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Special Report

Guidelines for the antiviral therapy of hepatitis C virus carriers with normal serum aminotransferase based on platelet counts

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Aim: We aimed to identify the candidates for antiviral therapy, among patients who are hepatitis C virus (HCV) carriers with normal serum aminotransferase (ALT), focused on the inhibition of hepatocellular carcinoma (HCC).

Methods: Four hundred and sixty-four HCV carriers with normal serum ALT and 129 HCV carriers with persistently normal ALT (PNALT) and platelet (PLT) counts ≥150 000/μL who received liver biopsies were enrolled. HCV carriers with normal serum ALT were divided into four groups according to their ALT levels (≤30 U/L or 31–40 U/L) and PLT counts (≥150 000/μL or <150 000/μL).

Results: In 129 HCV carriers with PNALT, the rate of progression of fibrosis stage was 0.05/year and no HCC was detected during the follow up for 10 years. Approximately 20% of patients with ALT ≤40 U/L and PLT counts ≥150 000/μL were at stage F2–3; however, approximately 50% of patients with ALT ≤ 40 U/L and PLT counts <150 000/μL were at stage F2–4. An algorithm for the management of HCV carriers with normal serum ALT was advocated based on ALT and PLT counts.

Conclusion: The combination of ALT and PLT counts is useful for evaluating the fibrosis stage in HCV carriers with normal serum ALT. Most patients with PLT counts <150 000/μL are candidates for antiviral therapy, especially those with ALT levels ≥31 U/L when we focus on the inhibition of the development of HCC.

Key words: antiviral therapy, chronic hepatitis C, hepatitis C virus carriers, normal serum aminotransferase, platelet count

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) caused by hepatitis C virus (HCV) infection usually develops in patients with advanced chronic hepatitis (CH) or liver cirrhosis. The antiviral treatment for chronic hepatitis C (CH-C) is useful for inhibiting hepatic inflammation and progression of hepatic fibrosis, and consequently the development of HCC.[1–6]

Serum aminotransferase (ALT) levels are within the normal ranges in 20–40% of patients with chronic HCV infection,[7–11] defining the upper limit of normal serum ALT as ≤40 U/L. Significant hepatic fibrosis (≥F2 by the METAVIR classification) has been demonstrated in 5–30% of such patients.[9,12–16] We reported previously

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that HCV carriers with persistently normal ALT (PNALT) had histological features ranging from normal to minimal CH[17,18]; they showed slow progression of liver fibrosis and were at very low risk of developing HCC.[18]

The National Institute of Health Consensus Development Conference reported that HCV carriers with normal serum ALT are candidates for antiviral therapy.[19] A controlled study for the treatment of HCV carriers with PNALT with pegylated interferon alpha and ribavirin (PEG-IFN/Riba) for 48 weeks led to the eradication of HCV RNA in 40% of patients with genotype 1 and high viral load,[20] which is similar to the results of CH-C patients with elevated ALT levels.[21,22] However, it remains controversial whether these patients are candidates for antiviral therapy because of the limited efficacy of treatment, post-treatment flare-up, various side-effects, high cost of treatment, and their good prognoses.

In many Western countries, the upper limits of normal serum ALT are below 40 U/L;[23] however, a recent report from Italy demonstrated that the upper limit in healthy individuals was less than 30 U/L for men and 19 U/L for women.[24] We attempted to draft therapeutic guidelines for the treatment of HCV carriers with normal serum ALT. The biochemical and histological analyses were performed in HCV carriers with serum ALT levels below 40 U/L. These patients were divided into two groups based on ALT levels and then further divided into two subgroups according to their platelet (PLT) counts. We proposed an algorithm for the treatment of HCV carriers with normal serum ALT, taking into consideration the risk of progression to cirrhosis and the development of HCC. The present study demonstrated that the ranges of serum ALT and PLT counts are useful for deciding the indication of antiviral therapy for HCV carriers with normal serum ALT.

METHODS

Eligibility and definition

TWELVE HEPATOLOGISTS BELONGING to the Japanese Study Group of the Standard Antiviral Therapy for Viral Hepatitis, supported by the Ministry of Health, Labour and Welfare of Japan, which was settled on April 2004, participated in the study. Hiromitsu Kumada (Toranomon Hospital, Tokyo, Japan) serves as a chief and Takeshi Okanoue served as a researcher responsible for drafting the guidelines for the treatment of HCV carriers with normal serum ALT. In the present study, we tentatively defined the upper limit of the normal serum ALT as ≤40 U/L.

Patients with hepatitis B virus surface antigen, previous IFN treatment, history of heavy alcohol abuse, antinuclear antibody or antismooth muscle antibody, overt diabetes mellitus, or obesity (body mass index; ≥25 kg/m^2) were excluded from the study.

All of the patients underwent liver biopsy (≥2.0 cm in length) within 6 months prior to antiviral therapy, at which time their serum ALT levels were ≤40 U/L. Informed consent was obtained from every patient prior to liver biopsy and antiviral therapy.

Another study was conducted from January 1990 to August 2004 at Kyoto Prefectural University of Medicine (Kyoto, Japan). HCV carriers with PNALT were defined by serum ALT levels ≤30 U/L on at least three different occasions over a 12-month period and PLT counts ≥150 000/μL as reported previously.[18]

Study design

Among the 580 HCV carriers with normal serum ALT (≤40 U/L), 116 patients were excluded from the study because of insufficient data. Thus, 464 patients who received antiviral therapy from 1995 to 2004 were enrolled in this study (Table 1). Formalin-fixed liver specimens were stained with hematoxylin–eosin, and with Masson's trichrome. The liver specimens ($n = 262$) were also stained with Perls' Prussian blue to study hepatic iron loading. The histological findings were scored according to the classification proposed by Desmet *et al.*[25] and Ishak *et al.*[26] Steatosis was defined as fat droplets in >10% of hepatocytes. The degree of iron loading was assessed using a Perls' score of 0–4+, based on the scoring system of MacSween *et al.*[27]

The serum ALT, blood glucose level, immunoreactive insulin (IRI), serum ferritin, PLT count, serum hyaluronic acid, amount of serum HCV RNA, and the HCV genotype were examined. The homeostasis model assessment–insulin resistance was calculated as follows: plasma fasting glucose (mg/dL) × IRI (ng/mL) ÷ 405. The serum HCV RNA levels were determined using an Amplicor GT HCV monitor (Roche Diagnostic Systems, Tokyo, Japan). HCV genotype 1 (G1) and 2 (G2) were determined by a serologic genotyping assay.[28] G1 and G2 in this assay correspond to genotype 1 (1a, 1b) and 2 (2a, 2b) proposed by Simmonds *et al.*[29]

All the patients received IFN monotherapy or IFN/Riba combination therapy for 12–36 weeks. The average

Table 1 Baseline of hepatitis C virus patients with normal serum aminotransferase (ALT) received antiviral therapy

	ALT ≤ 30 U/L (group A)	ALT 31–40 U/L (group B)	*P*-value
No. patients	255	209	
Age	51.6 ± 13.0	53.5 ± 13.2	0.548*
Sex (male/female)	112/143	117/92	0.01**
BMI (kg/m^2)	21.6 ± 2.9	22.8 ± 3.0	<0.001*
HOMA-IR	2.5 ± 3.2	5.2 ± 6.5	0.093*
Genotype: 1/2/others	127/127/1	112/96/1	0.881**
Viral load: low/high	138/117	99/110	0.203**
G1 (low/high)	114/125		
G2 (low/high)	161/62		
Histology			
F stage (0/1/2/3/4)	29/166/48/11/1	22/122/57/6/2	0.169**
Grade (0/1/2/3)	25/187/41/2	7/159/43/0	0.046**
Fatty change† 0–1/2–4	232/23	161/48	0.033**
Iron load‡ 0/1–4	101/15	97/19	0.458**
Ferritin (ng/mL)	83.9 ± 103.7	118.8 ± 135.3	0.006*
PLT count (/μL)	19.2 ± 5.4	18.4 ± 6.1	0.059*
≥150 000/<150 000	204/51	141/68	0.002**
Hyaluronate (ng/mL)	60.8 ± 73.7	69.1 ± 73.0	0.249*
Duration of antiviral therapy (weeks)	25.6 ± 12.0	26.1 ± 12.1	0.297*
Effects of therapy			
SVR/non-SVR	142/113	99/110	0.075**

**P*-values were calculated by Mann–Whitney-*U*-test. ***Fisher-exact-test. †0: no fatty change, 1: ≤10%, 2: 11–33%, 3: 34–66%, 4: ≥67% of hepatocyte; ‡no stain by 400×, 1: few stains by 250×, 2: stains by 100×, 3: stains by 25×, 4: stains by 10×. There were significant differences in sex distribution ($P = 0.01$), BMI ($P = 0.01$), frequency of steatosis ($P = 0.033$), serum ferritin level ($P = 0.006$), grade of hepatic inflammation ($P = 0.046$), incidence of fatty change ($P = 0.033$), serum ferritin level ($P = 0.006$), and the incidence of low PLT counts ($P = 0.002$) between groups A and B. Values are expressed as mean ± SD.
ALT, alanine aminotransferase; BMI, body mass index; HOMA-IR, homeostasis model assessment–insulin resistance; PLT, platelet; SVR, sustained viral responders.

duration of therapy between 1995 and 2003 was 26 weeks for IFN monotherapy and 24 weeks for IFN/Riba combination therapy. In principle, 6–10 MU IFN was administered daily for 2 weeks and three times per week subsequently. The daily dosage of ribavirin was 600–1000 mg depending on body weight. Sustained viral responders (SVR) were defined as patients who were negative for serum HCV RNA 6 months after the completion of antiviral therapy.

All of the patients were divided into two groups (group A: ALT ≤ 30 U/L, group B: 31 U/L ≤ ALT ≤ 40 U/L) which were further divided into two subgroups based on PLT counts: group A-1 and B-1 (PLT counts ≥150 000/μL) and groups A-2 and B-2 (PLT counts <150 000/μL).

One hundred and twenty-nine HCV carriers with PNALT were enrolled to determine their long-term prognosis. These patients showed normal serum ALT levels (≤30 U/L) over a 12-month period on least three different occasions (PLT counts ≥150 000/μL, and body mass index [BMI] <25 kg/m^2). Thirty-nine patients received serial liver biopsies. The mean follow-up period of the 129 patients was 7.2 ± 3.2 years on 15 November 2006.

Statistical analyses

Data are expressed as mean ± SD. We compared continuous variables using the Mann–Whitney *U*-test. A frequency analysis and comparison between the groups were performed using the χ^2-test or Fisher's exact test and the Mann–Whitney *U*-test. ANOVA and Tukey's HSD procedure was used to determine the difference between multiple groups. All tests were two-tailed and *P*-values of less than 0.05 were considered significant. All statistical analyses were performed using Statistical Package of Services Solutions software, version 11.0 (SPSS, Chicago, IL, USA).

Table 2 Baseline of hepatitis C virus patients with less than 30 U/L aminotransferase who received antiviral therapy

	PLT ≥ 150 000/μL (group A-1)	PLT < 150 000/μL (group A-2)	*P*-value
No. patients	204	51	
Age	48.4 ± 12.7	58.7 ± 7.5	<0.001*
Sex (male/female)	90/114	22/29	1.000**
BMI (kg/m^2)	21.6 ± 3.0	21.3 ± 2.4	0.514*
HOMA-IR	2.8 ± 3.5	1.2 ± 0.8	0.598*
Genotype: 1/2/others	101/101/2	25/26/0	0.952**
Viral load: low/high	112/92	26/25	0.574**
Histology			
F stage (0/1/2/3/4)	29/142/27/6/0	1/25/21/3/1	<0.001**
Grade (0–1/2,3)	179/25	33/18	<0.001**
Fatty change† 0–1/2–4	188/16	44/7	0.582**
Iron load‡ 0/1–4	82/12	17/3	0.762**
Ferritin (ng/mL)	86.0 ± 112.1	73.9 ± 46.6	0.204*
PLT count (/μL)	21.0 ± 4.4	12.1 ± 2.5	<0.001*
Hyaluronate (ng/mL)	41.8 ± 56.1	112.5 ± 109.9	<0.001*
Duration of antiviral therapy (weeks)	25.7 ± 10.3	27.0 ± 9.9	0.503*
Effects of therapy			
SVR/non-SVR	115/89	27/24	0.66**

**P*-values were calculated by Mann–Whitney-*U*-test. ***Fisher-exact-test. †0: no fatty change, 1: ≤10%, 2: 11–33%, 3: 34–66%, 4: ≥67% of hepatocyte; ‡no stain by 400×, 1: few stains by 250×, 2: stains by 100×, 3: stains by 25×, 4: stains by 10×. There were significant differences in age ($P < 0.001$), distribution of F stage ($P < 0.001$), grade of inflammatory activity ($P < 0.001$), PLT count ($P < 0.001$), and serum-hyaluronic acid ($P < 0.001$) between groups A-1 and A-2. Frequency of F2–4 patients was 16.2% in group A-1 and 51.6% in group A-2. Values are expressed as mean ± SD.
BMI, body mass index; HOMA-IR, homeostasis model assessment–insulin resistance; PLT, platelet counts; SVR, sustained viral responders.

RESULTS

Demographic, clinical, and histological features of 464 HCV carriers with normal serum ALT

THE CHARACTERISTICS OF the 464 HCV carriers with normal serum ALT are shown in Table 1. There were significant differences in sex, frequency of steatosis, serum ferritin levels, BMI, and the incidence of low PLT counts (<150 000/μL) between groups A and B.

There were significant differences in age, fibrosis (F) stage, inflammatory activity, PLT counts, and serum hyaluronate between groups A-1 and A-2 (Table 2). The frequency of stage F2–4 patients was 16.2% in group A-1, and 49.0% in group A-2 (Table 2). In group B, there were significant differences in age, F stage, PLT counts, and serum hyaluronate between groups B-1 and B-2 (Table 3). There were no F4 patients in group A-1 and B-1, and the frequency of F3 patients was very low compared with those in groups A-2 and B-2 (2.6% *vs* 7.6%). The PLT counts decreased in proportion to the progression of liver fibrosis as follows; F0 ($n = 51$); $20.7 \pm 5.2 \times 10^4$/μL, F1 ($n = 288$); $19.8 \pm 5.6 \times 10^4$/μL, F2 ($n = 105$); $16.9 \pm 5.3 \times 10^4$/μL, F3 ($n = 17$); $15.9 \pm 4.6 \times 10^4$/μL, and F4 ($n = 3$); $11.3 \pm 3.8 \times 10^4$/μL.

Of the 464 patients, the frequency of the F0–1 stages was 80.1% and that of the F2–4 stages was 19.9% in patients with PLT counts ≥150 000/μL, and it was 50.4% and 49.6%, respectively, in patients with PLT counts <150 000/μL. In patients with PLT counts $\geq 17.0 \times 10^4$/μL, 80.8% were in stages F0–1 and 19.2% were in stages F2–4, and in patients with PLT counts $< 17.0 \times 10^4$/μL, 60.1% were in stages F0–1 and 39.9% were in stages F2–4.

The SVR rates of IFN therapy were 52.4% in F0–1 patients, 49.5% in F2–4 patients ($P = 0.896$ by Fisher's exact test), and 58.0% and 43.8% ($P = 0.592$) in IFN/Riba therapy, respectively.

In patients with genotype 1b and high viral load, the SVR rate was 12.5%. The SVR rate in genotype 2 patients was 60.4% in the IFN group and 67.7% in the IFN/Riba combination therapy group.

Table 3 Baseline of hepatitis C virus carriers with 31–40 U/L aminotransferase who received antiviral therapy

	PLT ≥ 150 000/μL (group B-1)	PLT < 150 000/μL (group B-2)	*P*-value
No. patients	141	68	
Age	48.2 ± 11.9	57.9 ± 7.5	<0.001*
Sex (male/female)	80/61	37/31	0.751**
BMI (kg/m^2)	22.9 ± 3.1	22.7 ± 2.6	0.08*
HOMA-IR	3.0 ± 2.0	8.2 ± 9.5	0.8.8*
Genotype: 1/2/others	82/58/1	30/38/0	0.095**
Viral load: low/high	64/77	35/33	0.542**
Histology			
F stage (0/1/2/3/4)	17/91/31/2/0	4/30/26/6/2	<0.001**
Grade (0–1/2,3)	116/25	50/18	0.114**
Fatty change† 0–1/2–4	111/30	50/18	0.10**
Iron load‡ 0/1–4	67/12	30/7	0.762**
Ferritin (ng/mL)	114.4 ± 116.1	127.2 ± 167.8	0.869*
PLT count (/μL)	21.5 ± 4.9	12.2 ± 2.1	<0.001*
Hyaluronate (ng/mL)	46.9 ± 35.4	100.7 ± 0.98.1	<0.001*
Administration of IFN (weeks)	26.1 ± 11.9	27.7 ± 11.4	0.983*
Effects of therapy			
SVR/non-SVR	64/77	35/33	0.409**

**P*-values were calculated by Mann–Whitney-*U*-test. ***Fisher-exact-test. †0: no fatty change, 1: ≤10%, 2: 11–33%, 3: 34–66%, 4: ≥67% of hepatocyte; ‡no stain by 400×, 1: few stains by 250×, 2: stains by 100×, 3: stains by 25×, 4: stains by 10×. In group B, there were significant differences in age ($P < 0.001$), distribution of F stage ($P < 0.001$), PLT count ($P < 0.001$), and hyaluronic acid ($P < 0.001$) between B-1 and B-2. Frequency of F2–4 was 23.4% in B-1and 50.0% in B-2, respectively. Values are expressed as mean ± SD.
BMI, body mass index; HOMA-IR, homeostasis model assessment–insulin resistance; IFN, interferon; PLT, platelet counts; SVR, sustained viral responders.

Demographic, clinical, and histological features of 129 HCV carriers with PNALT

The demographic and clinical features of the 129 HCV carriers with PNALT who were followed up for 7.2 years are shown in Table 4. Normal liver histology was noted in 17 patients, 102 showed minimal to mild CH, and 10 had moderate CH. Steatosis was seen in 7% and iron loading was noted in 12%.[18]

Of the 78 patients followed longer than 7 years (mean follow-up period; 10.4 ± 3.1 years), 11 (14%) had continuously normal ALT (G-1), 43 (55%) showed a transient elevation of ALT (G-2), and 24 (31%) changed to CH with continuously elevated ALT (G-3).

Thirty-nine patients received repeated liver biopsies (2–4 times). Of the 39 patients, six were in G-1, 17 were in G-2, and 16 were in G-3. The intervals between the first biopsy and the last biopsy in these three groups were 7.1, 7.8, and 7.2 years, respectively. The progression of the F stage was noted in two of six in G-1, six of 17 in G-2, and seven of 16 in G-3. The median rates of fibrosis progression per year for these three groups were 0.05, 0.05, and 0.08 fibrosis unit. HCC was not detected in any patients during the follow-up periods.

Guidelines for the antiviral therapy of HCV carriers with normal serum ALT focused on the inhibition of the development of HCC

Considering the risk of progression to liver cirrhosis and the development of HCC, as well as the expected efficacy and various side-effects of antiviral therapy, an algorithm is needed for the management of HCV carriers with normal serum ALT. The progression rate of liver fibrosis stage was 0.05/year in HCV carriers with PNALT. The annual incidence of HCC in CH-C patients has been reported to be 0.5% at stages F0–F1, 1–2% at stage F2, 3–5% at stage F3, and 7% at stage F4.[4]

In principle, follow up without antiviral treatment is recommended for HCV carriers with PNALT (ALT ≤30 U/L) and PLT counts ≥150 000/μL, particularly in older patients (i.e. >65 years old), because over 90% show normal or minimal liver damage with good prognoses. However, antiviral therapy is not contraindicated for such patients since roughly 40% are infected with HCV genotype 2,[18] which suggests a high rate of SVR to the therapy with PEG-IFN/Riba.

As for the indication of antiviral therapy for HCV carriers with normal serum ALT (≤40 U/L), the PLT

Table 4 Characteristics of 129 HCV carriers with persistently normal ALT who received liver biopsy

	$n = 129$	Follow up over 5 years ($n = 78$)
Follow-up period (years)	7.2 ± 3.2	10.4 ± 3.1
Age (years)	48 (21–77)	45 (29–71)
Male ($n = 24$)	49.8 ± 16.4	42.3 ± 14.9
Female ($n = 105$)	47.2 ± 12.5	46.6 ± 11.6
Sex (male/female)	24/105	10/68
ALT (U/L)	8–30	9–30
Male ($n = 24$)	22.5 ± 5.7	21.1 ± 5.4
Female ($n = 105$)	21.6 ± 4.8	22.3 ± 5.1
PLT ($\times 10^4/\mu L$)	15–31	15–31
Ferritin (ng/mL)	5–225	5–225
Male ($n = 24$)	76.2 ± 53.5	84.6 ± 59.2
Female ($n = 105$)	60.0 ± 43.3	66.6 ± 52.5
HCV genotype	G1 ($n = 58$), G2 ($n = 45$) Mixed and unclassified ($n = 16$)	
BMI (kg/m^2)	16–27	16–27
Male	22.2 ± 1.7	21.9 ± 1.9
Female	21.3 ± 2.2	21.0 ± 2.4

Values are expressed as mean ± SD.
ALT, alanine aminotransferase; BMI, body mass index; HCV, hepatitis C virus; PLT, platelet.

count is a good indicator for discriminating as to whether or not they have minimal to mild fibrosis or moderate to advanced fibrosis. Serum hyaluronate levels were significantly higher in HCV carriers with 31–40 U/L ALT having less than 150 000/μL PLT (Table 3). Advanced hepatic F stage, an elevated ALT level, old age (>65 years old), and sex (male) are important risk factors for the development of HCC.[6,18,30] We advocated an algorithm for such patients (Fig. 1) taking into consideration the risk of the progression to cirrhosis and the development of HCC. Therapy with PEG-IFN/Riba is the first-line treatment; therapy for 48 weeks is recommended for genotype 1 patients with high viral load and 12–24 weeks therapy for genotypes 2 and 1 with low viral load.

DISCUSSION

OUR PREVIOUS STUDY in 129 HCV carriers with PNALT demonstrated a predominance of females, higher frequency of genotype 2, minimal to mild liver histology, and very slow progression of hepatic fibrosis.[18] However, over 30% of these patients advanced to CH-C with elevated ALT levels during the 7-year follow up.

There are many reports concerning the natural course of liver fibrosis in CH-C patients, including those who are HCV carriers with normal serum ALT.[19,31–39] More than half of CH-C patients show progression of F stage from F1 to F2–4 within 10 years, and it was reported that the progression of liver fibrosis in HCV carriers with normal serum ALT was more rapid than was observed in the present study.[23] The main reason for the discrepancy between the report by Puoti *et al.*[23] and our results might be due to the definitions used for the normal range of serum ALT. In our previous study, the patients were HCV carriers with PNALT (ALT ≤ 30 U/L) and PLT counts ≥150 000/μL. On the other hand, the patients in the study by Puoti *et al.* had ALT levels ≤40 U/L, irrespective of PLT counts, in which cirrhotic patients might be included.[23] However, recent studies have demonstrated that normal ALT levels are less than 30 U/L[24] or 25 U/L in men[40] and less than 19 U/L[24] or 22 U/L in women.[40]

The present study demonstrated that the different distribution of hepatic F stage became remarkable when the A and B groups were divided into two subgroups according to their PLT counts. In HCV carriers with ALT levels ≤30 U/L, the frequency of stages F2–3 was 16.2% among those with PLT counts ≥150 000/μL; however, the frequency of stages F2–3 was 49.0% in those with PLT counts <150 000/μL. Conversely, in HCV carriers with ALT levels between 31 and 40 U/L, the frequency of stages F2–4 was 23.4% among those with PLT counts ≥150 000/μL and 50.0% in those with PLT counts <150 000/μL. The PLT count is a useful marker in dis-

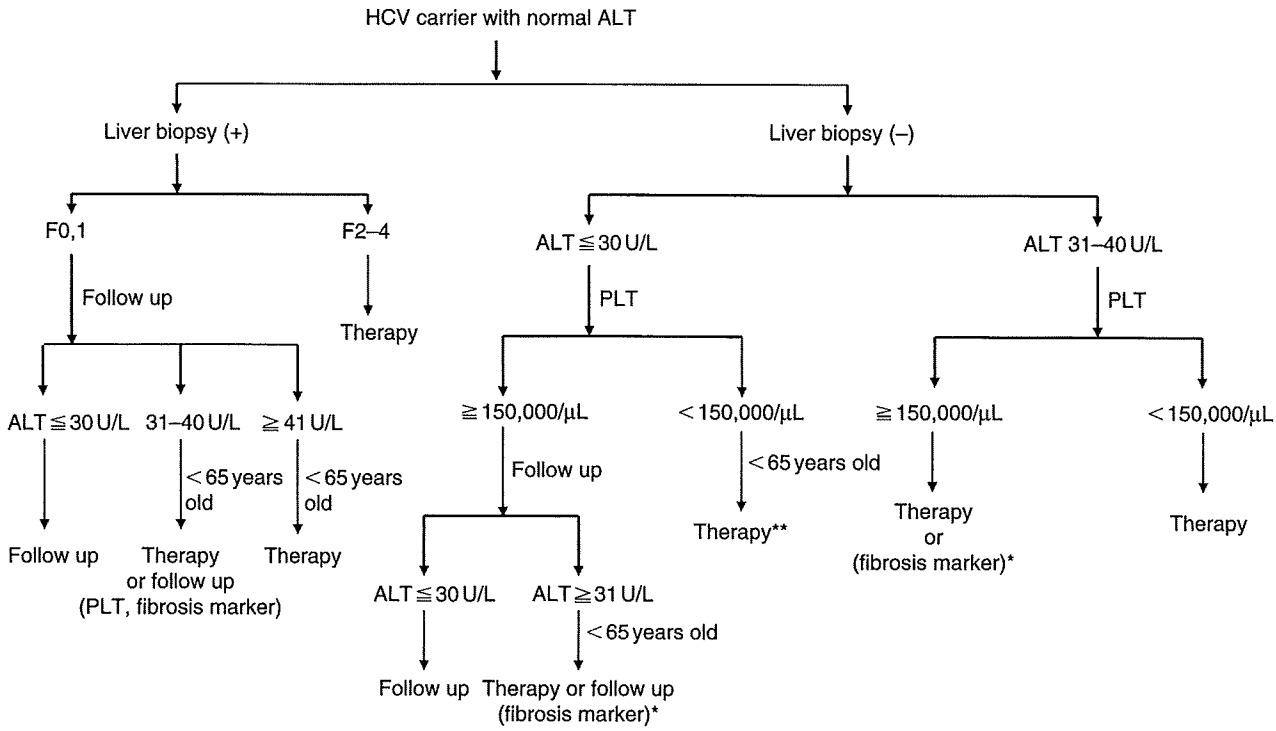

Figure 1 Algorithm for the management of hepatitis C virus (HCV) carriers with normal serum aminotransferase (ALT, ≤40 U/L) focused on the inhibition of the development of hepatocellular carcinoma. In patients who underwent liver biopsy, F0 and F1 patients younger than 65 years are candidates for antiviral therapy, especially those with genotype 2 after the elevation of serum ALT levels. In patients who did not undergo liver biopsy, ALT and platelet (PLT) levels are good indicators for determining candidates for antiviral therapy. Older patients (>65 years) and/or patients having uncontrolled hypertension, diabetes mellitus, or anemia should not be treated with pegylated interferon and ribavirin. Combination therapy with pegylated interferon and ribavirin for 48–72 weeks is recommended for patients with genotype 1 and high viral load, and 12–24 weeks therapy is suggested for patients with genotype 2 and genotype 1 with low viral load. *,**Serum fibrosis markers, such as hyaluronate, might be useful to decide whether patients are candidates for antiviral therapy or not.

criminating between stages F0–1 and F2–4 F in HCV carriers with normal serum ALT (≤40 U/L). In the present study, the mean PLT count in F2 and F3 patients was 16.9 ± 5.3 ($\times 10^4/\mu L$) and 15.9 ± 4.6 ($\times 10^4/\mu L$), respectively. The distribution of the F stage was not significantly different between patients with PLT counts $\geq 15 \times 10^4/\mu L$ versus $< 15 \times 10^4/\mu L$ and $\geq 17 \times 10^4/\mu L$ versus $< 17 \times 10^4/\mu L$.

The SVR rate for genotype 1 patients with high viral load treated with either IFN monotherapy or IFN/Riba were 12.5% and 37.7%, respectively. In genotype 2 patients with high viral load, the SVR rate in the present study was better than the data of Japanese CH-C patients with elevated ALT levels in our previous paper.[6] It was not reasonable to compare the SVR rates between HCV carriers with normal serum ALT and CH-C with elevated ALT in the present study, because the total dosage of IFN and the duration of treatment were significantly different.

The annual incidence of HCC is correlated with the progression of liver fibrosis, that is, the stage of liver disease.[2–4,6] Sustained low serum ALT levels are also associated with a lower incidence of HCC.[2,6,41] PEG-IFN/Riba therapy is expensive and induces various side-effects. The present results indicate that most HCV carriers with normal serum ALT (≤40 U/L) and PLT counts ≥150 000/μL have minimal to mild liver damage, indicating a low risk for the progression to cirrhosis and the development of HCC. This was more remarkable in patients with ALT levels ≤30 U/L and PLT counts ≥150 000/μL. However, nearly half of the patients with PLT count <150 000/μL have F2 or F3 F stages, indicating a certain risk for the progression to cirrhosis and the development of HCC. Fibrosis

progression is associated with age, baseline and follow-up ALT levels, inflammatory activity and steatosis in the initial liver biopsy, and alcohol consumption.[42] The present results indicate that most HCV carriers with PNALT have a good prognosis and a low risk of developing HCC.

Liver biopsy is a useful procedure for identifying the stage of liver fibrosis; however, it is invasive and may sometimes cause complications.[43,44] The error rate of predicting the F stage with this procedure can be estimated to be as high as 20%.[45] Recently introduced biochemical markers, such as FibroTest,[46] and FibroScan,[47–49] are excellent procedures for identifying liver fibrosis stage in CH-C patients.[50] The combined use of FibroScan and FibroTest is useful for accurately estimating moderate to severe liver fibrosis in most patients with CH-C, but not in F0 and F1 patients.[51]

Recently, Alberti proposed an individualized management algorithm for HCV carriers with PNALT with or without liver biopsy in which HCV genotype, patient age, motivation to receive antiviral therapy, and factors influencing side-effects were included.[52] The algorithm using a combination of serum ALT levels and PLT counts in the present study is simple, but it is useful because it focuses mainly on the inhibition of the progression to cirrhosis and the development of HCC.

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Original Article

Evidence of oxidative stress as a cofactor in the development of insulin resistance in patients with chronic hepatitis C

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Aim: The mechanisms by which metabolic disorders develop in patients with chronic hepatitis C are unknown. Our study aimed to test whether oxidative stress contributes to these mechanisms.

Methods: The index of homeostasis model assessment–insulin resistance (HOMA–IR) and serum and hepatic levels of thioredoxin (Trx), which are markers of oxidative stress, were evaluated in 203 biopsy-proven chronic hepatitis C patients with hepatitis C virus (HCV) genotype 1 or 2 infection. HOMA–IR and Trx levels were compared with baseline values after phlebotomy in 23 patients.

Results: HOMA–IR and serum Trx levels were significantly correlated with disease stage (HOMA–IR, $P < 0.00001$; Trx, $P < 0.0001$) and independently predicted fibrosis scores (HOMA–IR, $P < 0.05$; Trx, $P < 0.005$). Steatosis (%) was significantly correlated with HOMA–IR ($P < 0.00005$) and Trx ($P < 0.001$) stage ($P < 0.00001$). Serum Trx levels were significantly correlated with HOMA–IR ($P < 0.05$), even after adjustment for body mass index ($P < 0.05$). Furthermore, the mRNA levels of hepatic Trx were significantly correlated with HOMA–IR ($P < 0.05$) and independently-predicted HOMA–IR ($P < 0.05$). The alanine aminotransferase ($P < 0.00001$), Trx ($P < 0.05$), and HOMA–IR ($P < 0.05$) serum levels decreased significantly after phlebotomy; these effects were similar even in non-responders to interferon.

Conclusion: Oxidative stress contributed to the development of IR irrespective of obesity in patients with HCV genotype 1 or 2 infection. This study could contribute to our understanding of how metabolic disorders develop and how they should be treated in chronic hepatitis C patients.

Key words: hepatitis C virus, insulin resistance, oxidative stress, steatosis, thioredoxin

INTRODUCTION

CHRONIC HEPATITIS C progresses to cirrhosis and eventually to hepatocellular carcinoma (HCC).[1] Although interferon (IFN)-based antiviral therapy has achieved great advances, it can not eradicate hepatitis C virus (HCV) in approximately 50% of patients infected with the genotype 1 strain,[1] which is highly prevalent in Japan. Therefore, other therapeutic strategies remain important, and efforts to understand the pathogenesis are required.

Metabolic disorders have recently been implicated in the pathogenesis of chronic hepatitis C.[2–6] HCV-infected patients with hepatic steatosis exhibit clinical features associated with metabolic syndromes,[3] and glucose intolerance is considered to represent an extra-hepatic manifestation of HCV infection.[2,4] Furthermore, grades of steatosis are reported to predict rapid fibrosis progression,[5] and diabetes increases the risk of HCC.[6] From these findings, insulin resistance (IR), a central cause of metabolic syndromes,[7] has been described as a risk factor in advanced staged chronic hepatitis C patients,[2,8] as seen in non-alcoholic steatohepatitis.[9] Thus, insulin signaling could be an important target for

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the management of patients with HCV infection; however, how IR develops is not well understood.

In this study, we focused on the role of oxidative stress, another key player in progressive liver injury in patients with chronic hepatitis C infection[10] in the development of IR. Because steatosis results in the overproduction of reactive oxygen species (ROS),[11] and ROS may exacerbate hepatic insulin sensitivity,[12] we hypothesized a close relationship between IR and oxidative stress. Therefore, we retrospectively analyzed the index of IR[13] and the serum and hepatic levels of thioredoxin (Trx), which are markers of oxidative stress,[14] in 203 patients with HCV infection. We also investigated whether relieving hepatic oxidative stress could improve IR among these patients.

METHODS

Patients

CHRONIC HEPATITIS PATIENTS who underwent liver biopsies in our institute between April 2003 and March 2006 were selected according to the following criteria: no excessive alcohol intake (more than 40 g/week), as assessed by interview (at least on 3 occasions); positive serum HCV-RNA, as confirmed by reverse transcription–polymerase chain reaction (RT–PCR); infection with HCV genotype 1 (1a, 1b) or 2 (2a, 2b); no history of antiviral therapy nor treatment with steatosis-inducing drugs within the 12 months before the study; negativity for hepatitis B surface antigen or antibodies to HIV; and an absence of other forms of chronic liver disease. Anthropometry and laboratory data were collected from all patients at the time of the liver biopsy. The serum HCV-RNA level was determined using the AMPLICOR GT HCV Monitor (Roche Diagnostic Systems, Tokyo, Japan). HCV genotypes 1 and 2 were determined by a serologic genotyping assay.[15] Serogroups 1 and 2 in this assay correspond to genotypes 1 (1a, 1b) and 2 (2a, 2b). Informed written consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki approved by the Ethics Committee of the Kyoto Prefectural University of Medicine.

Laboratory determination

After a 12-h overnight fast, venous blood samples were drawn to determine alanine aminotransferase (ALT), γ-glutamylcysteine transpeptidases, fasting plasma glucose (FPG), insulin (IRI), triglyceride, and ferritin levels. These parameters were measured using standard techniques from clinical chemistry laboratories. The index of IR was calculated only in patients without overt diabetes (FPG >126 mg/dL), according to the homeostasis model assessment (HOMA).[13] The formula for IR was as follows: HOMA–IR = FPG (mg/dL) × IRI (μU/mL)/405. HOMA–IR was only calculated in patients without diabetes ($n = 189$).

Measurement of Trx

The levels of serum Trx were used as a marker of hepatic oxidative stress, as reported previously.[14] For the measurements of Trx concentrations, serum and liver biopsy specimens were stored at −80°C until use. The serum Trx levels were then measured using a commercial, sensitive enzyme-linked immunosorbent assay kit (REDOX BIOSCIENCE, Kyoto, Japan), as described previously.[14] All measurements were made in duplicate and average values were used for the statistical analysis.

The hepatic levels of Trx were measured by real-time PCR. Total RNA was isolated from biopsy specimens using the RNeasy kit (Qiagen, Hilden, Germany). The PCR mixture contained first-strand cDNA and specific primers for human Trx: sense, 5′-CTGCTTTTCAG GAAGCCTTG-3′ and antisense, 5′-ACCCACCTTTTGT CCCTTCT-3′. PCR was performed using the Light Cycler 2.0 System (Roche, Mannheim, Germany), and the mRNA levels of Trx were normalized to those of β-actin.

Histological evaluation

Formalin-fixed and paraffin-embedded liver biopsy specimens were stained with hematoxylin–eosin, Masson's trichrome, and Perl's Prussian blue. Degrees of hepatic fibrosis (stage) were scored as follows: F0 = none, F1 = portal expansion, F2 = bridging fibrosis, F3 = bridging fibrosis with lobular distortion, and F4 = cirrhosis. Degrees of inflammation (grade) were scored as follows: A0 = none, A1 = mild, A2 = moderate, and A3 = severe. Steatosis was assessed according to the percentage of hepatocytes containing fat droplets. The degree of iron loading was graded using a Perl's score of 0–4, as described previously.[16]

Phlebotomy

Phlebotomy was initiated to relieve iron-induced oxidative stress in 23 patients. All patients showed elevated serum ferritin levels and/or persistent abnormal ALT levels, and none showed anemia (hemoglobin <11.0 g/dL). They underwent phlebotomy (300–400 mL) either biweekly or monthly until serum ferritin levels were <20 ng/mL. Thereafter, the serum Trx levels and HOMA–IR were compared with baseline values in each

individual. However, treatments were terminated irrespective of serum ferritin levels when blood hemoglobin concentrations decreased to less than 10 g/dL.

Statistical analysis

The relationships between variables were analyzed using the Spearman's correlation coefficient by rank, and a partial correlation coefficient was calculated to remove the influence of confounding variables. Values after phlebotomy were compared with baseline values using a Wilcoxon rank sum test. All analyses were performed using SPSS software for Windows, version 14.0 (SPSS, Chicago, IL, USA). A *P*-value of less than 0.05 was considered significant.

RESULTS

General characteristics of and histological findings in patients

OF THE 309 HCV-infected patients who underwent liver biopsies, 203 patients met the criteria. Because many excessive drinkers among the male patients were excluded from the analysis, the number of females exceeded that of males in the study population. A summary of the clinical data for the liver biopsy findings in these patients is shown in Tables 1 and 2. Of the 203 patients that qualified, body mass index (BMI) was greater than 25 (kg/m^2) in 57 patients (28%), and 124 patients (61%) had a varying degree of hepatic steatosis, as shown in Table 2. Iron staining was performed in only 198 patients; a varying degree of iron loading was observed in 71 patients. Fourteen patients (7%) suffered from type 2 diabetes mellitus. The fibrosis scores of these patients were F1 in two patients, F2 in six, F3 in five, and F4 in only one. Seventy patients had received IFN-based antiviral therapy before the study and this treatment had failed to eradicate HCV.

Table 1 Baseline characteristics of patients

	Mean values of clinical data
Age	56.0 ± 11.9
Male/Female	73/146
BMI (kg/m^2)	22.9 ± 3.1
IFN: yes/no	70/133
ALT (IU/L)	75.5 ± 59.3
γ-GTP (U/L)	56.0 ± 51.0
FPG (mg/dl)	96.8 ± 13.1
HOMA-IR	2.3 ± 1.4
Ferritin (ng/ml)	174.2 ± 161.0
TG (mg/dl)	99.5 ± 50.5
Plt (×10^4/ml)	17.4 ± 5.3
HCV-RNA (KIU/ml)	1516 ± 1484.7
Serogroup 1/2	162/41
Trx (ng/ml)	30.4 ± 15.4

Data are expressed as mean ± standard deviation.
ALT, alanine aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; γ-GTP, γ-glutamylcysteine transpeptidases; HCV, hepatitis C virus; HOMA-IR, homeostasis model assessment-insulin resistance; IFN, interferon; Plt, platelet; TG, triglyceride; Trx, thioredoxin.

Table 2 Histological findings on liver biopsy

	No. patients
F0/F1/F2/F3/F4	3/72/71/51/6
A0/A1/A2/A3	2/79/89/33
Steatosis:	
None	79
<10%	54
<30%	53
<60%	17
Iron load:	
Grade 0/1/2/3	127/33/25/13

Data are expressed as number of patients.

Predictors of the fibrosis score

The stage was significantly correlated with age, BMI, grade, grades of steatosis, iron score, ALT levels, platelet counts, ferritin levels, HOMA-IR, and serum Trx levels (Table 3). In a multiple regression analysis, grade, HOMA-IR, and serum Trx levels were shown to be

Table 3 Variables correlated with fibrosis scores

	Coefficient	Univariate	Multivariate
Age	$r = 0.163$	$P = 0.019$	$P = 0.931$
BMI	$r = 0.199$	$P = 0.004$	$P = 0.920$
Grade	$r = 0.869$	$P < 0.00001$	$P < 0.00001$
Steatosis	$r = 0.412$	$P < 0.00001$	$P = 0.761$
Iron score	$r = 0.155$	$P = 0.030$	$P = 0.437$
ALT	$r = 0.416$	$P < 0.00001$	$P = 0.259$
Plt	$r = -0.376$	$P < 0.00001$	$P = 0.119$
Ferritin	$r = 0.189$	$P = 0.010$	$P = 0.227$
HOMA-IR	$r = 0.406$	$P < 0.00001$	$P = 0.043$
Trx	$r = 0.365$	$P = 0.00006$	$P = 0.003$

Multiple regression analysis was used to analyze variables independently correlated with fibrosis scores.
ALT, alanine aminotransferase; BMI, body mass index; HOMA-IR, homeostasis model assessment-insulin resistance; Plt, platelet; Trx, thioredoxin.

independently correlated with stage (Table 3). Although the grade of steatosis is reported to predict rapid fibrosis progression,[5] it was not an independent variable in the multivariate analysis. Considering that IR is a major cause of hepatic steatosis,[11] HOMA-IR should be more significant than steatosis in this model.

Relationship between grades of steatosis and HOMA-IR or serum Trx levels

Steatosis has been considered to independently contribute to the progression of fibrosis in patients with chronic hepatitis C.[5] Therefore, we focused on the relationships between steatosis and either IR or oxidative stress. We found that grades of steatosis were significantly correlated not only with HOMA-IR, but also with serum Trx levels (HOMA-IR; $r = 0.344$, $P = 0.00002$: Trx; $r = 0.3$, $P < 0.001$). These findings suggested that oxidative stress could have a significant role in fibrosis progression through steatogenesis. We then focused on the relationship between IR and oxidative stress.

Relationship between HOMA-IR and serum Trx levels

HOMA-IR was significantly correlated with serum Trx levels (Fig. 1a: $r = 0.262$, $P = 0.012$) and BMI (Fig. 1b: $r = 0.302$, $P = 0.0002$). After adjustment for the effect of each variable using a corrected correlation coefficient, a significant relationship with HOMA-IR still remained for both serum Trx levels ($r = 0.244$, $P = 0.02$) and BMI ($r = 0.284$, $P = 0.006$). These results indicated that IR was attributable to oxidative stress, irrespective of obesity.

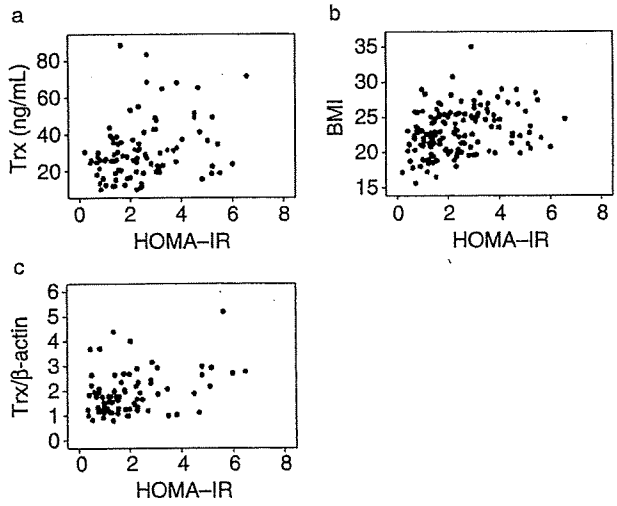

Figure 1 Correlation between homeostasis model assessment-insulin resistance (HOMA-IR) and serum levels of thioredoxin (Trx) (a), body mass index (BMI) (b), and mRNA levels of Trx (c). Both serum Trx levels and BMI were significantly correlated with HOMA-IR (serum Trx levels; $r = 0.262$, $P = 0.012$: BMI; $r = 0.302$, $P = 0.0002$). HOMA-IR was also significantly correlated with hepatic Trx levels ($r = 0.273$, $P = 0.014$).

Table 4 Factors correlated with HOMA-IR in subgroup patients ($n = 101$)

	Coefficient	Univariate	Multivariate
Hepatic Trx	$r = 0.273$	$P = 0.014$	$P = 0.011$
Grade	$r = 0.233$	$P = 0.038$	$P = 0.170$
Steatosis	$r = 0.286$	$P = 0.010$	$P = 0.251$
ALT	$r = 0.287$	$r = 0.010$	$r = 0.517$

Multiple regression analysis was used to analyze variables independently correlated with HOMA-IR.
ALT, alanine aminotransferase; HOMA-IR, homeostasis model assessment-insulin resistance; Trx, thioredoxin.

Relationship between HOMA-IR and hepatic Trx levels

Since Trx is known to be ubiquitously expressed,[17] we compared the mRNA levels of hepatic Trx with HOMA-IR in 101 patients whose liver biopsy specimens were available. The mRNA levels of Trx were significantly correlated with HOMA-IR (Fig. 1c: $r = 0.273$, $P = 0.014$). Among these patients, HOMA-IR also significantly correlated with grade, steatosis, and ALT levels (Table 4). In a multiple regression analysis, only the level of hepatic Trx was independently correlated with HOMA-IR (Table 4).

Effects of phlebotomy on ALT and serum Trx levels and HOMA-IR

All patients completed treatment without a significant change in body weight (age; 60.8 ± 10.8 kg, male/female; 15/8, BMI; 25.3 ± 2.6 kg/m^2, F0/F1/F2/F3/F4; 3/8/8/4, serogroup 1/2; 20/3). Nine patients had experienced IFN therapy before phlebotomy, whereas 14 patients had not experienced IFN therapy because of either old age or personal reasons. Changes in the serum levels of ALT, Trx, ferritin, HOMA-IR in the 23 patients that received phlebotomy are summarized in Table 5. Overall, the serum levels of ALT, Trx, and HOMA-IR were significantly decreased after phlebotomy compared with baseline values ($P < 0.00001$, $P = 0.023$, $P = 0.022$, respectively). These results indicated the efficacy of phlebotomy on insulin sensitivity as well as on liver function

Table 5 Changes in ALT, Trx, ferritin, and HOMA-IR after phlebotomy ($n = 23$)

	Before	After	Difference
BMI (kg/m^2)	23.6 (19.1–29.4)	24.0 (19.1–29.4)	NS
AST (IU/L)	67.0 (21–527)	51.0 (32–129)	$P < 0.005$
ALT (IU/L)	42.5 (27–121)	29.5 (24–53)	$P < 0.00001$
γ-GTP (IU/L)	89.0 (29–287)	60.5 (23–218)	$P < 0.0005$
Trx (ng/ml)	36.1 (20.2–79.4)	26.7 (18.1–32.7)	$P = 0.023$
Ferritin (ng/ml)	409.5 (125–1028)	20 (20–53)	$P < 0.00001$
HOMA-IR	3.5 (0.9–4.6)	2.4 (0.8–3.7)	$P = 0.022$

Data are expressed as medians (±range), Wilcoxon signed-ranks test.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γ-GTP, glutamylcysteine transpeptidases; HOMA-IR, homeostasis model assessment-insulin resistance; Trx, thioredoxin.

tests in chronic hepatitis C patients. Furthermore, we analyzed the effects of phlebotomy on HOMA-IR in patients with a history of past IFN therapy and found that there were significant decreases in HOMA-IR (from 4.2 [3.7–4.6] to 2.9 [2.3–3.7], $P = 0.043$).

DISCUSSION

THE PRESENT STUDY shows that oxidative stress is an independent factor in the development of IR in patients with chronic hepatitis C, and validates the beneficial effect of phlebotomy on insulin sensitivity. To our knowledge, our report is the first to show a direct relationship between IR and oxidative stress in patients with HCV. We excluded alcohol drinkers, patients treated with steatosis-inducing drugs, and patients infected with HCV genotype 3a[18] from our analysis, as these are confounding factors affecting steatosis.

In general, the development of IR and steatosis is due to host-associated factors (e.g. obesity). The molecular mechanism underlying IR involves dysregulation of insulin-stimulated tyrosine phosphorylation of insulin receptor substrates (IRS).[19] This is achieved by phosphorylation of serine/threonine residues in IRS by either increased or decreased levels of adipokines associated with obesity (such as tumor necrosis factor [TNF]-α and adiponectin), thereby inhibiting tyrosine phosphorylation.[19] However, a high prevalence (61%) of steatosis, despite a low prevalence (28%) of obesity (BMI >25 kg/m^2) or diabetes (7%), indicated that there are mechanisms regulating insulin sensitivity other than obesity. In our study, HOMA-IR was significantly correlated with serum Trx levels, independent of BMI. Furthermore, the hepatic Trx levels independently predicted HOMA-IR in subgroup patients. Thus, hepatic oxidative stress directly contributes to IR in chronic hepatitis C patients.

Our hypothesis is supported by the following findings. First, chronic hepatitis C is characterized by oxidative stress-induced liver injury.[10,14,20] The overproduction of ROS could result from inflammatory cells,[10] iron overload,[20] and presumably the direct association of HCV core protein with mitochondria in hepatocytes.[21] In addition, steatosis, a prominent feature of chronic hepatitis C,[2–5] could result in oxidative stress.[11] Second, the increased abundance of ROS inhibits tyrosine phosphorylation of IRS in hepatocytes via the activation of stress-sensitive pathways, such as the c-Jun N-terminal kinase (JNK)[12] and nuclear factor (NF)-κB[22] pathways. JNK directly phosphorylates serine/threonine residues in IRS,[12] while NF-κB inhibits tyrosine phosphorylation via the induction of TNF-α.[22] The failure of hepatic insulin signaling subsequently leads to systemic IR.[12]

The question arising from this correlation between IR and oxidative stress is how metabolic disorders and liver injury can develop simultaneously in patients with HCV infection. One possible mechanism could be an interaction between IR and oxidative stress. IR results in hepatic steatosis,[11] which leads to increased ROS production concomitant with an increase in the number of inflammatory cells[10] and/or iron overload.[23] Conversely, ROS could exacerbate insulin sensitivity to promote steatosis,[11,12] and could promote the recruitment of inflammatory cells and fibrosis through lipid peroxidation products.[24,25] Thus, IR, steatosis, and oxidative stress could be involved in a feedback loop that exacerbates liver injury. This hypothesis is supported by the findings that HOMA-IR was significantly correlated with the serum and hepatic Trx levels, and both the HOMA-IR and serum Trx levels were significantly correlated with grades of steatosis.

Finally, we employed phlebotomy to validate the interaction between IR and oxidative stress, because

phlebotomy is useful for reducing hepatic oxidative stress.[20] Although phlebotomy is known to improve liver function tests in patients with HCV infection, its efficacy on insulin metabolism has not been well documented. Therefore, our findings provide new insight into the efficacy of phlebotomy. Notably, phlebotomy significantly improved HOMA–IR, even in patients who had been refractory to IFN. However, the long-term outcome of phlebotomy was unclear in this study, and a follow-up study should be performed.

In conclusion, we demonstrated an association between oxidative stress and IR in patients infected with HCV genotype 1 or 2. Our findings will contribute to our understanding of how metabolic disorders can develop in patients with chronic hepatitis C. Antioxidative therapy is a promising treatment to improve the pathogenesis of HCV.

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Original Article

Early decline of hemoglobin can predict progression of hemolytic anemia during pegylated interferon and ribavirin combination therapy in patients with chronic hepatitis C

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Aim: Ribavirin, used to treat chronic hepatitis C, can induce hemolytic anemia, forcing the discontinuance of treatment. To establish a predictive measure to help circumvent this, we evaluated the relationship of hemoglobin (Hb) decline with the discontinuance of treatment during the progression of ribavirin-induced anemia.

Methods: One hundred and sixteen patients (71% male) with genotype 1 chronic hepatitis C were treated with pegylated interferon (PegIFN) α-2b and ribavirin. The mean age was 50.6 years and 55% were IFN naïve. A decline of Hb concentration by 2 g/dL at two weeks from the start of the treatment ("2 by 2" standard) was adopted as the predictive factor for the progression of anemia.

Results: By applying the "2 by 2" standard, with $\Delta Hb \geq 2$ g/dL (34%, $n = 39$), treatment was discontinued in 12 cases (31%), three of which (8%) because of severe anemia. For $\Delta Hb < 2$ g/dL (64%, $n = 76$), treatment was discontinued in 11 (14%) cases; none due to severe anemia. Ten percent (4/39) of patients showed the minimum $Hb \leq 8.5$ g/dL in the $\Delta Hb \geq 2$ g/dL group, with none in the $\Delta Hb < 2$ g/dL group ($P = 0.001$). Furthermore, the patients with minimum $Hb \leq 8.5$ g/dL were found only in the "2 by 2" standard-positive and low CL/F (<15) group (4/29, 14%).

Conclusion: Monitoring the Hb decline using the "2 by 2" standard can identify patients who are prone to developing severe anemia. Further prospective studies are needed using ribavirin reduction based on the "2 by 2" standard.

Key words: "2 by 2" standard, chronic hepatitis C, pegylated interferon and ribavirin combination therapy, progression of anemia

INTRODUCTION

THE AIM OF antiviral therapy for hepatitis C virus (HCV) is to obtain a sustained viral response (SVR) and to reduce the occurrence rate of hepatocellular carcinoma or hepatic disease-related mortality.[1,2] The current optimal therapy for patients with chronic hepatitis C is a combination of pegylated interferon (PegIFN) and ribavirin. This combination can significantly improve the SVR rate and is recommended as a standard regimen worldwide.[3–8] However, the SVR rates for the combination therapy of ribavirin with PegIFN for naïve patients with HCV genotype 1 has been reported to be 42–52%,[6,9,10] which means that eradication of HCV is not complete in approximately half of these patients. Recently, long-term treatment[11] and a higher dosage

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of drugs[12,13] have been used to try to raise the SVR rate for patients with HCV genotype 1. However, it remains to be established what constitutes satisfactory efficacy. In this study we focused on a treatment strategy to enable the prediction of severe side-effects in order to avoid the need to discontinue treatment and raise the SVR rate by PegIFN and ribavirin combination therapy. It is important that ribavirin, the key drug for eradicating HCV, is continued until the end of treatment in order to attain the maximum SVR rate. Hemolytic anemia induced by ribavirin is known as one of the most important adverse effects in the combination therapy of PegIFN and ribavirin.[14–17] To decrease the discontinuance rate of ribavirin due to severe anemia, epoetin alfa has been used for patients with progressing anemia, which can maintain the dose level of ribavirin as well as the quality of life of the patients.[18–20] However, from a cost-effectiveness standpoint, it would be difficult for this treatment strategy to become standard. Also, side-effects other than anemia arising from an overload of ribavirin mainly due to renal dysfunction cannot be avoided by the additional administration of epoetin alfa.

Hemolysis induced by ribavirin has been suggested to be related to a high plasma concentration of ribavirin.[21] The apparent clearance of ribavirin (CL/F), which reflects its plasma concentration at four weeks after the start of combination therapy, has been used as a predictive factor for ribavirin-induced hemolytic anemia before the start of treatment.[22–24] However, the progression of hemolytic anemia occurs due not only to hemolysis, but also impaired hematogenous function. On the other hand, hemoglobin (Hb) dynamics directly reflect the degree of progression of anemia. We have reported that the early decline of Hb correlates with the progression of anemia during IFN and ribavirin combination therapy.[25] It is necessary to verify that a similar early predictor for the progression of anemia can be adopted in PegIFN and ribavirin combination therapy, since PegIFN is known to induce less depression of bone marrow function than usual IFN.

In this study, we evaluated the utility of the early decline of Hb in comparison with the CL/F to predict the progression of anemia in the combination therapy of PegIFN and ribavirin.

METHODS

Patients

THIS STUDY WAS conducted at 12 institutions in Japan. A total of 116 patients with chronic hepatitis C were enrolled and treated with a combination of PegIFN and ribavirin. All patients were anti-hepatitis C virus antibody positive, had HCV-RNA detectable in their serum by the polymerase chain reaction (PCR) method, and showed elevated serum alanine transaminase (ALT) (above the upper limit of the normal), serum Hb concentration ≥12 g/dL, neutrocytes ≥1500/mm^3 and platelets ≥10^5/mm^3 within six months before the treatment. Exclusion criteria were the presence of hepatitis B surface antigen, antihuman immunodeficiency virus antibody and other forms of liver disease (alcoholic liver disease, hepatotoxic drugs, autoimmune hepatitis).

Table 1 Patient characteristics

Age (years)	50.6 ± 10.1 (24–70)
Gender (male/female)	82/34 (male 70.7%)
Body weight (kg)	64.5 ± 11.1
Previous IFN therapy (naïve/relapser/no responder)	64/38/14
HCV-RNA level (KIU/L) (<500/500–850/850<)	18/27/71
ALT (IU/L)	110 ± 60 (33–76)
Crnn (mg/dL)	0.9 ± 0.2
Liver histology	
Fibrosis (F1/F2/F3/unknown)	35/49/31/1
Activity (A1/A2/A3/A4)	15/33/56/12
WBC (/mm^3)	5317 ± 1207
Neutrocytes (/mm^3)	2778 ± 902
Platelets (×10^4/mm^3)	17.4 ± 4.0
RBC (×10^4/mm^3)	459 ± 41
Hemoglobin (g/dL)	14.5 ± 1.2

Data are given as the mean ± SD.
ALT, alanine transaminase; RBC, red blood cells; WBC, white blood cells.

The baseline characteristics of the patients are shown in Table 1. The mean age was 50.6 ± 10.1 years, and 71% (82 patients) were male. All patients had HCV-RNA with genotype 1 and high viral loads (more than 10^5 copies/mL serum by Amplicor-HCV monitor assay). The mean ALT level was 110 ± 60 IU/L. Sixty-four patients (55%) were IFN naïve and the others were undergoing retreatment.

Treatment schedule

All patients were treated with a combination of PegIFN α-2b (Pegintron; Schering-Plough, Kenilworth, NJ, USA) and ribavirin (Rebetol; Schering-Plough) for 48 weeks. PegIFN was administered at a mean of 1.5 μg/kg body weight subcutaneously once a week. Ribavirin was given orally twice a day for the total dose. Dosages of both medications were decided based on the

body weight of the patients: those with a body weight of 40–60 kilograms (kg) were given PegIFN 75 μg/body and ribavirin 600 mg/day, those with a body weight of 60–80 kg were given PegIFN 105 μg/body and ribavirin 800 mg/day, and those with a body weight of 80–100 kg were given PegIFN 135 μg/body and ribavirin 1000 mg/day. The PegIFN dose was reduced by 50% if the neutrocyte count was below 750/mm^3 or the platelet (Plt) count was below 8×10^4/mm^3. The PegIFN was discontinued if the neutrocyte count was below 500/mm^3 or the Plt count was below 5.0×10^4/mm^3. The ribavirin dose of 200 mg was reduced when the Hb concentration decreased to less than 10 g/dL and the ribavirin was discontinued when the Hb concentration decreased to less than 8.5 g/dL, in accordance with the drug information for ribavirin. No ferric medicine or erythropoietin to prevent anemia was administered.

Patients with persistently undetectable HCV-RNA six-months after the end of treatment were considered to have achieved SVR.

Blood tests

All patients were examined for serum HCV-RNA level, hematological and biochemical tests just before therapy, at the end of week 2 and every four weeks during the treatment. When the treatment was completed, the patients were assessed every four weeks up to 24 weeks after the end of treatment.

Total ribavirin clearance

Using the method of Kamar *et al.*, CL/F at the start of the treatment was calculated as follows: CL/F (L/h) = 32.3 × BW × (1 − 0.0094 × age) × (1 − 0.42 × sex)/Scr (BW, body weight; sex = 0 for male and 1 for female; Scr = serum creatinine).[17]

Definition of "severe anemia" leading to the discontinuance of ribavirin

In this study, the "discontinuance of ribavirin due to severe anemia" was defined as follows: discontinuance of ribavirin due to a decrease of Hb to less than 8.5 g/dL or clinical symptoms of anemia associated with a decrease of Hb of more than 3 g/dL from the start of the combination therapy.

Statistical analysis

Age, body weight, ribavirin dosage/body weight, white blood cell count, red blood cell count, Hb concentration, Plt, serum ALT levels and serum creatinine are expressed as mean ± SD. The SVR rate was evaluated using the intention-to-treat analysis (ITT analysis). The differences in proportions were tested by the χ^2-test and Mantel–Haenszel χ^2-test. A value of $P < 0.05$ (two-tailed) was considered to indicate significance. All calculations were performed by SAS program 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Frequency and reasons for dose reduction or discontinuance of PegIFN and/or ribavirin

OF THE 116 patients, 92 completed 48 weeks of therapy, but 24 patients (21%) had to discontinue both PegIFN and ribavirin. Thirty-nine patients (34%) completed the entire treatment schedule without reduction or discontinuance of either drug. The ribavirin dose was decreased for 39 patients (34%) and the PegIFN dose was decreased for 33 patients (28%), including 19 patients for whom both drugs had to be reduced. The reasons for discontinuance of both drugs included anemia, thyroid dysfunction, skin eruption and neutropenia, with the major reasons being anemia (17%) and thyroid dysfunction (17%).

Efficacy of the combination therapy with dose reduction or discontinuance of PegIFN and/or ribavirin

The SVR rate was 57% (66/116) for all according to ITT analysis. According to the category of response to previous IFN therapy, the SVR rates were 43% (6/14) in

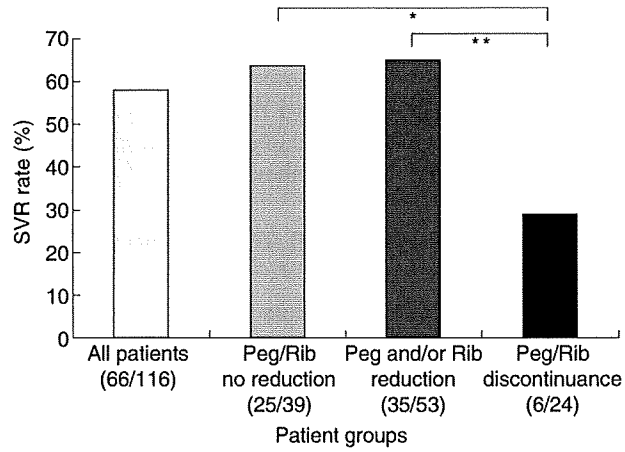

Figure 1 SVR rate due to PegIFN/ribavirin dose reduction or discontinuance. (□), All patients; (▨), patients without dose reduction; (▩), patients with dose reduction; (■), patients with drug discontinuance. Significant levels: *$P = 0.003$; **$P = 0.001$.