Epidemiological survey of oral lichen planus among HCV-infected inhabitants in a town in Hiroshima Prefecture in Japan from 2000 to 2003

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Abstract. The objective of our study was to evaluate the natural history of oral lichen planus (OLP) and other extrahepatic manifestations in the inhabitants of an area in Japan that is hyperendemic for hepatitis C virus (HCV) infection. Over 4 years, 224 adult inhabitants with HCV infection were examined for OLP by a single oral surgeon. All subjects were interviewed regarding the natural history of other extrahepatic manifestations they had developed. The antibodies to HCV (anti-HCV) and serum HCV RNA were determined. Anti-HCV were detected in sera from 224 subjects (100%); HCV RNA in 210 (93.8%). Of the 224, 88 had at least 1 oral examination for OLP during the 4-year period. In 2000, 2001, 2002 and 2003, OLP was observed in 8.5 (5/59), 14.8 (8/54), 20 (11/55) and 21.4% (12/56) of subjects, respectively. OLP prevalence increased as the subjects grew older. The incidence of OLP over the 4 years among all subjects with HCV infection was 17.0% (15/88, 2 men and 13 women). None experienced natural healing or the development of malignant transformations. Between 2000 and 2003, there was an increase in the prevalence of type 2 diabetes mellitus (DM), thyroid dysfunction, skin disease, renal disease and hypertension. Screening for extrahepatic manifestations should be conducted in patients with risk factors for HCV infection.

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Abbreviations: HCV, hepatitis C virus; OLP, oral lichen planus; HCC, hepatocellular carcinoma; anti-HCV, antibodies to HCV; DM, diabetes mellitus

Key words: lichen planus, hepatitis C virus, extrahepatic manifestations

Introduction

Hepatitis C virus (HCV) infection is a major health problem in Japan. It is highly prevalent in subjects with chronic liver disease and is strongly associated with hepatocellular carcinoma (HCC). HCV-related HCC accounts in large part for the recent increase in HCC and now constitutes about 80% of all HCC cases in Japan. HCV also incites many extrahepatic manifestations (1,2) of which lichen planus is the most common (3,4). Other associated diseases include cryoglobulinaemic nephropathy and glomerulonephritis (5), thyroid dysfunction (6), porphyria cutanea tarda (7) and type 2 diabetes mellitus (DM) (8).

We previously reported that the incidence of oral lichen planus (OLP) in subjects with HCV infection was significantly higher than in those without HCV. We reached this conclusion by mass screening 685 inhabitants of a hyperendemic area, H town, located in the Fukuoka prefecture of Northern Kyushu, Japan (Fig. 1) for HCV infection (9). The prevalence of other extrahepatic manifestations in subjects with antibodies to HCV (anti-HCV) was higher than in those without HCV (10).

We also conducted an epidemiological study of another HCV hyperendemic area, O town, in the northwest of the Hiroshima prefecture in Honshu, Japan (Fig. 1). The presence of HCV-associated extrahepatic manifestations was found in 66.1% (39/59) of those screened (11). These findings suggest that the high prevalence of various extrahepatic manifestations among HCV-infected subjects is not unique to specific areas.

In the present investigation, we annually examined extrahepatic manifestations in the inhabitants of O town from 2000 to 2003. The aim of this study was to evaluate the natural history of OLP and other extrahepatic manifestations in individuals with HCV infections.

Patients and methods

Patients. From 2000 to 2003, we studied a total of 224 adult inhabitants of O town, a hyperendemic area of HCV infection. All were HCV carriers, though the causes of viral transmission were unknown. In 2000, 2001, 2002 and 2003,

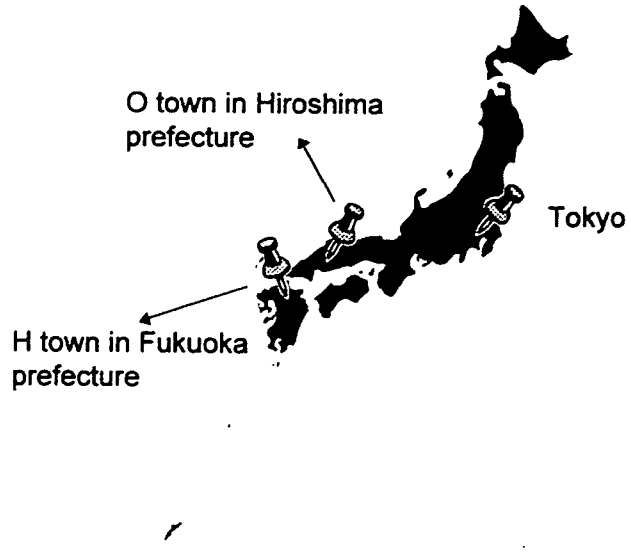

Figure 1. The research area. The location of O town in the northwest region of the Hiroshima prefecture in Honshu, Japan.

we examined 59, 54, 55 and 56 inhabitants, respectively (Table I). A single oral surgeon examined subjects for oral membrane diseases. A topographic classification of the oral mucosa, with location codes indicated, is shown in Fig. 2 (12). The diagnosis of OLP was made based on clinical and histopathological features.

All subjects were interviewed in person by 2 trained interviewers. We inquired about the following: cigarette smoking habits, present health condition and the presence of extrahepatic manifestations of HCV infection such as type 2 DM, rheumatoid arthritis, thyroid dysfunction, skin disease, renal disease, hypertension and extrahepatic malignant tumors.

Informed consent was obtained from all subjects once the purpose and methods of the study were explained.

Examination for anti-HCV and HCV RNA in serum. Sera were examined for the presence or absence of HCV. Anti-HCV were measured by a second-generation, enzyme-linked immunosorbent assay (Abbott HCV PHA 2nd Generation, Dainabot Co., Ltd., Tokyo, Japan). HCV RNA in the sera was detected using the Amplocore HCV test (Nippon Roche, Tokyo, Japan).

Examination of the prevalence of extrahepatic manifestations from 2000 to 2003. We have previously reported on the prevalence of extrahepatic manifestations in HCV infection, including OLP, for inhabitants of the same town (11). We now examined the prevalence of these extrahepatic manifestations from 2000 to 2003.

Results

Anti-HCV were detected in the sera of 224 subjects (100%) and HCV RNA in 210 subjects (93.8%), as shown in Table I. Of the 224, 88 had at least 1 oral examination over the course of the 4 years of the study (34 men and 54 women).

Table I shows the prevalence of OLP in all subjects. In 2000, 2001, 2002 and 2003 it was 8.5 (5/59), 14.8 (8/54), 20 (11/55) and 21.4% (12/56), respectively. The prevalence of OLP in HCV RNA positive subjects in 2000, 2001, 2002 and 2003 was 8.8 (5/57), 16 (8/50), 21.6 (11/51) and 23.1% (12/52), respectively. The prevalence of OLP increased with age. The incidence of OLP among all subjects with HCV infection over the 4-year period was 17.0% (15/88, 2 men and 13 women). A history of smoking was found in 1/15 OLP cases (6.7%) among inhabitants. Of the 15 cases, 2 had medical checkups once a year, 3 had them 3 times a year, 9 had them twice a year and 1 had a checkup just once in the 4-year period from 2000 to 2003 (Table II). No one had visited a clinic for the treatment of their OLP prior to our discovery of their OLP lesions. By far the most common site for OLP was the buccal mucosa. The predominant type in 53.3% of the 15 cases (8/15) was the reticular form of the disease. In 46.7% (7/15) it was the erosive form. Fig. 3 shows the erosive form (inhabitant No. 6 in Table II). Reticular lesions were generally asymptomatic. Two of the 15 cases had aggravated oral symptoms during the 4-year period. None experienced natural healing or developed malignant transformation.

From 2000 to 2003, there was an increase in the prevalence of type 2 DM, thyroid dysfunction, skin disease, renal disease and hypertension (Table I).

Discussion

HCV carriers in Japan are presumed to number 2 million (13). The growing incidence of HCC is expected to reach a plateau by around the year 2015. However, there are many people who are not aware that they are infected, some of whom will advance to liver cirrhosis or HCC (14). The incidence of HCC varies greatly among different regions. Epidemiological studies conducted by the Japanese Ministry of Health, Labour and Welfare showed that the mortality rate associated with HCC was high in several prefectures in Western Japan. Areas with high rates of anti-HCV, such as the Saga prefecture (3.9%), Hiroshima (1.8%), Fukuoka (1.7%) and Kagawa (1.7%), had high death rates for primary liver cancer of 43.1, 39.6, 39.8 and 31.9 per 100,000 people, respectively. These rates were higher than the national average (15).

HCV is associated with a wide range of extrahepatic manifestations. Zignego *et al* classified the extrahepatic manifestations of HCV into 4 main categories (16). The first category (A) includes extrahepatic manifestations characterised by a very strong association to HCV and supported by both epidemiological and pathogenetic evidence. Category A comprises mixed cryoglobulinaemia. The second category (B) includes disorders which are significantly associated with HCV infection, supported by adequate data. Category B comprises B-cell non-Hodgkin's lymphoma, monoclonal gammopathies, porphyria cutanea tarda and lichen planus. The third category (C) includes manifestations whose association with HCV still requires confirmation and/or a more detailed characterisation of similar pathologies of different aetiology or idiopathic nature. Finally, the fourth category (D) includes only anecdotal observations.

Lichen planus is a chronic inflammatory disease of the skin and mucous membranes that frequently involves the oral mucosa. In Japan, the age-adjusted incidence rate of OLP is 59.7 per 100,000 males and 188.0 per 100,000 females (17).

Table I. Prevalence of extrahepatic manifestations in adult inhabitants with HCV infection.

	2000	2001	2002	2003
Subjects	59	54	55	56
Age (mean years ± SD)	70.7±7.2	71.2±7.2	72.0±6.5	73.4±6.8
Sex (M/F)	21/38	22/32	23/32	24/32
% with history of smoking	18.6 (11/59)	11.1 (6/54)	12.7 (7/55)	14.3 (8/56)
% positive for anti-HCV	100 (59/59)	100 (54/54)	100 (55/55)	100 (56/56)
% positive for HCV RNA	96.6 (57/59)	92.6 (50/54)	92.7 (51/55)	92.9 (52/56)
Extrahepatic manifestations				
% positive for oral lichen planus	8.5 (5/59)	14.8 (8/54)	20.0 (11/55)	21.4 (12/56)
Age (mean years ± SD)	74.8±5.2	74.3±5.7	73.1±5.1	74.7±5.8
Sex (M/F)	1/4	2/6	2/9	2/10
% positive for anti-HCV	8.5 (5/59)	14.8 (8/54)	20.0 (11/55)	21.4 (12/56)
% positive for HCV RNA	8.8 (5/57)	16.0 (8/50)	21.6 (11/51)	23.1 (12/52)
% positive for DM	15.3 (9/59)	24.1 (13/54)	20.0 (11/55)	19.6 (11/56)
Age (mean years ± SD)	67.9±7.2	68.8±7.9	69.5±7.9	68.6±7.4
Sex (M/F)	5/4	10/3	8/3	7/4
% positive for anti-HCV	15.3 (9/59)	24.1 (13/54)	20.0 (11/55)	19.6 (11/56)
% positive for HCV RNA	14 (8/57)	20.0 (10/50)	15.7 (8/51)	15.4 (8/52)
% positive for rheumatoid arthritis	1.7 (1/59)	1.9 (1/54)	5.5 (3/55)	5.4 (3/56)
Age (mean years ± SD)	67.9±7.2	70.0±0	70.0±0.8	73.0±2.9
Sex (M/F)	5/4	0/1	1/2	1/2
% positive for Anti-HCV	15.3 (9/59)	1.9 (1/54)	5.5 (3/55)	5.4 (3/56)
% positive for HCV RNA	14 (8/57)	2.0 (1/50)	5.9 (3/51)	5.8 (3/52)
% positive for thyroid dysfunction	0	3.7 (2/54)	3.6 (2/55)	8.9 (5/56)
Age (mean years ± SD)	-	67.0±1.0	68.0±1.0	72.0±3.3
Sex (M/F)	-	1/1	1/1	1/4
% positive for anti-HCV	-	3.7 (2/54)	3.6 (2/55)	8.9 (5/56)
% positive for HCV RNA	-	4.0 (2/50)	3.9 (2/51)	9.6 (5/52)
% positive for skin disease	5.1 (3/59)	11.1 (6/54)	7.3 (4/55)	16.1 (9/56)
Age (mean years ± SD)	70.3±7.3	71.3±5.8	70.8±5.2	74.1±5.9
Sex (M/F)	0/3	1/5	1/3	4/5
% positive for anti-HCV	5.1 (3/59)	11.1 (6/54)	7.3 (4/55)	16.1 (9/56)
% positive for HCV RNA	5.3 (3/57)	12.0 (6/50)	7.8 (4/51)	15.4 (8/52)
% positive for renal disease	1.7 (1/59)	5.6 (3/54)	0	1.8 (1/56)
Age (mean years ± SD)	76.0±0	76.0±2.2	-	86.0±0
Sex (M/F)	1/0	1/2	-	1/0
% positive for anti-HCV	1.7 (1/59)	5.6 (3/54)	-	1.8 (1/56)
% positive for HCV RNA	1.8 (1/57)	6.0 (3/50)	-	1.9 (1/52)
% positive for hypertension	28.8 (17/59)	40.7 (22/54)	43.7 (24/55)	55.4 (31/56)
Age (mean years ± SD)	71.0±6.9	70.9±6.3	72.6±6.0	74.4±6.7
Sex (M/F)	6/11	7/15	10/14	13/18
% positive for anti-HCV	28.8 (17/59)	40.7 (22/54)	43.6 (24/55)	55.4 (31/56)
% positive for HCV RNA	26.3 (15/57)	42.0 (21/50)	41.8 (13/51)	57.7 (30/52)
% positive for extrahepatic malignant tumor	11.9 (7/59)	13 (7/54)	9.1 (5/55)	7.1 (4/56)
Age (mean years ± SD)	74.4±3.5	76.3±3.7	77.2±4.2	79.3±2.2
Sex (M/F)	2/5	3/4	3/2	3/1
% positive for anti-HCV	11.9 (7/59)	13 (7/54)	9.1 (5/55)	7.1 (4/56)
% positive for HCV RNA	12.3 (7/57)	14 (7/50)	9.8 (5/51)	7.7 (4/52)

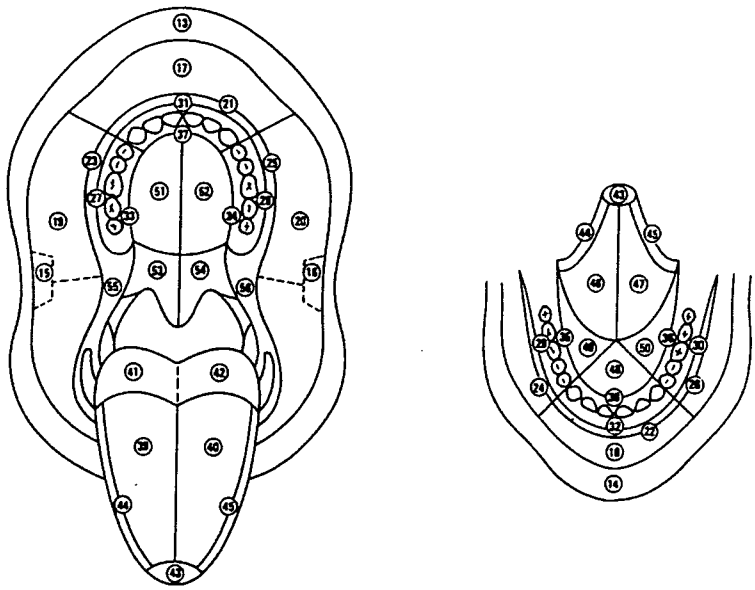

Figure 2. Topography of the oral mucosa modified from Roed-Petersen *et al* (12). The numbered locations are referred to in Table II.

Table II. Site involvement and clinical form of oral lichen planus (OLP) in subjects with HCV infection.

No.	Sex	Type	Smoking history	2000	2001	2002	2003	Course of OLP
1	F	Reticular	Negative	30	30	24, 30	24, 30	No change
2	M	Reticular	Negative	20	20	20	20	No change
3	F	Erosive	Negative	15, 16, 19-21, 23, 25, 27, 28, 31, 33, 34, 37, 39, 40, 46, 47	15, 16, 19-21, 23, 25, 27, 28, 31, 33, 34, 37, 39, 40, 46, 47	Not screened	Liver cirrhosis death	Unknown
4	F	Erosive	Negative	14, 19, 20	Not screened	Not screened	14, 19, 20	Exacerbation
5	F	Erosive	Negative	20, 45	Not screened	20, 45	Not screened	Unknown
6	F	Erosive	Negative	Not screened	19, 20, 47	Not screened	14, 19, 20, 46, 47, 51	Exacerbation
7	F	Reticular	Negative	ND	19, 55	Not screened	19, 55	No change
8	F	Reticular	Negative	Not screened	20, 26, 30	20, 26, 30	20, 26, 30	No change
9	M	Reticular	Negative	ND	46	29, 30, 35, 36, 46	29, 30, 35, 36, 46	No change
10	F	Reticular	Negative	Not screened	26, 30	26, 30	26, 30	No change
11	F	Erosive	Negative	ND	ND	14	14	No change
12	F	Erosive	Negative	Not screened	Not screened	17	17	Alleviation
13	F	Reticular	Negative	ND	Not screened	30	30	No change
14	F	Reticular	Negative	ND	ND	29	29	No change
15	F	Erosive	Positive	Not screened	Not screened	14-16, 19, 20, 24, 26, 32, 44, 45, 51, 52	Not screened	Unknown

ND, not detected. The numbers below the dates refer to locations in the oral mucosa as seen in Fig. 2.

Figure 3. A representative oral erosive lichen planus on the right buccal mucosa.

We conducted an epidemiological investigation to ascertain the possible correlation between OLP and HCV infection in patients living in Western Japan (9-11,18), where the prevalence of HCV infection is the highest in the country (15,19). We found the incidence of OLP in our patients to be higher than in the general population. OLP aside, the prevalence of other extrahepatic manifestations in subjects with anti-HCV was also higher than in those without HCV (10).

We previously reported a study on an HCV hyperendemic area, O town, with a population of approximately 3,900 in the northwest region of the Hiroshima prefecture in Honshu, Japan (11). The incidence there of subjects with 1 or more extrahepatic manifestations of HCV was 66.1%. In the present investigation, we examined extrahepatic manifestations in the same place over a 4-year period. Inhabitants with HCV infection had various extrahepatic manifestations, including OLP. The prevalence of OLP increased with the age of the subjects. This is consistent with an earlier study of inhabitants of the Fukuoka prefecture (20).

Patients with HCV-associated HCC in Japan are aging. People with chronic HCV infection should be monitored and followed carefully for extrahepatic manifestations. It is necessary for physicians and dentists to have an increased awareness of OLP in order for it to be detected at an early stage and treated promptly.

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HEPATOLOGY

Insulin resistance and lichen planus in patients with HCV-infectious liver diseases

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Abstract

Background and Aim: Hepatitis C virus (HCV) causes liver diseases and extrahepatic manifestations, and also contributes to insulin resistance and type 2 diabetes mellitus (DM). The aims of the present study were to examine the incidence of extrahepatic manifestations including lichen planus in HCV-infected patients and to evaluate the relationship between lichen planus and insulin resistance.

Methods: Of 9396 patients with liver diseases presenting to the study hospital, 87 patients (mean age 60.0 ± 11.5 years) with HCV-related liver diseases were identified and examined for the incidence of extrahepatic manifestations. Insulin resistance and the presence of *Helicobacter pylori* antibodies were also measured.

Results: The prevalence of DM was 21.8% (19/87), hypertension was 28.7% (25/87), thyroid dysfunction was 20.7% (18/87), and extrahepatic malignant tumor was 9.2% (8/87). The prevalence of lichen planus at oral, cutaneous, pharyngeal, and/or vulval locations was 19.5% (17/87). Characteristics of 17 patients with lichen planus (group A) were compared with 70 patients without lichen planus (group B). Prevalence of smoking history, presence of hypertension, extrahepatic malignant tumor, and insulin resistance (HOMA-IR) were significantly higher in group A than in group B. Significant differences were not observed for age, sex, body mass index, diagnosis of liver disease, alcohol consumption, presence of DM, thyroid dysfunction, liver function tests, or presence of *H. pylori* infection between the two groups.

Conclusions: Infection with HCV induces insulin resistance and may cause lichen planus. It is necessary for an HCV-infected patient to be assayed for insulin resistance, and to be checked for different extrahepatic manifestations of this infection, particularly lichen planus.

Introduction

The number of fatalities due to hepatocellular carcinoma (HCC) in Japan continues to increase, and it is estimated that this tendency will continue at least until 2015. Of the HCC cases in Japan, approximately 16% are caused by hepatitis B virus (HBV) infection and approximately 80% by hepatitis C virus (HCV) infection.[1] The average prevalence of HCV carriers in Japan is about 2%, with the absolute number estimated at 2 million.[2] The increase in HCC in Japan depends on the spread of HCV infection.[2]

Infection with HCV induces various extrahepatic manifestations as well as chronic liver diseases.[3,4] HCV infects cells or organs except hepatocytes and multiplies. Representative extrahepatic manifestations of HCV infection include lichen planus, diabetes mellitus (DM), malignant lymphoma, Sjögren's syndrome, cryoglobulinemia, and membranoproliferative glomerulonephritis. It has been reported that combined therapy using interferon and ribavirin is effective for different extrahepatic manifestations that are apt to be overlooked.[5,6]

At present, it has been shown that HCV multiplies in skin and oral mucosa leading to HCV-related lichen planus,[7,8] and that the risk of malignant transformation is higher in lichen planus with HCV infection than in lichen planus without HCV.[9] However, a mechanism for these extrahepatic manifestations has not been elucidated. Recently it was reported that there is a significant correlation between lichen planus and HCV and DM in southern Taiwan, particularly in HCV patients with elevated serum alanine aminotransferase (ALT) levels and atrophic-erosive oral lichen planus (OLP).[10] In our previous report, patients with lichen planus having DM were all found to be HCV-infected.[11]

In addition, it has been reported that DM is a risk factor for HCV-related hepatocarcinogenesis[12] and for decreased survival

among liver cirrhosis patients.[13] In addition, the incidence of diabetes in patients having HCV-related liver cirrhosis is higher than that in patients with HBV-related liver diseases.[14]

We recently showed molecular mechanisms for HCV core-induced insulin resistance.[15] HCV core up-regulates the suppressor of cytokine signaling (SOCS) 3, and inhibits insulin signaling by down-regulation of insulin receptor substrate (IRS) -1 and IRS-2 in hepatocytes. Moreover, in an epidemiological survey, we demonstrated that a significant increase in the incidence of diabetes occurs in subjects with high titers of HCV core compared to subjects who are negative for anti-HCV antibody[16] and concluded that HCV infection induces insulin resistance, which causes an increase in the incidence of extrahepatic manifestations in HCV-infected individuals.[17]

In the current study, we surveyed the incidence of abnormal glucose tolerance in patients with or without lichen planus in a study population with HCV-related chronic liver disease, and investigated the relationship between lichen planus and insulin resistance.

Methods

Patients

A total of 105 984 consecutive patients had checkups for chronic liver disease for the first time in the Digestive Disease Center at Kurume University Hospital from April 1988 to August 2005. In the Digestive Disease Center, physicians, surgeons, radiologists, and an oral surgeon hold full-time positions. One of us (M.S.) is a hepatologist and examined 9396 of these 105 984 patients. There were 522 patients who were HCV antibody positive and who thereafter continued with regular hospital visits until April 2006.

Exclusion criteria were the following: (i) other causes of chronic liver disease or disease other than chronic HCV infection; (ii) liver disease related to HBV infection; and (iii) patients treated with interferon therapy at the time of study inclusion.

We examined the presence of extrahepatic manifestations of chronic HCV infection in 87 patients. Informed consent was obtained from all patients after the purpose and methods of the study were explained. The 87 patients were 44 men and 43 women with a mean age of 60.0 ± 11.5 years.

The patients were monitored for the presence of extrahepatic manifestations of HCV infection such as lichen planus, DM, hypertension, thyroid dysfunction, and extrahepatic malignant tumor as well as liver disease. Biochemical tests were done and insulin values, blood glucose levels, and *Helicobacter pylori* antibody were measured in patient blood samples. Life histories were taken.

Clinical examinations

Patients received oral mucosa and cutaneous medical examinations by an oral surgeon and a dermatologist. The diagnosis of OLP was made on the basis of clinical and histopathological features. Diagnosis of type 2 DM was based on the American Diabetic Association (ADA) criteria of 1997.[18] Persons in whom diabetes was diagnosed before 30 years of age and who used insulin were categorized as type 1 DM and were excluded from our study.

The following definitions of cardiovascular disease were employed. Obesity was defined as a body mass index (BMI) >25 kg/m^2 or higher. Hypertension was defined as a systolic blood pressure (SBP) of 140 mmHg or higher, or a diastolic blood pressure (DBP) of 90 mmHg or higher according to the criteria of JNC-VI of the International Hypertension Society.[19] Thyroid hormones such as FT3, FT4 and thyroid stimulating hormone were measured for all patients, and thyroid echography examination was performed for some patients. Examination of the upper gastrointestinal tract or lower digestive tract was performed on patients for whom it was deemed clinically necessary.

We also took a history of smoking and alcohol consumption.

Serological assays

Serum samples from the 87 patients were collected and tested for platelets (PLT) and for the following liver function tests: serum ALT, aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (γ-GTP), lactate dehydrogenase (LDH), total bilirubin (TBil), direct bilirubin (DBil), thymol turbidity test (TTT), zinc sulfate turbidity test (ZTT), total cholesterol (TC), total protein (TP), and albumin (Alb). Sera were also examined for the presence or absence of HCV or HBV infection. Anti-HCV was measured by a chemiluminescent enzyme immunoassay kit (Lumipulse II HCV, Fujirebio, Tokyo, Japan). HCV RNA in serum was detected using the Amplicore HCV test (Roche, Tokyo, Japan). Hepatitis B virus surface antigen (HBsAg) was assayed using a chemiluminescent immunoassay kit (Architect, HBsAg QT, Dainabot, Tokyo, Japan). Ultrasonographic examination for all patients was performed in order to investigate the shape of the liver and lesions occupying the liver. Computed tomography and liver biopsy were performed in some patients. Most patients underwent endoscopy for detection of esophagogastric varices. We used other possible predictors of liver cirrhosis progression, including serum albumin, TBil, prothrombin time, and PLT.

Plasma glucose levels were measured by a glucose oxidase method for all subjects and serum insulin levels were measured using a sandwich enzyme immunoassay kit (Eiken Chemical, Tokyo, Japan). Insulin resistance (IR) was calculated on the basis of fasting levels of plasma glucose and insulin, according to the homeostasis model assessment (HOMA-IR) method.[20] The formula for the HOMA-IR is: HOMA-IR = fasting glucose (mg/dL) × fasting insulin (μU/mL)/405.

The presence of serum IgG antibodies against *H. pylori* antibody were measured by the SRL (Tokyo) using E Plate *H. pylori* antibody produced by Eiken Chemical.

Statistical analysis

The chi-squared test and the unpaired Student *t*-test were used for statistical analyses. Differences were judged significant for $P < 0.05$ (two-tailed). This study was approved by the Institutional Review Board/Ethics Committee of our Institution.

Results

Among 87 patients with HCV-related liver diseases, the prevalence of lichen planus was 19.5% (17/87), DM was 21.8% (19/87),

Table 1 Clinical characteristics of 87 patients with HCV-related liver diseases according to presence of lichen planus (LP)

Clinical characteristic	All patients	Group A (with LP)	Group B (without LP)	*P*-value (A *vs* B)
No. subjects	87	17	70	–
Age (years)	60.0 ± 11.5	63.7 ± 10.6	59.1 ± 11.6	NS
Sex (M/F)	44/43	11/6	33/37	NS
BMI (kg/m^2)	22.8 ± 2.9	23.9 ± 2.8	22.5 ± 2.9	NS
Smoking history	32 (36.8)	10 (58.8)	22 (31.4)	0.0356
Alcohol consumption percentage	50 (57.5)	10 (58.8)	40 (57.1)	NS
Diagnosis of liver disease				
Past history of HCV infection	1	0	1	NS
Chronic hepatitis C	69	11	58	
HCV-related liver cirrhosis	9	3	6	
HCV-related HCC	8	3	5	
Comorbidities				
Diabetes mellitus	19 (21.8)	4 (23.5)	15 (21.4)	NS
Hypertension	25 (28.7)	10 (58.8)	15 (21.4)	0.0022
Thyroid dysfunction	18 (20.7)	5 (29.4)	13 (18.6)	NS
Extrahepatic malignant tumor	8 (9.2%)	5 (29.4)†	3 (4.3)‡	0.0013

Values shown as *n* (%) or mean ± SD. BMI, body mass index; F, female; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; M, male; NS, not significant.

†Tumors were: gastric cancer (two), tongue cancer (one), larynx cancer (one), and renal and colon cancer (one). ‡Tumors were: gastric cancer (one), colon cancer (one), and gallbladder cancer (one).

hypertension was 28.7% (25/87), thyroid dysfunction was 20.7% (18/87), and extrahepatic malignant tumor was 9.2% (8/87).

We compared characteristics of 17 patients who had lichen planus (group A) and 70 patients who did not have lichen planus (group B). The mean age in group A was 63.7 ± 10.6 years; there were 11 men and six women. The mean age in group B was 59.1 ± 11.6 years; there were 33 men and 37 women. Table 1 shows clinical features of groups A and B. The diagnoses of liver diseases in group A were chronic hepatitis C infection (11 patients), HCV-related liver cirrhosis (three patients), and HCV-related HCC (three patients). Those of group B were chronic hepatitis C infection (58 patients), HCV-related liver cirrhosis (six patients), HCV-related HCC (five patients) and past history of HCV infection (one patient) (Table 1).

The prevalence of smoking history ($P = 0.0356$), hypertension ($P = 0.0022$), and extrahepatic malignant tumor ($P = 0.0013$) were significantly higher in group A than in group B (Table 1). Diagnoses of extrahepatic malignant tumors in group A were: tongue cancer (one squamous cell carcinoma), larynx cancer (one squamous cell carcinoma), gastric cancer (one adenocarcinoma, one signet ring cell carcinoma), renal and colon cancer (one renal cell carcinoma). Diagnoses of extrahepatic tumor in group B were: gastric cancer (one adenocarcinoma), colon cancer (one adenocarcinoma), and gallbladder cancer (one adenocarcinoma). Significant differences were not observed for age, sex, BMI, liver disease, alcohol consumption, presence of DM, or thyroid dysfunction between these two groups.

We analyzed for differences between these two groups in liver assays, blood platelets, insulin, blood glucose, HOMA-IR, and presence of *H. pylori* infection. The laboratory data of both groups are shown in Table 2. Prevalence of insulin ($P = 0.0076$) and HOMA-IR ($P = 0.0113$) were significantly higher in group A than in group B (Table 2). Significant differences were not observed for serum AST, ALT, LDH, γGTP, TP, Alb, TBil, DBil, TTT, ZTT, TC, blood platelets, blood glucose, or presence of *H. pylori* infection between these two groups.

Seventeen patients had OLP at a total of 24 sites. The site of occurrence was: buccal mucosa in 13 (76.5%), lower lip in six (35.3%), upper lip in two (11.8%), gingiva in one (5.9%), tongue in one (5.9%), and floor of mouth in one (5.9%) (Table 3). The sites of lichen planus except oral mucosa were lower leg in four (23.5%), antebrachium in one (5.9%), skin extremities in two (11.8%), hypopharynx in one (5.9%), and vulva in one (5.9%). Biopsies of hypopharyngeal lichen planus were performed by an otolaryngologist, and of vulvar lichen planus by a gynecologist. The erosive and reticular variety, respectively, was found to be the prevalent form (Table 3).

Discussion

We performed an epidemiological survey for extrahepatic manifestations and HCC in an HCV hyperendemic area in Japan.[21,22] Anti-HCV positivity among residents of this area in 1990 was 23.6%.[23] We found that the prevalence of extrahepatic manifestations among individuals with HCV infection was higher than among those without HCV,[22] and found an association between HCV core, insulin resistance, and the development of type 2 DM.[16] Recently, we reported that insulin resistance in inhabitants who have an extrahepatic manifestation including OLP with HCV infection shows significantly greater increases than for inhabitants who have neither an extrahepatic manifestation nor HCV infection.[17] By the results of these epidemiological surveys we think that insulin resistance induced by HCV infection causes an increase in the incidence of extrahepatic manifestations in HCV-infected individuals.

In this study, we did long-term follow up for insulin resistance from the standpoint of lichen planus among patients who we identified as having HCV-related chronic liver disease at our hos-

Table 2 Laboratory data of 87 patients with HCV-related liver diseases according to presence of lichen planus (LP)

Laboratory assay	All patients	Group A (with LP)	Group B (without LP)	*P*-value (A *vs* B)
AST (IU/L)	61.1 ± 38.1	60.9 ± 33.5	61.2 ± 39.3	NS
ALT (IU/L)	68.2 ± 46.7	62.4 ± 39.6	69.6 ± 48.5	NS
LDH (IU/L)	216.8 ± 62.8	205.8 ± 72.1	219.6 ± 60.6	NS
γ-GTP (IU/L)	64.1 ± 68.4	63.5 ± 50.0	64.2 ± 72.5	NS
TP (g/dL)	7.7 ± 0.5	7.7 ± 0.5	7.7 ± 0.5	NS
Alb (g/dL)	4.1 ± 0.5	3.9 ± 0.5	4.2 ± 0.5	NS
PLT (/mm^3)	13.8 ± 5.1	12.5 ± 5.0	14.1 ± 5.09	NS
TBil (mg/dL)	1.1 ± 0.6	1.2 ± 0.9	1.0 ± 0.5	NS
DBil (mg/dL)	0.2 ± 0.2	0.2 ± 0.3	0.2 ± 0.2	NS
TTT	16.2 ± 6.7	18.4 ± 4.7	15.8 ± 7.0	NS
ZTT	20.6 ± 6.9	21.8 ± 5.8	20.3 ± 7.2	NS
TC (mg/dL)	172.3 ± 35.8	164.3 ± 41.9	174.1 ± 34.4	NS
Insulin (μU/L)	23.3 ± 42.0	47.3 ± 87.8	17.4 ± 15.4	0.0076
Blood glucose (mg/dL)	97.4 ± 30.1	103 ± 33.2	96.1 ± 29.5	NS
HOMA-IR	7.1 ± 18.8	17.4 ± 40.0	4.6 ± 6.0	0.0113
Helicobacter pylori antibody (*n* (%))	58 (66.7)	10 (58.8)	48 (68.6)	NS

Values shown as mean ± SD. Alb, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; DBil, direct bilirubin; γ-GTP, gamma-glutamyl transpeptidase; HOMA-IR, homeostasis model assessment; LDH, lactate dehydrogenase; NS, not significant; PLT, platelets; TBil, total bilirubin; TP, total protein; TTT, thymol turbidity test; TC, total cholesterol; ZTT, zinc sulfate turbidity test.

Table 3 Location of lichen planus in 17 patients with hepatitis C virus-related liver diseases

No	Sex	Age (years)	Liver disease	Lichen planus location			Type
				Cutaneous	Oral	Other	
1	M	71	CH	Antebrachium	–	–	–
2	M	60	CH	Extremities	–	–	
3	F	70	LC	–	Gingiva	–	Erosive
4	M	72	LC	–	Lower lip	–	Reticular
5	F	64	LC	Leg	Buccal mucosa, upper lip, lower lip	–	Erosive
6	M	66	CH	Leg	Buccal mucosa, upper lip, lower lip	–	Erosive
7	M	59	CH	–	Buccal mucosa (reticular)	Pharynx (erosive)	Erosive + reticular
8	M	66	CH	Leg	Buccal mucosa, lower lip	–	Reticular
9	M	57	CH	–	Buccal mucosa	–	Reticular
10	M	50	CH	–	Buccal mucosa, tongue, lower lip	–	Erosive
11	F	77	CH	–	Buccal mucosa	–	Atrophic
12	F	75	CH	–	Buccal mucosa	–	Reticular
13	M	62	HCC	–	Buccal mucosa, lower lip	–	Erosive
14	F	83	HCC	Leg	Buccal mucosa (atrophic)	Vulva (erosive)	Atrophic + erosive
15	M	41	CH	–	Buccal mucosa	–	Reticular
16	M	58	HCC	Extremities	Buccal mucosa, floor of mouth	–	Erosive
17	F	53	CH	–	Buccal mucosa	–	Reticular

CH, chronic hepatitis C; F, female; LC, HCV-related liver cirrhosis; HCC, HCV-related hepatocellular carcinoma; M, male.

pital. Although there was no significant difference in fasting glucose levels and BMI between patients with and without lichen planus, fasting insulin levels and HOMA-IR values, an indicator of insulin resistance, were significantly higher in patients who had lichen planus than in those who did not.

In the present study, insulin levels (17.4 ± 15.4 μU/L) and HOMA-IR values (4.6 ± 6.0) in patients having HCV infection without lichen planus (group B) were higher than the normal range. Normal values for insulin are 3.06–16.9 μU/L, and for HOMA-IR are less than 2. Therefore, the significantly higher insulinemia in patients such as those in group A (among HCV infectious patients) might cause lichen planus.

In Japan, it is known that the prevalence of HCV infection in patients with lichen planus is high;[11] therefore, interferon therapy is often administered to patients with lichen planus and a persistent HCV infection. However, it has been reported that patients cannot

complete interferon therapy because of aggravation of lichen planus.[24,25] The measurement of insulin resistance as well as a search for lichen planus may be useful before performing interferon therapy. A large series of patients with OLP was evaluated for extraoral involvement by Eisen *et al.*[26] They concluded that any patient with OLP should undergo a through history and examination as part of an investigation of potential extraoral manifestations, because a high percentage of patients with OLP develop extraoral manifestations. In our 17 cases of lichen planus, cutaneous lichen planus was diagnosed in seven (41.2%), hypopharynx in one (5.9%), and vulva in one (5.9%). The simultaneous appearance of extraoral and oral lesions was noted among six (35.3%). Because the majority of OLP patients suffer from lichen planus of the genitalia,[27] clinicians should follow OLP patients with sufficient attention to the presence of extraoral manifestations.

Sikuker *et al.* evaluated an association between HCV infection and extrahepatic malignancies. Extrahepatic malignancies were found in 14.6% of anti-HCV positive patients.[28] The incidence of extrahepatic malignant tumor in our subjects was 9.2% (8/87). The insulin-like growth factor family of proteins plays a key role in cellular metabolism, differentiation, proliferation, transformation and apoptosis, during normal development and malignant growth.[29] The hyperinsulinemia that HCV infection causes may induce an extrahepatic malignant tumor as well as HCC.

Many studies have shown that *H. pylori* is involved in the pathogenesis of gastric cancer.[30] The seroprevalence of *H. pylori* is 71% in Japanese aged 50–59 years, and is 81% in those aged 60–69 years.[31] This is almost the same as the seroprevalence of our patients, which was 66.7% (58/87) overall and 82.6% (19/23) in those aged 60–69 years. Seroprevalence of *H. pylori* in our three subjects with gastric cancer was 66.7%. In our study, we did not find an association between *H. pylori* and lichen planus in patients with HCV-infectious liver diseases.

In conclusion, we investigated the association of insulin resistance and lichen planus among patients with HCV-infected chronic liver diseases. The significant factors for development of lichen planus were smoking history, presence of hypertension, extrahepatic malignant tumor, and insulin resistance (HOMA-IR). This supports our previous conclusion that insulin resistance in patients who have an extrahepatic manifestation of HCV infection increases more than insulin resistance of patients who have neither an extrahepatic manifestation nor HCV infection. HCV-infected patients with lichen planus should pay attention to the development of an extrahepatic malignancy. Cooperation with an oral surgeon and a hepatologist is vital for early diagnosis and treatment of any extrahepatic manifestations.

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Original Article

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/mL (group A-1)	PLT < 150 000/mL (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/mL (group B-1)	PLT < 150 000/mL (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 ($\times 10^4$/mL)	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-

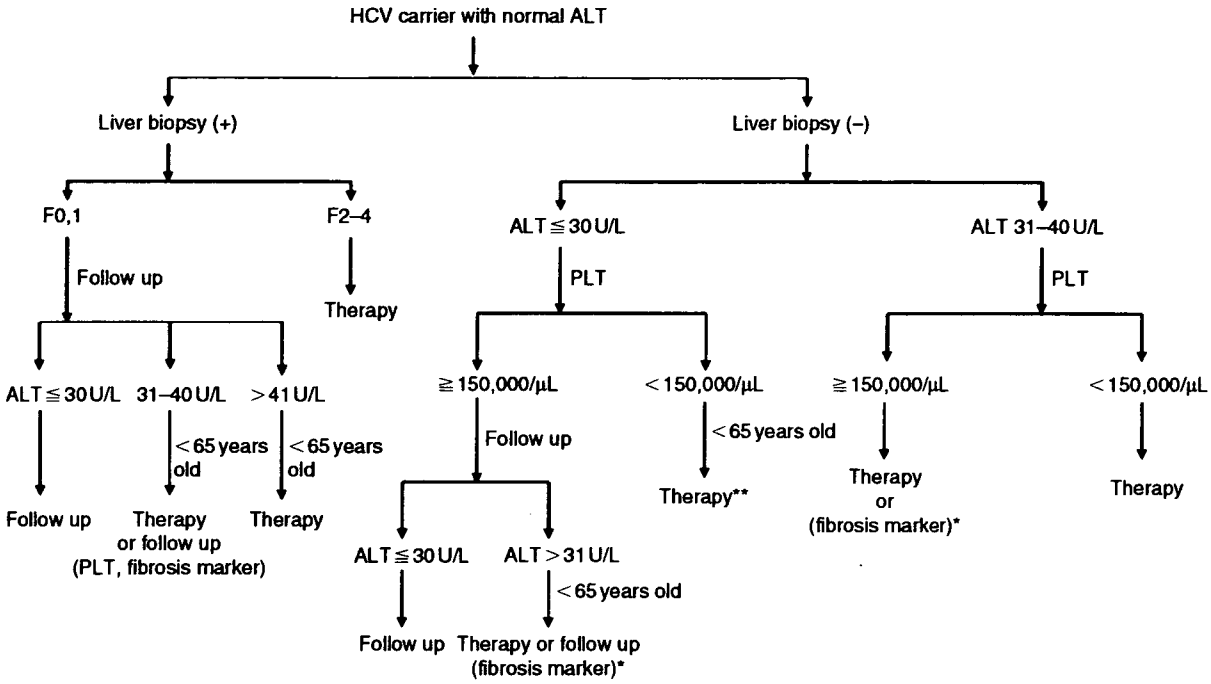

Figure 1 Algorithm for the management of hepatitis C virus (HCV) carriers with normal serum aminotransferase (ALT, ≤40 U/L) focused on the inhibition of the development of hepatocellular carcinoma. In patients who underwent liver biopsy, F0 and F1 patients younger than 65 years are candidates for antiviral 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 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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