

Table 4 FibroScan® values subgrouped by clinical variable in patients with chronic HBV and HCV infection

Variable		Chronic HBV infection			Chronic HCV infection		
		n	Mean ± SE (kPa)	P-value	n	Mean ± SE (kPa)	P-value
Age (years)	≥60	12	8.6 ± 1.5	0.4216	72	11.8 ± 1.3	0.0057
	<60	56	7.4 ± 0.6		89	8.0 ± 1.0	
Alanine aminotransferase (IU/L)	≥45	12	11.4 ± 0.6	0.0016	52	15.8 ± 1.6	<0.0001
	<45	56	6.8 ± 1.6		109	6.8 ± 1.6	
Aspartate aminotransferase (AST; IU/L)	≥45	15	10.1 ± 1.6	0.0187	56	13.1 ± 1.5	0.0017
	<45	53	6.9 ± 0.6		105	7.4 ± 0.5	
γ-Glutamyltranspeptidase (IU/L)	≥50	19	12.0 ± 1.2	<0.0001	50	13.4 ± 1.5	0.002
	<50	49	6.0 ± 0.4		110	8.7 ± 0.7	
Total bilirubin (mg/dL)	≥2.0	2	10.7 ± 6.9	0.35	4	31.2 ± 9.9	<0.0001
	<2.0	66	7.5 ± 0.6		157	9.5 ± 0.6	
Platelet count (PLT; 10 <sup>9</sup> /L)	<100	9	14.5 ± 2.0	0.0008	40	16.3 ± 2.0	<0.0001
	100–150	17	8.3 ± 1.1		58	10.0 ± 1.0	
	>150	42	5.9 ± 0.4		63	6.3 ± 0.4	
AST-to-PLT ratio index†	≥1.5	2	17.4 ± 8.3	<0.0001	21	22.2 ± 3.2	<0.0001
	<1.5	66	7.2 ± 0.5		140	8.6 ± 0.5	
Prothrombin time (%)	<80	22	10.3 ± 1.2	0.011	43	15.9 ± 1.9	<0.0001
	≥80	32	6.9 ± 0.8		91	7.6 ± 0.4	
Albumin (g/dL)	≥3.5	2	17.6 ± 8.1	0.0018	9	29.9 ± 5.0	<0.0001
	>3.5	66	7.3 ± 0.5		152	9.0 ± 0.6	
Serum hyaluronic acid (ng/mL)	≥130	7	15.1 ± 2.8	<0.0001	36	16.6 ± 1.7	<0.0001
	<130	39	7.4 ± 0.6		84	7.7 ± 0.6	
Serum type IV collagen (ng/mL)	≥250	4	14.5 ± 4.1	0.001	22	21.6 ± 2.6	<0.0001
	<250	45	7.7 ± 0.7		108	7.9 ± 0.5	
Serum HBV-DNA level (log copies/mL)‡	≥4.0	39	8.2 ± 0.8	0.2638			
	<4.0	29	6.9 ± 0.8				
Serum HCV-RNA level (kIU/mL)	≥2000				99	10.8 ± 0.8	0.376
	<2000				53	9.5 ± 1.3	

†Calculated as AST (/upper limit of normal range) × 100/PLT (10<sup>9</sup>/L).

‡Logarithmic transformed copies/mL.

Data are shown as mean ± standard error (SE).

HBV, hepatitis B virus; HCV, hepatitis C virus.

## DISCUSSION

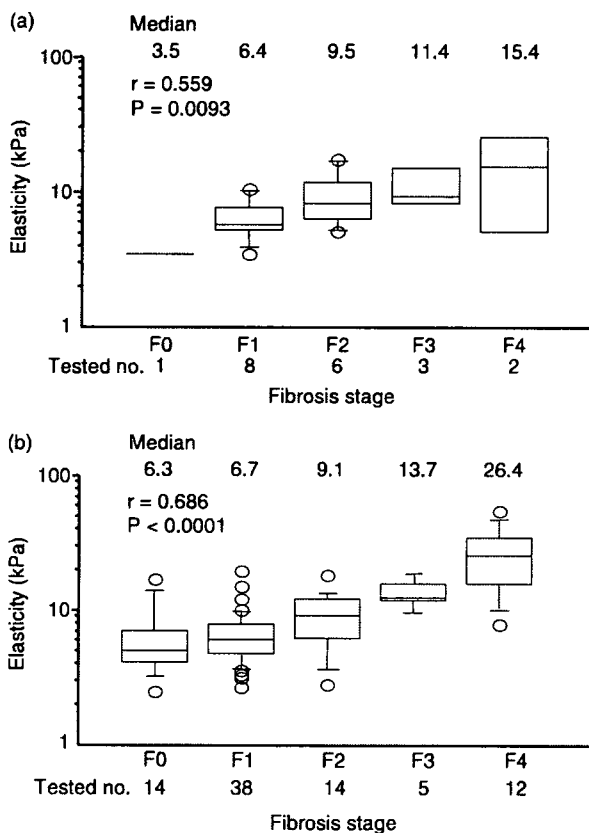
THE PRESENT STUDY provided data about the reliability of FibroScan for the quantitative assessment of liver fibrosis with chronic HBV and HCV infection. The findings showed a good correlation between the fibrosis stage assessed by histopathological examination of a liver biopsy sample and the elasticity measurements performed using the FibroScan.

Until now, needle liver biopsy has been used as the 'gold standard' for the assessment of liver fibrosis.<sup>23</sup> However, liver biopsy is an invasive procedure that may cause pain, bleeding, pneumothorax, hemothorax, bile peritonitis, hemobilia, puncture of the kidney and intestine, infection, anxiety, and even death.<sup>6,24,25</sup> These risks

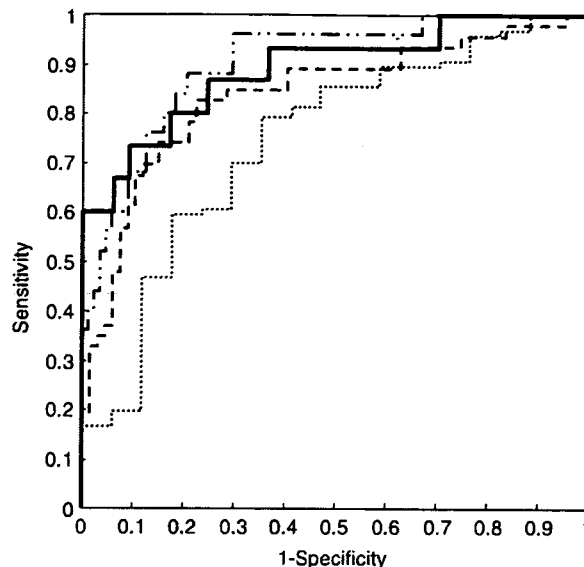
are not deemed to be entirely avoidable and the risk of complications is increased in the presence of cirrhosis, even if liver biopsy is done by highly skilled persons. Moreover, a biopsy specimen represents at most 1/50 000 of the total liver mass<sup>6</sup> and distribution of fibrosis in the liver parenchyma is heterogeneous, which may result in some sampling error.<sup>7–9</sup> Regev *et al.* showed that 33% of 124 patients had at least one fibrosis stage of difference between the right and left lobes of the liver,<sup>7</sup> and Siddique *et al.* showed a difference 45% of at least one fibrosis stage between two specimens obtained at the same puncture site.<sup>26</sup> Conversely, FibroScan measures liver stiffness in a volume that is a cylinder of approximately 1 cm in diameter and 4 cm long, between 25 and 45 mm below the skin's surface,

an area at least 100 times bigger than the biopsy specimen, which makes it more representative of the hepatic parenchyma.

Several serum markers of hepatic fibrosis have been proposed as an alternative to liver biopsy for the evaluation of fibrosis in chronic hepatitis. Hyaluronic acid, type IV collagen, procollagen III peptide, and laminin, which are extracellular matrix markers of fibrosis, are the most studied in serum.<sup>27,28</sup> However, fibrosis is not specific to the liver and an autoimmune disease, impaired metabolism, or lung fibrosis could influence the blood levels of these markers. Some standard indicators (AST/ALT ratio, prothrombin time, platelets, AST-to-PLT ratio index) have long been recognized as indirect markers of



**Figure 2** FibroScan values for each fibrosis stage by (a) chronic hepatitis B virus and (b) hepatitis C virus infection status. The vertical axis is a logarithmic scale. The top and bottom of the boxes are the first and third quartiles. The length of the box represents the interquartile range within which are located 50% of the values. The lines through the middle of the boxes represent the median. The error bars are the minimum and maximum values.



**Figure 3** Receiver operator characteristic curves for liver stiffness measurements for different fibrosis thresholds. Mild fibrosis or more ( $\geq F1$ ), moderate fibrosis or more ( $\geq F2$ ), severe fibrosis or more ( $\geq F3$ ), cirrhosis (F4). (—),  $\geq F4$  (0.89); (---),  $\geq F3$  (0.90); (- -),  $\geq F2$  (0.84); (· · · · ·),  $\geq F1$  (0.74).

extensive fibrosis.<sup>16,29-31</sup> For several years, scores have been calculated with algorithms that combine several indicators determined simultaneously to assess fibrosis in patients with chronic HCV infection and sometimes other chronic liver diseases. Of these, the Fibrotest is the most widely used validated predictive index.<sup>15,32</sup> In the study by Halfon *et al.* with liver stiffness measurements using FibroScan, the area under the ROC curve for the diagnosis of severe fibrosis was 0.90.<sup>32</sup> Our data were consistent with their results. Major advantages of FibroScan as compared with fibrosis markers and biochemical scores are that it directly measures a quantitative assessment of the liver and there is no interference from extrahepatic disorders. The FibroScan was confirmed to be a better method than liver biopsy for assessing liver fibrosis in Japanese patients with chronic HBV or HCV infection.

Ultrasonographic quantitative description of the liver surface and parenchyma was related with the severity of fibrosis. Shen *et al.* reported that among the quantitative ultrasonographic parameters, the cut-off value of spleen length (12.1 cm) had a sensitivity of 0.600 and a specificity of 0.753 and that the portal vein (12 mm) had a diagnostic sensitivity of 0.767 and a diagnostic specificity of 0.446 for the diagnosis of liver cirrhosis.<sup>33</sup> With

liver stiffness measurements by FibroScan, there is no intra- or interobserver variability and the technique is reproducible and independent of the operator.<sup>13</sup>

The limitations to FibroScan should also be considered. It cannot be used for patients with ascites. The presence of ascites indicates cirrhosis and the elastic waves do not propagate through liquids. FibroScan is also more usable for patients with narrow intercostal spaces or with severe obesity, although probes of smaller size are currently available for the former. In obese patients, the fatty thoracic belt attenuates both elastic waves and ultrasound. Specific probes are also being developed for obese patients. In the case of fatty liver, fatty tissues are softer than healthy liver parenchyma, so they would be expected to induce a decrease of liver elasticity.<sup>13</sup>

In conclusion, liver stiffness measurement with FibroScan is a good method for the diagnosis of fibrosis. Liver stiffness measurement may be the most accurate method in current use for assessing advanced fibrosis or cirrhosis. FibroScan appears to be a simple and reliable, non-invasive method for evaluating fibrosis and should be a promising device for assessing the prognosis of patients with chronic HBV and HCV infection who undergo antiviral treatment.

## ACKNOWLEDGMENT

THIS STUDY WAS supported by a grant from the 21st Century COE program of the Japanese Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan.

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## Original Article

## High molecular weight form of adiponectin levels of Japanese patients with chronic hepatitis C virus infection

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**Aim:** The aim of the present study was to clarify the correlation between serum adiponectin level and the properties of hepatitis C virus (HCV).

**Methods:** A meal test was carried out for insulin resistance assessment in 81 patients with chronic HCV infection. Blood samples were taken before and after the test to measure serum insulin and plasma glucose (PG). The adiponectin level was measured by enzyme-linked immunosorbent assay in each patient.

**Results:** Serum adiponectin levels were significantly correlated with the area under the insulin curve (AUC-insulin)

during the meal test and with serum HCV-RNA level. Multiple regression analysis showed age to be a significant independent parameter associated with an increased adiponectin level, whereas male sex, fasting insulin, and serum HCV-RNA level were significant independent parameters associated with a decreased adiponectin level.

**Conclusion:** It is possible that insulin resistance in patients with chronic HCV infection is related to adiponectin secretion.

**Key words:** adiponectin, hepatitis C virus, insulin resistance

## INTRODUCTION

THE HEPATITIS C virus (HCV) is a major cause of chronic liver disease, with 170 million persons infected worldwide. The severity of disease varies widely from asymptomatic chronic infection to cirrhosis and hepatocellular carcinoma (HCC).<sup>1,2</sup> In addition, several extrahepatic diseases have been associated with chronic HCV infection and, in most cases, appear to be directly related to viral infection. These include hematological diseases such as cryoglobulinemia, autoimmune disorders such as thyroiditis, and the presence of autoantibodies, renal diseases, and dermatological conditions such as lichen planus and porphyria cutanea tarda.<sup>3–6</sup> Concerning these extrahepatic diseases related to HCV infection, clinical and epidemiological data have shown that chronic HCV infection

plays a significant role in the development of insulin resistance, supporting earlier findings of a correlation between diabetes mellitus type 2 and chronic HCV infection.<sup>7,8</sup> Moreover, animal experiments have shown that HCV infection directly leads to insulin resistance and diabetes mellitus.<sup>9</sup>

Adiponectin is a novel, recently discovered 244-amino acid and adipose-specific plasma protein belonging to the collectin family that is specially expressed from fat tissue and is found abundantly present in the peripheral circulation.<sup>10–13</sup> The level of adiponectin has no circadian variation of daily profile, both in diabetes patients and subjects without diabetes.<sup>14</sup> Hypoadiponectinemia is associated with an increased risk of diabetes mellitus type 2<sup>15</sup> and the degree of insulin resistance and hyperinsulinemia, rather than with the degree of adiposity and glucose intolerance.<sup>16</sup>

The above findings raise the issue of which factors may be associated with the serum adiponectin level and insulin resistance of patients with chronic HCV infection. The aim of the present study was to analyze the impact of the HCV-RNA level on the serum adiponectin level and insulin resistance of patients with chronic HCV infection.

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Received 22 February 2007; revision 18 April 2007; accepted 7 May 2007.

## METHODS

### Patients

THE ANALYSIS INCLUDES the data of 81 consecutive patients (36 men and 45 women, mean age 56.9 years, age range 22–67 years) who visited the Department of General Medicine, Kyushu University Hospital between January 2005 and December 2005 with chronic HCV infection and who were chronically positive for antibody to HCV (anti-HCV) and HCV-RNA positive for over 6 months (Table 1). Of the 81 studied

Table 1 Characteristics of 81 patients with chronic HCV infection†

Characteristic	
Age (years)	56.9 ± 11.1
Male/Female	36/45
Body mass index (kg/m <sup>2</sup> )	23.1 ± 3.1
Fasting plasma glucose (PG; mg/dL)	99.2 ± 21.4
PG at 120 min by meal test (mg/dL)	143.2 ± 49.4
HOMA-IR	2.3 ± 1.6
AUC-insulin‡ (μU/mL·hr)	86.8 ± 39.3
AUC-insulin × AUC-PG§ (μU/mL·mg/dL·hr <sup>2</sup> )	24 718.3 ± 15 667.8
Hemoglobin A1c (%)	5.2 ± 0.7
Triglyceride (mg/dL)	103.3 ± 44.0
Total cholesterol (mg/dL)	173.3 ± 30.3
HDL cholesterol (mg/dL)	50.8 ± 12.6
LDL cholesterol (mg/dL)	99.9 ± 26.4
Alanine aminotransferase (IU/L)	65.3 ± 41.1
γ-Glutamyltranspeptidase (IU/L)	52.1 ± 77.3
HCV genotype 1b/2	64/17
Serum HCV-RNA level (kIU/mL)	1677.1 ± 1272.4
Serum HCV core antigen level (fmol/L)	8206.9 ± 7110.5
Histology at biopsy	
Stage of fibrosis	
F0/F1/F2/F3/F4	9/33/15/15/9
Grade of inflammation	
A0/A1/A2/A3	0/28/36/17
Steatosis	
None/Mild/Moderate/Severe	48/20/11/2

†Total no. patients = 81.

‡Area under the serum insulin level curves at 0, 60 min, and 120 min during the meal test.

§Area under the plasma glucose level curves at 0, 60 min, and 120 min during the meal test.

Data are shown as mean ± standard deviation.

AUC, area under curve; HCV, hepatitis C virus; HDL, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment-insulin; LDL, low-density lipoprotein-cholesterol.

patients, 64 (79.0%) with genotype 1b and 17 (21.0%) with genotypes 2a and 2b were enrolled into the study. No patients had genotype 3. The mean levels of alanine aminotransferase (ALT) and γ-glutamyltranspeptidase at entry were 65.3 IU/L and 52.1 IU/L, respectively. To clarify the possibility of a direct correlation between chronic HCV infection and insulin resistance, we excluded from the study patients who were obese or who had been diagnosed with diabetes mellitus type 2 or hypertension. None of the studied patients had a body mass index (BMI) over 28 kg/m<sup>2</sup> or fasting plasma glucose (PG) level over 126 mg/dL. Needle biopsy of the liver was done for each patient at the time of enrollment for histological analysis, and two pathologists examined each biopsy specimen independently without prior knowledge of the patient. Histological findings showed nine patients (11.1%) with cirrhosis (F4) and no patients with grade A0 inflammation.

Patients were excluded if they had: (i) BMI over 28 kg/m<sup>2</sup>; (ii) advanced cirrhosis, identified by large esophageal varices (F2 or F3), history of gastrointestinal bleeding, ascites, encephalopathy, or HCC; (iii) a fasting PG level over 126 mg/dL or antidiabetic treatment; (iv) severe hyperlipidemia (cholesterol over 300 mg/dL or triglyceride over 300 mg/dL) or antihyperlipidemic treatment; (v) evidence of hypertension (systolic blood pressure over 140 mmHg, diastolic blood pressure over 90 mmHg) or antihypertensive treatment; (vi) active alcohol consumption over 80 g/day; (vii) positive for hepatitis B virus surface antigen or antibody to human immunodeficiency virus; (viii) hepatocellular injury such as autoimmunity or drug-induced liver disease; or (ix) antiviral or corticosteroid therapy within the 12 months prior to inclusion.

All patients were Japanese and informed written consent was obtained. The study was done in accordance with the principles of the Declaration of Helsinki and its appendices and was approved by the ethics committee of Kyushu University Hospital.

### Clinical and laboratory assessment

Body mass index was calculated as weight in kilograms/height in square meters. Blood samples were taken in the morning after 12 h overnight fasting on the day the liver biopsy was performed. Serum levels of ALT, γ-glutamyltransferase, cholesterol, triglycerides, and high-density lipoprotein (HDL)-cholesterol, PG, hemoglobin A1c (HbA1c), and platelet count were measured by standard laboratory techniques at a commercial laboratory (MBC Laboratory, Tokyo, Japan).

### Meal test for insulin resistance assessment

After a 12-h overnight fast, a meal test was done after insuring that each patient had not had any unusual exercise or alcohol intake the day before the test, as suggested in recent reports.<sup>17–19</sup> This meal test, using cookies, was reported to provide more information about insulin resistance and have fewer side-effects than the oral glucose tolerance test.<sup>20</sup> One pack of 10 cookies (ABILIT, Osaka, Japan) consisted of 75 g carbohydrate (85% flour starch, 15% maltose), 25 g butter fat, and 7 g protein for a total of 553 kcal. The cookies were ingested in 15 min with tea or cold water. Very short walks were allowed, but no other exercise or smoking was permitted during the time the blood samples were being taken. The baseline time was the time when the cookies were ingested. Blood samples were taken before (minute 0) and 60 and 120 min after eating the cookies for the measurement of serum insulin and PG levels. Serial levels of serum insulin were determined by radioimmunoassay at a commercial laboratory (MBC Laboratory, Tokyo, Japan). Insulin resistance was determined by the homeostasis model assessment (HOMA) method by using the following equation:<sup>21</sup>

$$\text{HOMA-IR} = \frac{\text{fasting insulin } (\mu\text{U/mL}) \times \text{fasting PG (mg/dL)}}{405}$$

HOMA-IR  $\geq$  2.5 was defined as insulin resistance in the present study. The area under the PG and serum insulin curves (AUC-PG and AUC-insulin) during the meal test was calculated by the linear trapezoidal method, because AUC-PG, AUC-insulin, and AUC-PG  $\times$  AUC-insulin have been recommended for determining the possibility of insulin resistance in non-diabetic patients.<sup>20,22</sup>

### Quantification of the level of serum adiponectin in the high molecular weight form

The level of adiponectin in the high molecular weight form was determined using an enzyme-linked immunosorbent assay (ELISA; Human Adiponectin ELISA Kit<sup>®</sup>; Otsuka Pharmaceutical, Tokyo, Japan). The range of the linear relationship provided was 0.375  $\mu\text{g/mL}$  to 12.0  $\mu\text{g/mL}$ .

### Virological assessment

Testing for anti-HCV was carried out using a commercial ELISA (AxSYM HCV version 3.0; Abbott Laboratories, Chicago, IL, USA). Serum HCV-RNA was detected by two-stage polymerase chain reaction (PCR) with

primers from the 5' non-coding region of the HCV genome, as previously described.<sup>1</sup> The HCV genotype was determined by two-stage PCR using universal and type-specific primers from the putative core gene of the HCV genome by a modification of the method of our previous reports.<sup>1,2</sup> The genotype nomenclature was based on the system proposed by Simmonds *et al.*<sup>23</sup> The serum HCV-RNA level was determined by the second-generation Cobas Amplicor HCV Monitor assay (Amplicor-M; COBAS v2.0<sup>®</sup>; Roche Diagnostics Systems, Meylan, France). The range of the linear relationship provided was  $5 \times 10^3$  international units per milliliter (kIU/mL) to 5100 kIU/mL. For quantification, serum HCV core antigen was measured by chemiluminescent enzyme immunoassay (Lumipulse Ortho HCV Ag<sup>®</sup> [Lumipulse-Ag], Ortho Clinical Diagnostics, Tokyo, Japan) as specified by the manufacturer. The range of detection is 20–50 000 fmol/L.

### Assessment of hepatic histology

A minimum 15 mm length of liver biopsy specimen or the presence of at least 10 complete portal tracts was required. For each specimen, the stage of fibrosis and the grade of activity were established according to the following criteria.<sup>24</sup> Fibrosis was staged on a scale of 0–4: F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous septa without cirrhosis; F4, cirrhosis. The grading of activity, including the intensity of necroinflammation, was scored as follows: A0, no histological activity; A1, mild activity; A2, moderate activity; A3, severe activity. An average percentage steatosis was then determined for the entire specimen. Steatosis was graded according to the scoring system of Brunt<sup>25</sup> based on the percentage of hepatocytes in the biopsy involved, as follows: 0 (no steatosis), 1 (<30% of hepatocytes affected; mild steatosis), 2 (33–66% of hepatocytes affected; moderate steatosis), or 3 (>66% of hepatocytes affected; severe steatosis).

### Statistical analysis

Continuous data were expressed as mean values, mean  $\pm$  standard deviation (SD), or values  $\pm$  standard error (SE) of the mean. Statistical differences in the continuous data were determined by paired *t*-test, unpaired *t*-test, Mann–Whitney *U*-test, or Kruskal–Wallis test, and categorical data were compared by chi-squared test and Fisher's exact test. Backward stepwise multiple linear regression analysis was done to evaluate the independent relationship of the studied variables for the adiponectin levels among the studied patients, using a commercially available software package (BMDP Statis-

tical Software, Los Angeles, CA, USA) for the IBM 3090 computer system (Yorktown Heights, NY, USA). A *P*-value less than 0.05 was regarded as statistically significant.

## RESULTS

### Incidence of insulin resistance in patients with chronic HCV infection

BASED ON WORLD Health Organization diabetes criteria in 2003, we identified seven (8.6%) diabetes mellitus and 30 (37.0%) impaired glucose tolerance patients among the 81 studied patients, none of whom had been diagnosed before the present study. Of the diabetes and impaired glucose tolerance patients, all had fasting PG levels < 126 mg/dL. Moreover, we found 24 (29.6%) of the 81 patients had insulin resistance with HOMA-IR  $\geq$  2.5. Patients with HOMA-IR  $\geq$  2.5 had significantly higher mean values  $\pm$  SE in the following markers than those with HOMA-IR < 2.5: fasting PG (114.1  $\pm$  6.6 mg/dL vs 92.9  $\pm$  1.2 mg/dL), fasting insulin (14.3  $\pm$  0.7  $\mu$ U/mL vs 6.6  $\pm$  0.3  $\mu$ U/mL), AUC-insulin (113.5  $\pm$  9.9  $\mu$ U/mL·h vs 75.6  $\pm$  3.8  $\mu$ U/mL·h), AUC-PG  $\times$  AUC-insulin (36 826.8  $\pm$  4392.2  $\mu$ U/mL·mg/dL·hr<sup>2</sup> vs 19 620.1  $\pm$  1117.9  $\mu$ U/mL·mg/dL·hr<sup>2</sup>), triglycerides (130.3  $\pm$  10.7 mg/dL vs 89.1  $\pm$  4.8 mg/dL),  $\gamma$ -GTP (57.5  $\pm$  7.2 IU/L vs 39.2  $\pm$  3.9 IU/L), and BMI (25.6  $\pm$  0.6 kg/m<sup>2</sup> vs 22.7  $\pm$  0.4 kg/m<sup>2</sup>). Moreover, patients with HOMA-IR  $\geq$  2.5 had significantly more progressive hepatic histology such as F3 to F4 (12 of 24, 50.0% vs 12 of 57, 21.1%) and A2 to A3 (19 of 24, 79.2% vs 24 of 57, 42.1%) than those with HOMA-IR < 2.5. No significant difference was found in age, gender, ALT level, HCV genotype, HCV-RNA level, or hepatic steatosis between patients with and without HOMA-IR  $\geq$  2.5. The mean  $\pm$  SE of serum adiponectin level was lower in patients with HOMA-IR  $\geq$  2.5 (6.20  $\pm$  0.78  $\mu$ g/mL) than in those with HOMA-IR < 2.5 (7.37  $\pm$  0.62  $\mu$ g/mL), but no significant difference was found.

### Correlation between adiponectin and other characteristics

The serum adiponectin levels by subgrouped characteristics of the 81 patients are shown in Table 2. A lower serum adiponectin level was significantly associated with male sex, higher AUC-insulin, lower HDL-cholesterol, a lower HCV-RNA level, and lower HCV core antigen by univariate analysis. Male patients had significantly lower adiponectin levels (4.64  $\mu$ g/mL)

than female patients (8.93  $\mu$ g/mL). Patients with AUC-insulin  $\geq$  85  $\mu$ U·Eh/mL had a significantly lower adiponectin level (5.73  $\mu$ g/mL) than those with AUC-insulin < 85  $\mu$ U·Eh/mL (8.11  $\mu$ g/mL), suggesting that adiponectin was correlated with insulin resistance. Patients with HDL-cholesterol < 40 mg/dL had a significantly lower adiponectin level (4.34  $\mu$ g/mL) than those with HDL-cholesterol  $\geq$  40 mg/dL or over (7.90  $\mu$ g/mL). A low adiponectin level was accompanied by a high HCV-RNA level, and a high adiponectin level was seen with a low HCV-RNA level. In contrast, the serum adiponectin level was not correlated with BMI, fasting PG, lipid profiles, ALT,  $\gamma$ -glutamyl-transferase, HOMA-IR, or HCV genotype. No significant correlation was found between the serum adiponectin level and either the stage of fibrosis or the grade of inflammation when crude values were considered (*P* = 0.3888 and *P* = 0.9040, respectively). Because of the limited number of cases, steatosis was not significantly correlated with adiponectin level (*P* = 0.1161), although, in contrast with the other patients, the lowest level was found in patients with severe steatosis (3.19  $\mu$ g/mL).

### Factors correlated with insulin resistance

Serum adiponectin levels were significantly correlated with AUC-insulin (*r* = -0.220, *P* = 0.0482) (Fig. 1a) and serum HCV-RNA levels (*r* = -0.364, *P* = 0.0008) (Fig. 1b). The adiponectin level decreased as the values of both AUC-insulin and HCV-RNA increased. The values of AUC-insulin were significantly correlated with serum HCV-RNA levels (*r* = 0.261, *P* = 0.0181) (Fig. 1c). AUC-insulin increased with an increased HCV-RNA level. Serum adiponectin level was also negatively correlated with HCV core antigen level (*r* = -0.337, *P* = 0.0019). There was a significant relationship between the HCV-RNA level and the HCV core antigen level (*r* = 0.768, *P* < 0.0001). The values of fasting PG, fasting insulin, and HOMA-IR were not significantly correlated with the HCV-RNA level.

### Multiple linear regression analysis of serum adiponectin levels

Age was an independent parameter significantly correlated with an increased serum adiponectin level, but male sex, fasting serum insulin, and serum HCV-RNA level were independent parameters significantly associated with a decreased serum adiponectin level (Table 3).



Table 2 Serum adiponectin levels by subgrouped characteristics of 81 patients with chronic HCV infection

Characteristic	No. patients	Mean $\pm$ SE ( $\mu\text{g/mL}$ )	P-value
Age			
<58 years	41	6.20 $\pm$ 0.67	0.0805
$\geq$ 58 years	40	7.87 $\pm$ 0.71	
Sex			
Male	36	4.64 $\pm$ 0.69	<0.0001
Female	45	8.93 $\pm$ 0.45	
Body mass index			
<22 kg/m <sup>2</sup>	20	8.26 $\pm$ 1.15	0.4571
22-25 kg/m <sup>2</sup>	39	6.57 $\pm$ 0.62	
$\geq$ 25 kg/m <sup>2</sup>	22	6.71 $\pm$ 0.99	
Fasting plasma glucose (PG)			
<110 mg/dL	67	7.21 $\pm$ 0.57	0.5405
$\geq$ 110 mg/dL	14	6.14 $\pm$ 0.85	
PG at 120 min by meal test			
<200 mg/dL	72	7.13 $\pm$ 0.52	0.4615
$\geq$ 200 mg/dL	9	6.14 $\pm$ 1.55	
HOMA-IR			
<2.5	57	7.37 $\pm$ 0.62	0.2962
$\geq$ 2.5	24	6.20 $\pm$ 0.78	
Fasting serum insulin ( $\mu\text{U/mL}$ )			
<8	38	6.93 $\pm$ 0.73	0.5138
$\geq$ 8	43	7.13 $\pm$ 0.66	
AUC-insulin <sup>†</sup> ( $\mu\text{U/mL}\cdot\text{hr}$ )			
<85	41	8.11 $\pm$ 0.69	0.0079
$\geq$ 85	40	5.73 $\pm$ 0.65	
AUC-insulin $\times$ AUC-PG <sup>‡</sup> ( $\mu\text{U/mL}\cdot\text{mg/dL}\cdot\text{hr}^2$ )			
<23 000	41	7.61 $\pm$ 0.53	0.1300
$\geq$ 23 000	40	6.36 $\pm$ 1.40	
Triglyceride			
<150 mg/dL	72	7.16 $\pm$ 0.50	0.3834
$\geq$ 150 mg/dL	9	5.92 $\pm$ 0.60	
Total cholesterol			
<240 mg/dL	79	7.11 $\pm$ 0.71	0.2475
$\geq$ 240 mg/dL	2	3.71 $\pm$ 0.60	
HDL-cholesterol			
<40 mg/dL	20	4.34 $\pm$ 0.71	0.0004
$\geq$ 40 mg/dL	61	7.90 $\pm$ 0.57	
LDL-cholesterol			
<120 mg/dL	69	7.21 $\pm$ 0.55	0.3385
$\geq$ 120 mg/dL	12	5.96 $\pm$ 1.11	
Alanine aminotransferase			
<35 IU/L	12	5.96 $\pm$ 0.64	0.3655
36-50 IU/L	22	7.40 $\pm$ 1.05	
51-74 IU/L	22	9.00 $\pm$ 1.36	
$\geq$ 75 IU/L	25	6.32 $\pm$ 0.85	
$\gamma$ -Glutamyltranspeptidase			
<30 IU/L	39	7.60 $\pm$ 0.63	0.1826
$\geq$ 30 IU/L	42	6.49 $\pm$ 0.79	
HCV genotype			
1b	64	7.04 $\pm$ 0.51	0.4440
2a + 2b	17	6.98 $\pm$ 1.36	

Table 2 Continued

Characteristic	No. patients	Mean $\pm$ SE ( $\mu\text{g/mL}$ )	P-value
Serum HCV-RNA level			
<100 kIU/mL	9	10.05 $\pm$ 1.72	0.0183
100-999 kIU/mL	19	8.71 $\pm$ 1.23	
$\geq$ 1000 kIU/mL	53	5.91 $\pm$ 0.48	
Serum HCV core antigen levels			
<1000 fmol/L	13	9.82 $\pm$ 1.34	0.0089
1000-9999 fmol/L	37	7.44 $\pm$ 0.75	
$\geq$ 10 000 fmol/L	31	5.36 $\pm$ 0.61	
Histology at biopsy			
Stage of fibrosis			
F0	9	5.78 $\pm$ 1.44	0.3888
F1	33	6.68 $\pm$ 0.79	
F2	15	7.91 $\pm$ 0.93	
F3	15	8.68 $\pm$ 1.45	
F4	9	8.53 $\pm$ 0.67	
Grade of inflammation			
A1	28	7.06 $\pm$ 0.86	0.9040
A2	36	6.75 $\pm$ 0.69	
A3	17	7.65 $\pm$ 1.27	
Steatosis			
None	48	7.30 $\pm$ 0.64	0.1161
Mild	20	7.77 $\pm$ 0.98	
Moderate	11	5.18 $\pm$ 1.36	
Severe	2	3.19 $\pm$ 2.08	

†Area under the serum insulin level curves at 0, 60 min, and 120 min during the meal test.

‡Area under the plasma glucose level curves at 0, 60 min, and 120 min during the meal test.

Data are shown as mean  $\pm$  standard error.

AUC, area under curve; HCV, hepatitis C virus; HDL, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment-insulin; LDL, low-density lipoprotein-cholesterol.

## DISCUSSION

THERE IS GROWING interest in the relationship between insulin resistance and chronic HCV infection. Hypoadiponectinemia may be involved in the pathogenesis of various disorders comprising metabolic diseases.<sup>26</sup> Moreover, the adiponectin receptor has been identified in human liver, and it is conceivable that hypoadiponectinemia might be related to the pathogenesis of liver diseases.<sup>27</sup> The present study was the first study of ethnic Japanese that has shown hypoadiponectinemia to be independently associated with insulin resistance, younger age, and higher HCV-RNA levels in patients with chronic HCV infection, but not with hepatic histology, including steatosis.

Adiponectin is present in serum in a number of forms, often a trimer or hexamer of high molecular weight (HMW).<sup>28</sup> Human studies investigating the role of adiponectin in chronic liver disease have focused predomi-

nantly on the role of serum total adiponectin in patients with non-alcoholic fatty liver disease where insulin resistance and reduced serum adiponectin levels have been implicated as factors in disease progression.<sup>29,30</sup> However, the HMW form of adiponectin appears to be the form most responsible for hepatic and whole-body insulin sensitivity and anti-inflammatory effects, but not total adiponectin or low molecular weight forms such as trimer and hexamer.<sup>31,32</sup> For these reasons, we investigated the serum level of the HMW adiponectin form for this analysis of Japanese patients with chronic HCV infection.

The present study showed no association between adiponectin level and HOMA-IR in patients with genotypes 1b and 2a, as reported by Wang *et al.*<sup>32</sup> The HOMA-IR indicates insulin resistance but has limitations in proving it, because HOMA-IR is composed of the quotient of fasting-PG and fasting-insulin. As our study showed, it may be important to measure the levels of PG

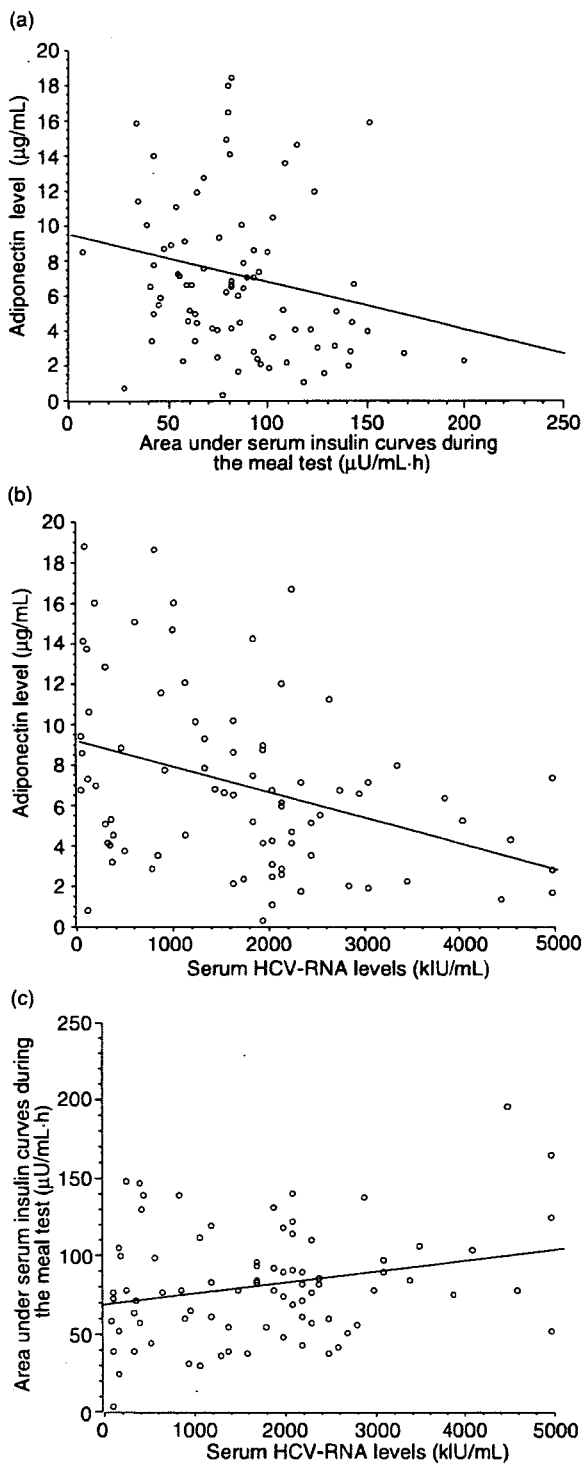


Figure 1 (a) Correlation between the adiponectin level and the area under the serum insulin curve during a meal test for 81 patients with chronic hepatitis C virus (HCV) infection ( $r = -0.220$ ,  $P = 0.0482$ ). (b) Correlation between the adiponectin and serum HCV-RNA levels of 81 patients with chronic HCV infection ( $r = -0.364$ ,  $P = 0.0008$ ). (c) Correlation between the area under the serum insulin curve during a meal test and the serum HCV-RNA levels of 81 patients with chronic hepatitis C virus infection ( $r = 0.261$ ,  $P = 0.0181$ ).

and insulin after a meal in patients with chronic liver disease. We showed a significant correlation between hypo adiponectinemia and fasting serum insulin and insulin resistance (the area under the serum insulin curves; AUC-insulin) during a meal test. The AUC-insulin assessment method has been recommended for determining possible insulin resistance in non-diabetes subjects.<sup>20,22</sup> We also documented a significant correlation between the level of the HMW form of adiponectin and insulin resistance in Japanese patients with chronic HCV infection.

Clinically, hypo adiponectinemia has been observed in patients with obesity and diabetes mellitus type 2, and adiponectin levels have been shown to increase during weight reduction.<sup>13,14,28</sup> In addition, adiponectin is downregulated in obesity.<sup>13</sup> In obese or lipoatrophic mice, giving adiponectin decreases the degree of insulin resistance associated with these conditions.<sup>33</sup> Considering the exclusion of patients with diabetes and alcohol abuse and the very low number of both obese patients and patients with associated hyperlipidemia in the present study, we found a significant reverse correlation between serum HCV-RNA and the adiponectin levels of Japanese patients with chronic HCV infection, similar to that found in a report of Taiwanese patients.<sup>34</sup>

Several assays for the detection of HCV core antigen, which closely track HCV-RNA dynamics, have been developed in recent years.<sup>35</sup> Serum HCV-RNA and HCV core antigen were both correlated with hepatic steatosis,<sup>36</sup> indicating that steatosis may be secondary to a viral cytopathic effect. *In vitro* studies and a transgenic mouse model have both suggested that HCV core antigen (protein) is sufficient to induce lipid accumulation in hepatocytes and that overexpression of the core protein further stimulates the formation of lipid droplets.<sup>3,9</sup> Moreover, serum adiponectin is invariably secreted by adipocytes, but not hepatocytes, and acts within the liver via adiponectin receptors.<sup>27,34</sup> Liu *et al.*<sup>34</sup> reported that the intrahepatic gene expression of adiponectin receptors was inversely correlated with serum adiponec-

Table 3 Multiple linear regression analysis for serum adiponectin levels

Adiponectin level vs independent variable†	Regression coefficient (standard error)	F-value	P-value
Age (years)	0.1413 (0.0459)	9.48	0.0031
Male‡	-4.5756 (0.9596)	22.74	<0.0001
Fasting serum insulin (mg/dL)	-0.2702 (0.1049)	6.64	0.0124
Serum HCV-RNA levels (kIU/mL)	-0.0009 (0.0004)	4.86	0.0312

†Total no. = 81.

‡Compared with female.

HCV, hepatitis C virus.

tin levels. These findings suggest a direct involvement of the extraviral activities of HCV proteins and adiponectin levels with insulin resistance in patients with chronic HCV infection. Several possibilities should be considered regarding the association between serum adiponectin and viral factors. It is unclear whether HCV replication may lead to the downregulation of the receptors or if adiponectin itself suppresses HCV replication. However, other mechanisms merit further study.

Adiponectin stimulates peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ), which is thought to accelerate fatty acid oxidation and upregulate the expression of insulin-receptor substrate, increasing insulin utility in various organs expressing adiponectin receptors, including the liver.<sup>27</sup> Thus, hypoadiponectinemia results in fat accumulation in the liver, fatty liver, that causes increased aminotransferase activities that lead to an insulin resistance state via the attenuation of these events. The present study showed no significant correlation between adiponectin and hepatic inflammation, fibrosis, or steatosis in our patients with chronic HCV infection. This finding can be explained by the fact that none of our patients had diabetes or abused alcohol and by the very small number of obese patients and patients with associated hyperlipidemia, in comparison with other studies.<sup>32,34</sup>

Hepatic steatosis is often found in chronic HCV infection, occurring in 30–70% of such patients.<sup>8</sup> Although the mechanism underlying steatosis in HCV infection is not known, it is thought to be multifactorial. A recent survey from Italy showed that hepatic steatosis accelerates the development and progression of fibrosis in non-diabetic European patients with HCV genotype 1, associated with hyporesponsiveness to interferon treatment.<sup>37</sup> High BMI, HCV genotype 3, and hepatic fibrosis have been independently associated with steatosis.<sup>38–40</sup> Serum HCV-RNA level was correlated with hepatic steatosis,<sup>7,41</sup> indicating that steatosis may be secondary to a

viral cytopathic effect. Moreover, hypoadiponectinemia is significantly associated with the development of hepatic steatosis in French patients.<sup>42</sup> However, we found a significant relationship between a high HCV-RNA level and hypoadiponectinemia but no significant relationship between the HCV-RNA level and steatosis in Japanese patients.

Although younger age was one of the significantly independent factors for hypoadiponectinemia in patients with chronic HCV infection, the finding seems not to be peculiar for HCV infection. An inverse relationship between adiponectin and creatinine clearance is found in essential hypertensives.<sup>43</sup> Also, adiponectin level is positively associated with abnormal renal function in diabetic patients,<sup>44</sup> suggesting that a decrease in adiponectin clearance in the kidney may be a cause of hyperadiponectinemia. In fact, an epidemiological study with a large number of subjects from the general population showed that adiponectin levels increased with age due to a decrease in adiponectin clearance in the kidney.<sup>45</sup>

A limitation of our study is the cross-sectional study design that only demonstrates an association between adiponectin, insulin resistance, and HCV viral loads. Further longitudinal studies with repeated measurements, especially after anti-HCV treatment, such as interferon and pegylated interferon, are needed to clarify the causal relationship between these factors. Although the correlation between serum adiponectin level and the properties of HCV remains unclear, our study contains interesting and potentially important information helpful for physicians and researchers from the standpoint of improving their understanding of the correlation between adiponectin level and HCV viral load in patients with chronic HCV infection. After this analysis, all of the patients in this study were treated with a combination treatment of pegylated interferon and ribavirin. It will be interesting and informative if we can

provide AUC-insulin and adiponectin data taken during or after this treatment, particularly in those with sustained viral response. The present study did not include patients with metabolic abnormalities such as diabetes, hypertension, and hyperlipidemia. Further study is needed that includes these patients with metabolic abnormalities.

In conclusion, hypoadiponectinemia was found to be highly associated with insulin resistance, younger age, and higher HCV-RNA levels in our patients with chronic HCV infection, but not with hepatic histology, including steatosis. These findings suggest a direct relationship between the level of HCV-RNA and the adiponectin level and insulin resistance in patients with chronic HCV infection. Therefore, it is possible that insulin resistance in patients with chronic HCV infection is related to adiponectin secretion.

## ACKNOWLEDGMENTS

WE THANK DR Kyoko Okada for research assistance with the present study. This study was supported by grants from the 21st Century Center of Excellence program of the Ministry of Education, Culture, Sports, Science and Technology of Japan, and from the Ministry of Health, Labor and Welfare.

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## Antibody to the Human T-Lymphotropic Virus Type 1 (HTLV-1) Envelope Protein Gp46 in Patients Co-infected with HCV and HTLV-1

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**Abstract.** Human T-lymphotropic virus type 1 (HTLV-1) infection is known to affect hepatitis C virus (HCV) clearance and to accelerate the development of hepatocellular carcinoma in HCV-infected patients. In this study, we found the prevalence and titer of an antibody recognizing the central region of the HTLV-1 Gp46 protein to be associated with the severity of chronic liver disease. The antibody prevalence was significantly correlated with the stage of chronic liver disease ( $P < 0.0001$ ): 3 (14.3%) of 21 patients with minimal–mild chronic hepatitis, 12 (24%) of 50 with moderate–severe chronic hepatitis, 7 (87.5%) of 8 with liver cirrhosis, and 13 (100%) of 13 with hepatocellular carcinoma. These results indicate that the antibody may be a useful marker of the deterioration of liver disease in patients co-infected with HCV and HTLV-1. This antibody may be useful for the diagnosis of liver diseases and the development of more effective treatments.

### INTRODUCTION

Human T-lymphotropic virus type 1 (HTLV-1) is an etiologic agent of malignant CD4<sup>+</sup> T lymphoproliferation, adult T-cell leukemia/lymphoma (ATLL), and a chronic progressive neurologic disorder termed HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP).<sup>1,2</sup> In addition to playing a pathogenic role in these diseases, HTLV-1 infection has been reported to be associated with a number of other diseases, including uveitis, polymyositis, and chronic inflammatory arthropathy as a result of its immunomodulating effects.<sup>3</sup> HTLV-1 causes impairment of host immunity and induces functional impairment of cellular immune response.<sup>4–6</sup> The majority of HTLV-1-infected individuals remain asymptomatic during their lifetime, and ≈ 5–7% of asymptomatic HTLV-1-infected carriers will develop either ATLL or HAM/TSP, depending on unknown cofactors.

Our previous study showed that the gp46-197 region, corresponding to the Asp197 to Leu216 region of envelope protein gp46 (gp46-197), functions as a binding site for cell-surface receptor molecules in cell-to-cell infection by HTLV-1.<sup>7,8</sup> The prevalence of the antibody to the gp46-197 region is remarkably increased in the sera of patients with HTLV-1-associated diseases. Also, the antibody titer of ATLL and HAM/TSP patients is significantly higher than that of asymptomatic carriers.<sup>8</sup> The appearance of anti-gp46-197 antibody is independent of the fluctuation of the total anti-HTLV-1 antibody, meaning that this antibody might be a unique predictor for the onset of HTLV-1-associated diseases.

We previously demonstrated that HTLV-1 infection influences hepatitis C virus (HCV) clearance both in the natural course and by IFN- $\alpha$  treatment. Notably, the sustained response to IFN- $\alpha$  in patients with HCV and HTLV-1 co-infection was significantly lower than in patients with HCV infection alone, and forward logistic multiple-regression analysis indicated that HTLV-1 was a negative predictive marker of IFN- $\alpha$  treatment of chronic hepatitis C.<sup>9</sup> It is also well known that patients with HCV infection are at risk

of developing chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC).<sup>10</sup> Boschi-Pinto et al.<sup>11</sup> reported that rates of liver disease and death from HCC were higher in patients with HCV and HTLV-1 co-infection than in those with HCV infection alone.

To search for clues to the mechanisms of the influence of HTLV-1 infection on HCV infection, we tested for anti-gp46-197 antibody among patients with HCV and HTLV-1 co-infection.

### MATERIALS AND METHODS

**Study procedures.** The study area, Iki, is an isolated island in southwestern Japan that has a population of ≈ 32,000. The main sources of income are fishing and farming, and the lifestyle is similar to that of persons residing elsewhere in Japan. This island is endemic for both HCV and HTLV-1.<sup>9</sup> A free public health examination was given that consisted of a general physical examination, a questionnaire, a blood cell count, and a blood chemistry analysis that included antibody to HCV (anti-HCV), HCV RNA, and antibody to HTLV-1 (anti-HTLV-1). Written informed consent was obtained from all residents and patients included in this survey. The study was approved by the Kyushu University Hospital ethics committee and was conducted in accordance with the human experimentation guidelines of the U.S. Department of Health and Human Services. Informed consent was obtained from all participants before the examination.

**Subjects.** The study included 2,245 residents of Iki Island (773 men and 1,472 women, age range 19–100 years, average age 63.0 years). All were volunteers who underwent a medical examination sponsored by the local public health office in June 2005.

At Mitsutake Hospital, on Iki Island,<sup>9</sup> 243 patients with HCV-related liver disease were also examined (104 men and 139 women, age range 30–92, average age 65.3 years). Of these patients, HCV genotype 1 was found in 195 (80.2%) and genotype 2 was found in 48 (19.8%). Liver biopsy was done for classification of histology using the histologic activity index (HAI).<sup>12</sup> Abdominal ultrasonography and computed tomography revealed that 45 patients had minimal–mild chronic hepatitis (CH) (scores of 1–8), 73 had moderate–severe CH

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(scores of 9–18), 14 had liver cirrhosis (LC) (a staging (fibrosis) score of 4 on HAI), and 19 had HCC.

HCV infection was defined as positive for both anti-HCV and HCV RNA for > 6 months, and HTLV-1 infection was defined as anti-HTLV-1 positive. Individuals positive for hepatitis B virus surface antigen were excluded from this study.

**Detection of anti-HTLV-1 antibody.** Screening for anti-HTLV-1 was done by the passive particle agglutination (PA) method (Fujirebio, Inc, Tokyo, Japan) for all samples. Positive results were confirmed by Western blot analysis (Fujirebio, Inc.), according to the manufacturer's protocol. Samples for which the results of both methods were positive were classified as positive; therefore, those determined to be positive for anti-HTLV-1 by PA alone were considered to be negative.

**Detection and titration of anti-gp46-197 antibody.** A modified peptide ELISA without blocking by goat serum and casein was used to detect anti-gp46-197 antibody in all residents and patients with HTLV-1 infection. Briefly, serum samples (1  $\mu$ L per well) were added to 99  $\mu$ L of PBS in each well with immobilized synthetic gp46-197 peptide, and the preparation was incubated for 1 h at 37°C. Then, 100 ng of horseradish peroxidase (HRP)-conjugated anti-human IgG (MBL, Nagoya, Japan) in PBS containing 5% BlockAce (Dainippon Seiyaku, Tokyo, Japan) per well was added, followed by incubation for 45 min at 37°C. Color development was done by *ortho*-phenylenediamine (Sigma-Aldrich, St. Louis, MO), and absorbance at 492 nm after addition of 50  $\mu$ L of 2.5 M H<sub>2</sub>SO<sub>4</sub> per well was measured in all anti-gp46-197 antibody-positive serum samples. The titer was calculated by multiplying the dilution value by the corresponding absorbance value.<sup>13</sup>

**Testing for HCV markers.** Anti-HCV was examined by ELISA (HCV EIA 2; Abbott Laboratories, North Chicago, IL) in all serum samples.<sup>14</sup> All anti-HCV-positive samples were tested for serum HCV RNA by two-stage polymerase chain reaction (PCR).

HCV RNA was extracted from 50  $\mu$ L of serum by Sep Gene RV (Sanko Junyaku, Tokyo, Japan). Complementary DNA was synthesized by use of random primers and reverse transcriptase (Super Script; Life Technologies, Gaithersburg, MD). HCV RNA was detected by two-stage PCR with primers from the 5' NC of the HCV genome, as described elsewhere.<sup>15,16</sup>

The serum HCV RNA level was determined by the second-generation Cobas Amplicor HCV Monitor assay (COBAS v2.0, Roche Diagnostics System, Meylen, France (Amplicor monitor). The range of the linear relationship provided was 5–5,000 kIU/mL for the Amplicor monitor.

The HCV RNA genotype was determined by two-stage PCR, using universal and type-specific primers from the putative C gene of the HCV genome, with modifications of the methods of Okamoto et al.<sup>17</sup> and Hayashi et al.<sup>16</sup>

**Blood cell counts and blood chemistry analysis.** Platelet count and alanine aminotransferase (ALT) were determined by a commercial blood chemistry analysis machine in a professional laboratory.

**Statistical analysis.** The Mann-Whitney *U*-test and  $\chi^2$  test were used to analyze the characteristics of patients co-infected with HCV and HTLV-1. The Cochran-Armitage test was used to compare differences in the prevalence and titer of

anti-gp46-197 antibody between patients with liver diseases and residents who had undergone a medical examination. For all tests,  $P < 0.05$  was considered to have statistical significance.

Correlations between the anti-gp46-197 antibody titer and the ALT level and platelet count were analyzed by BMDP statistical software for the IBM 3090 system computer (BMBD Statistical Software, Inc., Los Angeles, CA).

## RESULTS

We examined the age- and sex-specific prevalences of HCV and HTLV-1 of the 2,245 residents of this HTLV-1-endemic area. HCV RNA was detected in 2.7% ( $N = 61$ ) and anti-HTLV-1 in 20.3% ( $N = 456$ ) of the residents. The prevalence of both HCV and HTLV-1 increased significantly with age. The prevalence of HTLV-1 infection was significantly higher among women (22.1%) than among men (16.2%;  $P = 0.0009$ ), but the difference in HCV infection among men (4.2%) and women (1.9%) was not ( $P = 0.577$ ).

Of the 2,245 residents, the prevalence of HCV infection alone was 2.2% ( $N = 49$ ), HTLV-1 infection alone 19.8% ( $N = 444$ ), and HCV and HTLV-1 co-infection 0.5% ( $N = 12$ ). Moreover, of the 243 patients with HCV-related liver disease, the prevalence of HCV infection alone was 62.1% ( $N = 151$ ), and that of HCV and HTLV-1 co-infection was 37.9% ( $N = 92$ ). We tested for anti-gp46-197 antibody in residents and HCV-related liver disease patients with HTLV-1 infection. Of 444 residents with HTLV-1 infection alone (121 men, 323 women), only one resident was positive for anti-gp46-197 antibody. Of 12 residents with HCV and HTLV-1 co-infection (7 men, 5 women), this antibody was detected only in a 70-year-old female resident (8.3%) who had mild liver abnormality but no hematological abnormalities. Of 92 HCV-related liver disease patients with HTLV-1 co-infection (37 men, 55 women), this antibody was detected in 35 patients (16 men, 19 women, 38.0%). The anti-gp46-197 antibody was not found in any of the patients without HTLV-1. None of the 456 residents or 92 HCV-related liver disease patients with HTLV-1 infection had HTLV-1-associated diseases. Anti-gp46-197 antibody-positive patients showed no deviation in age, sex, or HCV RNA level.

Of 195 genotype 1 patients and 48 genotype 2 patients with HCV-related liver disease, HTLV-1 was detected in 39.5% ( $N = 77$ ) and 31.3% ( $N = 15$ ), respectively. Of 77 genotype 1 patients and 15 genotype 2 patients with HCV and HTLV-1 co-infection, anti-gp46-197 antibody was detected in 36.3% ( $N = 28$ ) and 46.7% ( $N = 7$ ), respectively. Among 92 chronic liver disease patients with HCV and HTLV-1 co-infection, the HCV RNA level was  $1,010.2 \pm 192.5$  kIU/mL in 35 anti-gp46-197 antibody-positive patients and  $1,336.1 \pm 184.5$  kIU/mL in 57 negative patients ( $P = 0.164$ ). The status of HTLV-1 and anti-gp46-197 antibody were not related to the status of HCV infection.

The relationships of anti-gp46-197 antibody with the ALT level and platelet count were analyzed (Figure 1). In 92 HCV-related liver disease patients with HTLV-1 co-infection, the ALT level was not different between the 35 anti-gp46-197 antibody-positive patients ( $60.1 \pm 8.7$  IU/L) and the 57 negative patients ( $51.0 \pm 5.6$  IU/L) ( $P = 0.12$ ). On the other hand, a positive correlation between the titer of anti-gp46-197 an-



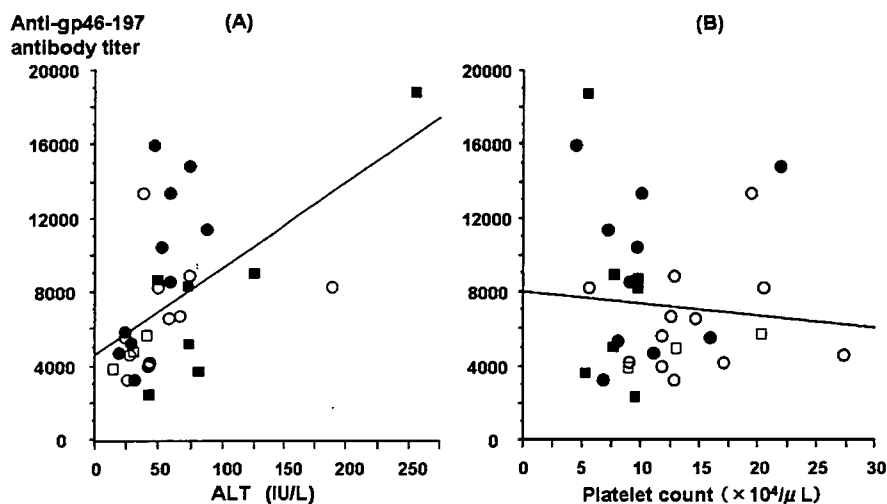


FIGURE 1. Relationship between anti-gp46-197 antibody titer and alanine aminotransferase level (ALT) (A) and platelet count (B) in anti-gp46-197-positive patients with hepatitis C virus and human T-lymphotropic virus type 1 co-infection: (A)  $P = 0.008$ ,  $r = 0.552$ ; (B)  $P = 0.644$ ,  $r = -0.086$ ; □, patients with minimal-mild chronic hepatitis; ○, patients with moderate-severe chronic hepatitis; ■, patients with liver cirrhosis; ●, patients with hepatocellular carcinoma.

tibody and ALT level was demonstrated in 35 positive patients ( $r = 0.552$ ,  $P = 0.008$ ). The platelet count was significantly lower in anti-gp46-197 antibody-positive patients ( $[12.2 \pm 0.9] \times 10^4/\mu\text{L}$ ) than in negative patients ( $[16.7 \pm 0.7] \times 10^4/\mu\text{L}$ ) ( $P = 0.004$ ). However, the platelet count did not show a significant correlation with the titer of anti-gp46-197 antibody ( $r = -0.086$ ,  $P = 0.644$ ).

The histologic status of the liver disease of 35 anti-gp46-197 antibody-positive patients found among 92 HCV-related liver disease patients with HTLV-1 co-infection is shown in Table 1. Anti-gp46-197 antibody was found in 3 of 21 (14.3%) patients with minimal-mild CH, 12 of 50 (24.0%) with moderate-severe CH, 7 of 8 (87.5%) with LC, and 13 (100%) with HCC, indicating a significant relationship between the presence of the anti-gp46-197 antibody and the severity of HCV-associated liver disease (Cochran-Armitage test,  $P < 0.0001$ ).

The relationship between the titer of anti-gp46-197 antibody and the histologic status of 92 HCV-related liver disease patients with HTLV-1 co-infection is shown in Table 1. The average titers of anti-gp46-197 antibody were  $4,715 \pm 529$  in minimal-mild CH,  $6,343 \pm 830$  in moderate-severe CH,  $7,835 \pm 2,045$  in LC, and  $8,132 \pm 1,127$  in HCC. The anti-gp46-197 antibody titer increased with the severity of liver disease.

## DISCUSSION

We previously demonstrated a high prevalence of both HCV and HTLV-1 infection among 2,280 residents of Iki

TABLE 1

Relationship between the anti-gp46-197 antibody positive rate and histological status of patients co-infected with HCV and HTLV-1\*

HAI category	Total	Anti-gp46-197 antibody positivity (%)	Anti-gp46-197 antibody titer (mean $\pm$ SE)
MM-CH	21	3 (14.3)	4715 $\pm$ 529
MS-CH	50	12 (24.0)	6343 $\pm$ 830
LC	8	7 (87.5)	7835 $\pm$ 2045
HCC	13	13 (100)	8132 $\pm$ 1127

\* MM-CH, minimal-mild chronic hepatitis; MS-CH, moderate-severe chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma; data  $\pm$  SE are shown.

Island in a study done between 1996 and 1999. The prevalence of anti-HCV was 13.9% and the prevalence of anti-HTLV-1 was 23.2%.<sup>9</sup> Our earlier study indicated that blood transfusions could be a common route for the spread of both HCV and HTLV-1.<sup>18</sup> Anti-HCV is the most sensitive marker for HCV infection, including past infection, and the percentage HCV RNA-positive to anti-HCV-positive is generally 80–85%.<sup>19</sup> The prevalence of HCV RNA in the present study seems to be lower than the usually estimated rate. Because we have informed the residents of the risk of HCV infection and the importance of testing and treatment, most residents with HCV infection reported to hospitals for testing. Most of the HCV-infected residents with liver abnormalities in the present study were followed at the single hospital. Therefore, the prevalence of HCV RNA was only 2.7% in the present study, despite the area being endemic for HCV.

We also reported that the higher rate of HCV RNA positivity found among Iki Island residents who tested positive for anti-HTLV-1 and anti-HCV may be due to an immunosuppressive effect of HTLV-1.<sup>9</sup> Moreover, the significantly lower rate of sustained response to interferon treatment in patients with HCV and HTLV-1 co-infection, compared with that in those with HCV infection alone, and the results of forward logistic multiple regression analysis showed that HTLV-1 negativity was predictive of the success of interferon treatment of chronic hepatitis C,<sup>9</sup> indicating that HTLV-1 infection might affect the elimination of HCV.

Chronic hepatitis, cirrhosis, and HCC are known sequelae of chronic HCV infection. We also reported that persistent liver damage plays an important role in the development of HCC in patients with chronic HCV viremia, as shown by results indicating that HCC developed more often in patients with consistently abnormal ALT levels than in those with consistently normal ALT levels.<sup>10</sup> Moreover, HTLV-1 infection contributes to the development of HCC in patients with HCV infection.<sup>11</sup> It seems possible that HTLV-1 affects the deterioration of liver disease in chronic hepatitis C patients. However, we have no evidence concerning the precise mechanisms by which HTLV-1 influences HCV infection.

The gp46-197 region on the gp46 envelope protein of

HTLV-1 is the essential domain for cell-to-cell infection.<sup>8</sup> The anti-gp46-197 antibody is a biomarker for the development of adult T-cell leukemia and HTLV-1-associated myelopathy from a carrier state.<sup>8</sup> The biologic correlates and pathologic significance of this antibody production are not clear at the present time. In the present study, we assessed the relationship between the prevalence of anti-gp46-197 antibody and the deterioration of liver disease in patients with HTLV-1 and HCV co-infection. The anti-gp46-197 antibody was found significantly more often in patients with HCV-associated liver diseases (35 of 92, 38.0%) than in residents (1 of 12, 8.3%) co-infected with HCV infection and HTLV-1. On the other hand, 1 of 444 (0.2%) residents with HTLV-1 infection alone showed positive for gp46-197 antibody. This prevalence was significantly lower than that of patients with HTLV-1 infection alone (7 of 23, 30.4%; data not shown). Notably, in this study, individuals in whom the antibody was detected did not have any HTLV-1-related diseases. The antibody seems to appear with the onset of liver disease in patients co-infected with HCV and HTLV-1.

The anti-gp46-197 antibody titer was closely associated with the severity of the liver disease. Patients co-infected with HCV and HTLV-1 had liver deterioration with lower HCV RNA levels than did patients with HCV alone. This suggests that HTLV-1 co-infection might influence the onset of liver disease and deterioration, meaning that the expression of HTLV-1 structural protein gp46 accelerates the activity of the host immune system in viral carriers.

Many investigators have reported that antibody production does not directly damage the liver<sup>20-22</sup> and that immune response of cytotoxic CD8<sup>+</sup> T lymphocytes (CTL) is the main reason for the development of hepatitis.<sup>20-22</sup>

Although the exact mechanism of HCV-associated liver damage has not been established, it is widely accepted that immune-mediated mechanisms, particularly HCV-specific CTL and helper CD4<sup>+</sup> T lymphocytes, are associated with the pathogenesis of HCV-induced liver damage.<sup>22,23</sup> We previously reported that the frequency of IFN- $\gamma$  producing CD4<sup>+</sup> (Th1) and CD8<sup>+</sup> (Tc1) T cells is increased in the peripheral blood of chronic hepatitis C patients, suggesting that these Th1 and Tc1 cells are involved with liver damage by HCV infection.<sup>24</sup> As for HTLV-1, cell-mediated immune response, especially CTL response to HTLV-1, is involved in the severity of HTLV-1-associated diseases.<sup>25</sup> Interaction between the responses to HCV and HTLV-1 are poorly understood in patients with HCV and HTLV-1 co-infection. The increase in the titer of anti-gp46-197 antibody suggests that the expression of the Gp46 protein increases in HTLV-1-infected CD4<sup>+</sup> T cells.

These results indicate that the appearance and titer of the anti-gp46-197 antibody may be practical markers of deterioration of the liver disease of patients co-infected with HCV and HTLV-1. This antibody may be useful for diagnosis and for increasing the effectiveness of treatment of liver diseases. In the future, it will be important to examine direct and/or indirect influences of the production of this antibody on the deterioration of liver disease associated with HCV/HTLV-1 co-infection. To further clarify the role of the anti-gp46-197 antibody, we intend to do an extensive cohort study to examine the relationship between the time of appearance of the antibody and the timing of seroconversion in both prospective and retrospective studies.

Received October 24, 2006. Accepted for publication January 15, 2007.

Acknowledgments: In addition to the authors, the following investigators were involved in the present study: Yoko Tomonari and Hiroshi Manabe, Fukuoka Red Cross Blood Center; Chikushino, Fukuoka, Dr. Arahito Mitsutake, and Dr. Takeshi Kuga, Mitsutake Hospital, Iki, Nagasaki.

Financial support: The research was supported by a grant from the 21st Century COE Program of the Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan.

Disclaimer: None of the authors have a commercial or other association that might pose a conflict of interest.

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HEPATOLOGY

**Association between the treatment length and cumulative dose of pegylated interferon alpha-2b plus ribavirin and their effectiveness as a combination treatment for Japanese chronic hepatitis C patients: Project of the Kyushu University Liver Disease Study Group**

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**Key words**

hepatitis C virus, pegylated interferon alpha-2b, ribavirin.

Accepted for publication 8 November 2007.

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**Abstract**

**Aim:** The aim of the present study was to investigate the association between the length of the treatment period and the cumulative dose of pegylated interferon alpha-2b (peg-IFN alpha-2b) plus ribavirin (RBV) and their effectiveness in the treatment of chronic hepatitis C.

**Methods:** Seven hundred and fifteen patients received peg-IFN alpha-2b plus RBV treatment for 48 weeks and 24 weeks for genotypes 1 ( $n = 586$ ) and 2 ( $n = 129$ ), respectively.

**Results:** Sustained virological responses (SVR), defined as serum hepatitis C virus (HCV)-RNA undetectable at 24 weeks after the end of treatment, were 42.4% and 74.4% in genotypes 1 and 2, respectively, on an intention-to-treat analysis. SVR significantly increased with treatment length (4.7%, 36.4%, and 51.8% for < 24 weeks, 24–47 weeks, and 48 weeks, respectively, for genotype 1; and 28.6%, 57.1%, 78.3% for < 12 weeks, 12–23 weeks, and 24 weeks, respectively, for genotype 2). SVR significantly increased with total cumulative treatment dose (21.1%, 36.5%, and 52.9% with < 60%, 60–79%, and  $\geq 80\%$  in peg-IFN dose; 29.6%, 51.1%, and 59.2% with < 60%, 60–79%, and  $\geq 80\%$  in RBV dose) in genotype 1, although it did not differ significantly for genotype 2.

**Conclusions:** In peg-IFN alpha-2b plus RBV treatment for chronic hepatitis C, it is important to complete the target length of treatment and to continue the target dosage to achieve SVR, especially for genotype 1 patients.

**Introduction**

Hepatitis C virus (HCV) is a major cause of chronic liver disease, with perhaps 200 million persons infected worldwide. Approximately 1.8 million patients have chronic HCV infection in Japan. The severity of disease varies widely, from asymptomatic chronic infection to cirrhosis and hepatocellular carcinoma (HCC).<sup>1,2</sup> Eradication of HCV by antiviral treatment improves liver histology and patient survival.<sup>3</sup> A currently popular antiviral treatment regimen for the treatment of chronic HCV infection worldwide is pegylated interferon alpha (peg-IFN alpha) in combination with

ribavirin (RBV). The combination treatment has resulted in a higher rate of sustained virological response (SVR), over 50% in Caucasian patients, than standard interferon (IFN) monotherapy.<sup>4,5</sup> However, there are no data concerning the response and safety of the combination treatment for a large number of Japanese patients with chronic HCV infection because this treatment was only approved by the Japanese Ministry of Health, Labor and Welfare in December 2004.

The HCV genotype has been reported to be the most important predictor of IFN treatment response.<sup>4–14</sup> Patients infected with genotypes 2 and 3 achieved approximately 65% SVR in a 24-week