on this evidence for HCV carriers with normal ALT, and to hasten the commencement of interferon therapy so as to decrease the risk of developing cirrhosis or HCC.

Recently, it was reported that SVR rate was 50-60% in chronic hepatitis C patients with genotype 1b using combination therapy with peginterferon alpha-2a or 2b

plus ribavirin [23,24,25,26]. With regard to the previous issue of HCV genotype 1b carriers with normal ALT levels, Zeuzem described an SVR rate of 52% with peginterferon alpha-2a plus ribavirin combination therapy for 48 weeks [27,28]. There are some published works focused on HCV carriers with normal ALT levels in Japanese patients using interferon mono-therapy or interferon and ribavirin combination therapy [29,30], but few articles have delved into the topic of treatment for such patients with PEG-IFN plus ribavirin combination therapy in Japan [21,31,32]. In this current study, the rates of SVR were 36.3% in HCV genotype 1b and 70% in genotype 2a/2b patients with normal ALT levels. Although these datum portray slightly lower SVR rates than those of other studies, the rates of SVR were similar to that of HCV genotype 1b patients with elevated ALT levels treated at our institution (data not shown). Furthermore, we evaluated the potential predictable factors that could contribute to successfully achieving SVR with PEG-IFN plus ribavirin combination therapy in HCV carriers with normal ALT levels. In the univariate analysis of the 43 patients overall, predictable factors for SVR were lower body mass index (BMI; p=0.0253) before the treatment and the achievement of early virological response (EVR; p=0.0041) upon treatment. In this current study, the most important factor for SVR was the achievement of EVR (p=0.02, odds ratio=12.1, 95% CI=2.1-126). We considered that the most important predicting the therapeutic response in such patients were similar to the cases of patients with elevated ALT levels that most patients without achievement of EVR don't expect to achieve SVR [33,34,35], and suggested that such patients without achievement of EVR should be required changing the therapeutic regimen such as extension of therapeutic period (for example 72 week extension) [36,37,38].

Conclusion

In this current study, we revealed that age over 55 years old and a platelet count less than 150,000 / μL are strongly related to progression of fibrosis in HCV-positive patients with normal serum ALT levels and antiviral therapy should be recommended.

By using peginterferon plus ribavirin combination therapy, the SVR rate is similar to that seen in chronic hepatitis C patients with elevated ALT levels. A crucial component to achieve sustained virological response (SVR) is the achievement of early virological response (EVR) in such patients undergoing treatment with peginterferon plus ribavirin combination therapy in Japan.

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