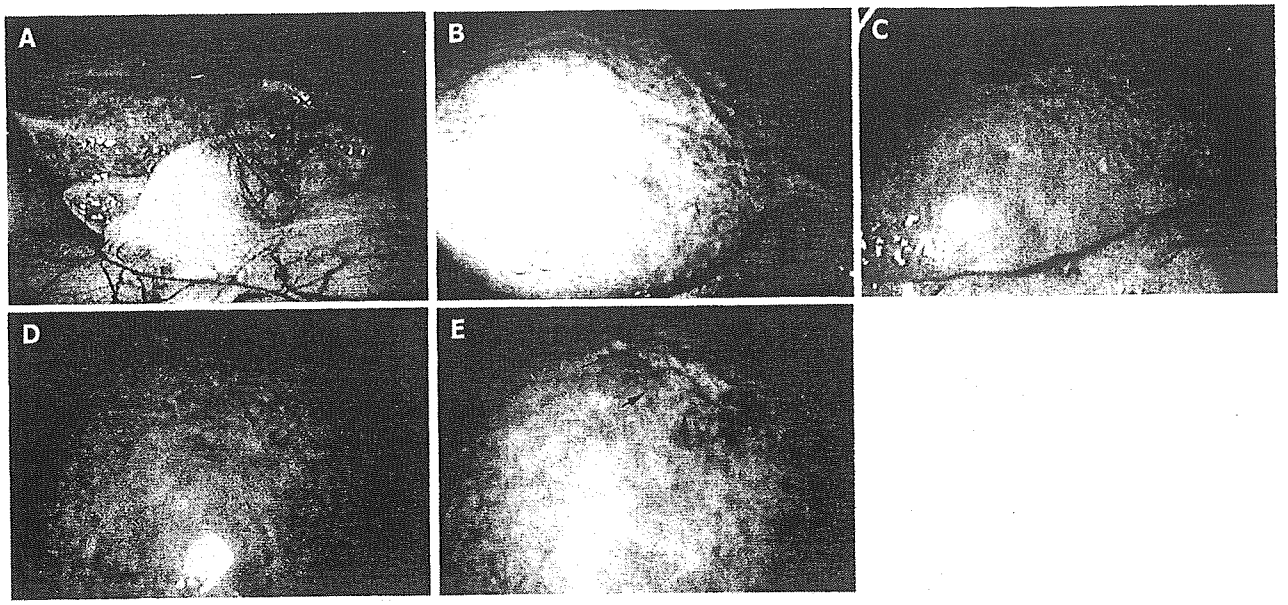

Figure 2 Irregular and deformed liver surface with multiple indentations and shallow linear depressions (**A** and **B**: right lobe, **C** and **D**: left lobe). A wide scar on the surface of S_2 at the site where metastatic tumor existed before chemotherapy (**E**, arrowheads).

over 200 μg/dL. The patient presented a rapid downhill course and died of respiratory failure due to lymphangitis carcinomatosa on March 20, 2004.

DISCUSSION

When metastatic breast carcinoma in the liver does not form space-occupying lesions, its radiological diagnosis is difficult. There are some case reports on diffuse infiltration of breast cancer cells found at autopsy, which was not diagnosed even with the most advanced modalities like CT scan, ultrasonography and magnetic resonance imaging[5-8]. In the present case, radiological diagnosis was difficult because dynamic study by CT scan only demonstrated uneven hepatic blood supply, which is also seen in chronic or acute liver injury. Indeed, definitive diagnosis was made only by laparoscopy-assisted liver biopsy.

Carcinomatous involvement of the liver mimicking cirrhosis is a rare complication of metastatic carcinoma, most frequently observed with scirrhous carcinoma of the breast[1-4]. Borja *et al.*[1], reported a case of metastatic breast cancer, which presented various clinical manifestations of liver failure grossly characterized by a distorted liver surface caused by multifocal portal obstruction with cancer cells.

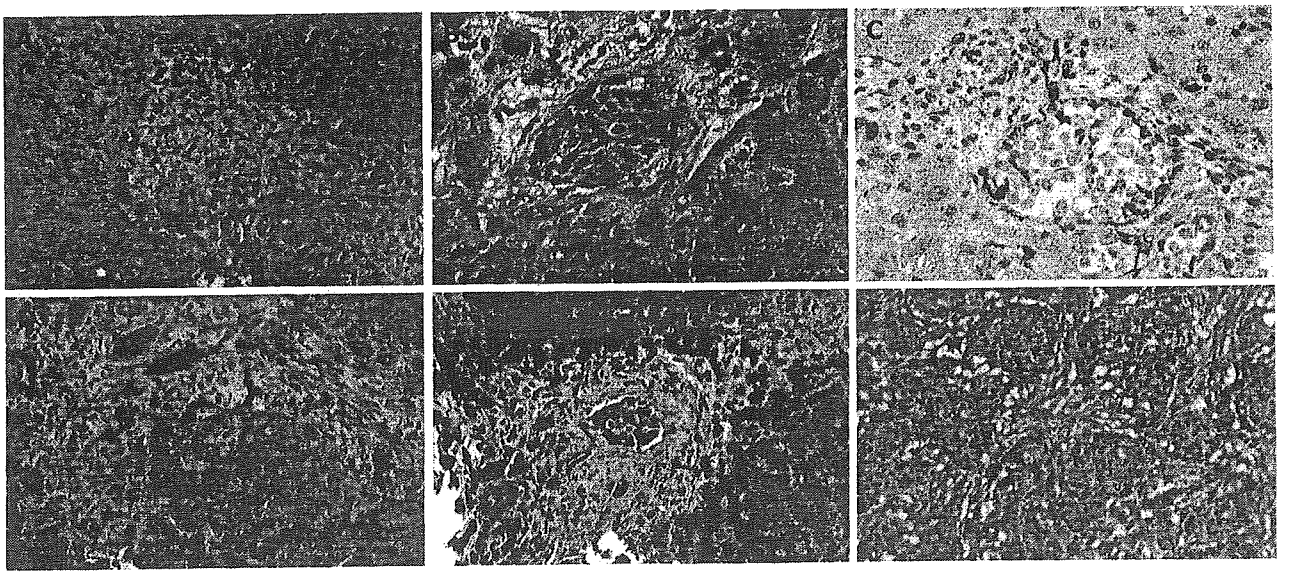

Figure 3 **A**: Laparoscopic liver biopsy from a wide scar lesion showed residual cancer cells scattered in a wide fibrous band in one area (hematoxylin and eosin (HE) stain). **B** and **C**: In another area intraportal tumor thrombi were clearly demonstrated. Endothelial cells were stained brown by CD31 immunohistochemistry (**B**, HE stain, **C**, CD31 immunohistochemical stain). **D** and **E**: Cancer cells showed desmoplastic change around them, which extended toward the sinusoids (**D**, HE stain, **E**, Masson's trichrome stain). F: Original breast cancer. Note desmoplastic change around the tumor cells (HE stain).

Busni *et al.*[9], first introduced the term hepar lobatum carcinomatosum (HLC), which was defined as an acquired hepatic deformity consisting of an irregularly lobulated hepatic contour caused by intravascular infiltration of metastatic carcinoma. To our knowledge, only nine cases of HLC have been reported in the literature to date[9-13].

In the present case, although radiological examinations revealed neither distorted nor irregularly lobulated liver surface, laparoscopy presented a slightly irregular and deformed liver surface with multiple indentations and shallow linear depressions. A biopsy sample from the wide fibrous scar revealed the intraportal infiltration of cancer cells surrounded by fibrous tissues. As speculated by Gravel *et al.*[13], multifocal carcinomatous obstruction of portal veins caused circulatory deficiency leading to multiple scattered and linear depressions on the liver surface, which were interpreted as fibrous scarring. In addition, desmoplastic change caused by carcinoma cells may have also contributed to the formation of fibrous scar tissue. These pathologic conditions are basically the same as those of HLC, and therefore, our case can be categorized as the same entity, though it was not so far advanced in terms of hepatic deformity.

Combination chemotherapy with cyclophosphamide and epirubicin was performed in this case. Various chemotherapeutic agents were given to eight out of the nine previously reported HLC patients, but most of the agents, including cyclophosphamide and doxorubicin (the same group as epirubicin) are not known to induce fibrogenesis by themselves or even synergistically. Hence, some authors[10-14] speculate that fibrosis in HLC is not caused by direct effect of chemotherapeutic agents, but is actually scar contraction after tissue collapse by chemotherapy-induced tumor necrosis or regression. In the present case, this condition occurred after chemotherapy which caused the disappearance of metastatic liver tumors. Furthermore, a wide scar was observed at the site where the metastatic tumor existed before chemotherapy. These findings also support the possibility of chemotherapy as another important but indirect cause of fibrous scarring.

In this case, drowsiness, disorientation and insomnia, and a high level of serum ammonia were suggestive of hepatic encephalopathy. However, lactulose and branched-chain amino acid supplementation were not effective, and serum ammonia level stayed over 200 μg/dL. In addition, leg edema with low serum albumin level mimicked the clinical manifestation of cirrhosis. These clinical symptoms and laboratory data indicate that rapidly progressive and diffuse circulatory disturbance due to carcinomatous obstruction of portal veins can cause some symptoms of hepatic failure[15-18].

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A follow-up study to determine the value of liver biopsy and need for antiviral therapy for hepatitis C virus carriers with persistently normal serum aminotransferase

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***Background/Aims*: Long-term follow-up study was performed to identify the candidates for antiviral therapy for hepatitis C virus (HCV) infection among carriers with persistently normal aminotransferase (ALT ≤ 30 U/L) levels (PNAL).**

***Methods*: One hundred and twenty-nine HCV carriers with PNAL who underwent liver biopsy and had platelet count over 150,000/μl were entered and 69 were followed for over 5 years. Thirty-five patients underwent serial liver biopsies. Serum ferritin and thioredoxin levels were also determined.**

***Results*: Seventeen patients had normal liver histology, 10 had moderate chronic hepatitis and the remainder 102 had mild hepatitis. Serum ferritin and thioredoxin levels were normal. The mean follow-up period for the 69 patients was 8.5 years. Of these 69 patients, 10 had persistently normal ALT levels (group A), 39 had transient elevation of ALT (group B), and 20 changed to symptomatic chronic hepatitis (group C). The rate of progression of fibrosis for groups A, B, and C were 0.05, 0.04, and 0.08, respectively. Hepatocellular carcinoma was not diagnosed in any of the patients.**

***Conclusions*: Around 90% of HCV carriers with PNAL have normal to mild liver histology. This long-term follow-up study demonstrated that 30% of such carriers became candidates for antiviral therapy within 5 years.**

***Keywords*: Hepatitis C virus; Chronic hepatitis C; Asymptomatic HCV carrier; Normal serum ALT; Interferon**

1. Introduction

An estimated 170 million individuals are infected with hepatitis C virus (HCV) worldwide and chronic hepatitis C has recently become the leading cause of liver cirrhosis and hepatocellular carcinoma (HCC) in many countries including Japan. Most HCC develop in patients with advanced staged chronic hepatitis or cirrhosis, and rarely from mild chronic hepatitis type C.

It is thought that type C liver cirrhosis and HCC develop over 20–35 years following HCV infection [1], however, around 25% of patients with chronic HCV infection have normal serum aminotransferase (ALT) levels [2,3]. We reported previously that asymptomatic HCV carriers were predominant among females and that most of them had histologically minimal to mild chronic hepatitis [4]. In that paper, we defined asymptomatic HCV carriers as persistently HCV RNA positive patients with normal serum ALT levels (≤30 U/L) over 1 year. However, it has been reported that HCV carriers with normal serum ALT level had more advanced liver histology compared to HCV carriers with elevated serum ALT [5]. This discrepancy might be attributed to differences in the definition of the normal range of serum ALT used by various centers, however, it is very important to clarify whether HCV carriers with persistently normal ALT level (PNAL) are candidates for antiviral therapy.

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The current normal limit of serum ALT is 40 U/L, however, a recent report from an Italian group demonstrated that the healthy ranges for serum ALT were 30 U/L for men and 19 U/L for women, respectively [6], which are lower than the current values that have been used over the past 15 years. This criterion of normal serum ALT might be reasonable because a few cirrhotic patients have from 30 to 40 U/L of ALT [7].

In Japan, the number of HCC patients with HCV infection has increased since 1975. Antiviral treatment for chronic hepatitis C resulted in the inhibition of hepatic inflammation and progression of hepatic fibrosis and as a consequence the inhibition of the development of HCC [8–13]. Thus, inhibition of HCC is a very important issue in the treatment of patients with chronic hepatitis C. It remains controversial whether asymptomatic HCV carriers are candidates for antiviral therapy because of the low efficacy of treatment and flare-ups post treatment. However, taking into consideration the recent progress in antiviral therapy for chronic hepatitis C patients, the National Institute of Health Consensus Development Conference reported that patients with hepatitis C with normal serum ALT levels are candidates for interferon and ribavirin therapy [14]. Recently, a multicenter, randomized, controlled study for the treatment of patients with chronic hepatitis and persistently normal ALT levels with pegylated interferon alpha and ribavirin for 48 weeks led to eradication in 40% of patients infected with genotype 1b patients [15], which is similar to the results for symptomatic chronic hepatitis C patients [16,17]. However, most HCV carriers with PNAL have minimal to mild liver histology and their prognosis might be very good. Thus, there is some doubt, whether they are candidates for antiviral treatment to inhibit the progression of liver disease and hepatocarcinogenesis.

Recently, it has been reported that oxidative stress is an important factor in the development of HCV-related HCC [18–22] and the HCV core protein may generate oxidative stress via mitochondrial injury [23,24]. It is also demonstrated that iron overload generates oxidative stress, resulting in hepatic injury, and DNA damage and consequently this becomes an important factor for hepatocarcinogenesis [22,25,26].

We report here the biochemical and histological results of 8.5 years of follow-up of HCV carriers with PNAL. The data were analyzed according to the definitions of normal range ($\leq$30 U/L) of serum ALT and platelet count (PLT) over 150,000 μl/ml. We also analyzed the status of oxidative stress using serum ferritin and thioredoxin levels. These results demonstrate the importance of the normal range of serum ALT, oxidative stress and follow-up study to decide the indication for antiviral therapy of HCV carriers with PNAL.

2. Patients and methods

2.1. Eligibility and definition

This study was conducted from January 1990 to August 2004.

HCV carriers with persistently normal ALT levels (PNAL) were defined as those patients who were HCV RNA positive by reverse transcriptase polymerase chain reaction (RT-PCR), had normal serum ALT levels ($\leq$30 U/L) over a 12-months period and on least three different occasions and platelet count of over 15×10^4 μl/ml. Patients positive for hepatitis B surface antigen (HBsAg), previous interferon (IFN) treatment, a history of heavy alcohol abuse, anti-nuclear antibody (ANA) and anti-smooth muscle antibody (ASMA) positivity, patients with overt Diabetes mellitus and obesity (body mass index; over 30 kg/m^2) were excluded from this study.

The study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki, and approved by the Ethics Committee of Kyoto Prefectural University of Medicine. Informed consent was obtained from every patient.

2.2. Quantification and determination of HCV RNA and genotyping

Frozen-stored sera from 129 individuals were tested. Serum HCV RNA levels was determined using the AMPLICOR GT HCV MONITOR (Roche Diagnostic Systems, Tokyo, Japan). The detection range of this assay was between 0.5 and 850 KIU/ml, and each sample was measured again after dilution with distilled water. HCV genotypes 1 and 2 were determined by a serologic genotyping assay [27]. Genotypes 1 and 2 in this assay correspond to genotype 1 (1a, 1b) and 2 (2a, 2b) proposed by Simmonds et al. [28].

2.3. Study design

Of the 129 patients who underwent liver biopsy, 69 patients enrolled in this study and followed over 5 years (8 males, 61 females). These patients received blood tests every 4 months for an initial 2 years and then received blood tests every 6 months when they remained still normal ALT. αfetoprotein (AFP) was measured every years in all patients, and all patients underwent ultrasonography every year to detect HCC.

All patients submitted to a liver biopsy using a Menghini needle guided by ultrasonography prior to entry. Formalin-fixed liver specimens were stained with hematoxylin and eosin for morphological evaluation, with Masson's trichrome stain for assessment of fibrosis, and with Perls' Prussian blue stain (from February 1998) for assessment of iron loading. Histological follow-up studies were carried out for 35 patients 3.4–13.4 years (mean: 6.8 years) after the first biopsy.

The histological findings of HCV carriers with PNAL were interpreted and scored according to the classification proposed by Desmet et al. [29] and Ishak et al. [30]. Steatosis is defined having fat droplets in over 10% of hepatocytes. The degree of iron loading was assessed using a Perls' score of 0 to 4+, based on the scoring system of MacSween et al. [31].

Fasting blood samples were collected in the morning. Serum ALT, blood glucose level, serum ferritin, platelet count (PLT), serum HCV RNA level and HCV genotype were examined in the laboratory of our university hospital, using the standard analytical method; the ULA ALT value was 30 U/L. Serum thioredoxin (TRX) levels were measured with a sensitive sandwich ELISA kit (Fujirebio, Inc., Tokyo, Japan) as described previously [26,32] and of the 129 patients 47 were available for this assay. Blood chemistry was done every 4–6 months during the follow-up period.

2.4. Statistical analysis

Data values are expressed as medians with interquartile ranges. We compared continuous variables using the Mann–Whitney *U*-test. The Kruskal–Wallis test was used for multiple group comparisons, and Spearman correction coefficients were used to examine the relationship between groups. Frequency analysis was performed with the χ^2 test, and Fisher's exact test. *P* values of less than 0.05 were considered significant.

3. Results

3.1. Demographic and clinical features

The demographic and clinical features of the 129 HCV carriers with PNAL are shown in Table 1. Twenty-four were male and 105 were female. No significant differences were noted in age, serum ALT, PLT, and follow-up period between males and females. Serum ferritin levels were 76.1±53.4 ng/ml in male and 60.0±43.3 ng/ml in female. Serum HCV RNA levels were significantly ($P=0.0012$) higher in G1 compared with G2 (648.7±622.5 KIU/ml vs 356.2±628.8 KIU/ml (Table 1).

Characteristics of the 69 patients followed over 5 years are also shown in Table 1. Their mean follow-up period was 8.5±2.4 years.

Of the 105 female patients, 44 had serum ALT levels ≦19 U/L and 61 had serum ALT levels of 20–30 U/L at entry. There were no significant differences in their ages, platelet count, serum ferritin levels, serum HCV RNA levels, or BMI (Table 2).

Serum thioredoxin (TRX) levels in these patients were within the normal range, and significantly lower than those of patients with chronic hepatitis and cirrhosis (Table 3).

Table 1
Characteristics of 129 HCV carriers with persistently normal ALT who underwent liver biopsy

	$N=129$	Followed over 5 years ($N=69$)
Follow-up period (years)	5.7±3.6	8.5±2.4
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	46.63±11.6
Sex (M/F)	24/105	8/61
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
Male ($N=24$)	20.3±4.4	20.9±5.3
Female ($N=105$)	21.8±4.4	21.2±4.0
Ferritin (ng/ml)	5–225	5–225
Male	76.2±53.5	84.6±59.2
Female	60.0±43.3	66.6±52.5
HCV RNA (KIU/ml)	6–3350	22–2100
G1 ($N=58$)	648.9±622.57*	595.1±561.1** ($N=32$)
G2 ($N=45$)	356.2±628.8	211.0±219.2 ($N=27$)
Mixed and unclassified	6–1994	
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 were expressed as mean±SD. P values were calculated by Mann–Whitney U-analysis with correction for tie. *$P=0.0012$ (G1 vs G2); **$P=0.0006$ (G1 vs G2).

Table 2
Baseline of female patients between HCV carriers having ≦19 U/L of ALT and HCV carriers showing 20–30 U/L of ALT

	ALT≦19 (U/L)	20<ALT≦30 (U/L)	P value
Number of patient	44	61	
Age (y.o)	44.9±12.5	48.8±12.2	
ALT (U/L)	16.0±2.4	24.3±2.9	<0.0001
PLT ($\times 10^4$/μl)	22.0±4.4	21.6±4.3	
HCV RNA (KIU/ml)	400.2±555.1	500.7±541.1	0.3896
BMI (kg/m^2)	21.2±2.3	21.4±2.2	

Values were expressed as mean±SD. P values were calculated by Mann–Whitney U-analysis with correction for tie.

3.2. Liver histology

The results of liver histology for the first biopsy are described in Table 4. Normal liver histology was noted in 17 (14%) subjects, 102 (79%) showed minimal to mild chronic hepatitis, 10 (8%) had moderate chronic hepatitis.

Steatosis was seen in nine patients (7%) and iron loading was noted in 6/50 (12%).

3.3. Follow-up study of laboratory data

Of the 69 patients followed over 5 years (mean±SD: 8.5±2.4 years), 10 (14%) had continuously normal ALT (group A), 39 (57%) showed transient elevation of ALT (group B), and 20 (29%) changed to chronic hepatitis with continuously abnormal serum ALT (group C) (Table 5). Of the 61 female patients, eight were group A, 34 were group B, and 19 were group C. There were no significant differences in age, ferritin levels, serum HCV RNA levels, or BMI among the three groups. However, serum ALT levels were significantly lower in group A compared with group B and C (Table 6). The number of patients having ALT levels ≦19 IU/L in these three groups were seven (7/8:87.5%) in group A, 12 (12/34:35.3%) in group B, and three (3/19:15.8%) in group C.

Table 3
Serum thioredoxin (TRX) levels in 47 HCV carriers with PNAL at liver biopsy

	Serum thioredoxin (ng/ml)
HCV carriers with PANL ($n=47$)	27.7 [9.1–38.5]
Chronic hepatitis ($n=124$)	34.5 [8.6–135.6][a]+
Liver cirrhosis ($n=24$)	42.5 [21.4–97.2][a]++
Control ($n=15$)	24.9 [1.3–50.7][a]

*$P=0.0012$ when compared with G2. The overall significance of differences between four groups according to non-parametric Kruskal–Wallis analysis of variance was $P<0.001$. Therefore, the significance of differences between groups was determined by Scheffe's method: +$P<0.01$; ++$P<0.001$, compared to HCV carriers with PNAL.

[a] These data were reported in J Hepatol 2000; 33: 616–622.

Table 4
Liver histology of 129 carriers at the first biopsy

Grade	Stage of liver fibrosis				Total number of patients
	F0	F1	F2	F3	
A0	17 (11)	3 (1)	0	0	20 (12)
A1	24 (21)	75 (62)	2 (2)	0	101 (85)
A2	0	6 (5)	2 (2)	0	8 (7)
A3	0	0	0	0	0
Total	41 (32)	84 (68)	4 (4)	0	129 (104)

Numbers of female patients are given in parentheses.

The stage of liver fibrosis in the 22 female patients with ALT levels≦19 IU/L at entry were F0 ($N=10$) or F1 ($N=12$). The frequency of stage F0 liver histology was slightly higher in group A and B patients compared with group C. However, there were no significant differences among the three groups.

Seven patients from group C had ALT levels over 100 U/L during the follow-up period and received antiviral therapy (five received interferon monotherapy and two received interferon plus ribavirin therapy), and five had a sustained virological response.

3.4. Follow-up study of liver histology

Thirty-five patients submitted to repeat biopsies and five of them a third biopsy. Of the 35 patients, 5 were in group A, 16 in B, and 14 in C. The intervals between the first biopsy and the last biopsy in these three groups were 7.3±2.1 years (group A), 6.8±2.0 years (group B), and 6.1±2.3 years (group C). The changes in stage of live fibrosis are shown in Fig. 1 (group A), 2 (group B), and 3 (group C). Progression of fibrosis stage was noted in 2 of 5 in group A, 5 of 16 in group B, and 6 of 14 in group C, as shown in Figs. 1–3. The median rates of fibrosis progression per year for these three groups were 0.05, 0.04, and 0.08 fibrosis unit, respectively. There were no significant differences in the rate of fibrosis progression per year between group A and B, B and C, and A and C (A vs B; $P=0.6643$, B vs C; $P=0.0699$, A vs C; $P=0.3512$).

Of the 32 female patients who received serial biopsies, 10 had ALT levels≦19 U/L at entry, in four of whom had F0 stage progress to F1. One F0 and five F1 patients showed no changes in their stages during the follow-up periods.

Table 5
Changes of serum ALT in 69 patients followed over 5 years

	No. of patients
Persistently normal (group A)	10 (14%)
Transient elevation (group B)	39 (57%)
Continuous elevation (group C)	20 (29%)

Group A, continuously normal serum ALT during the follow-up period. Group B, serum ALT transiently over 31 U/L during the follow-up period. Group C, serum ALT became continuously abnormal during the follow-up period.

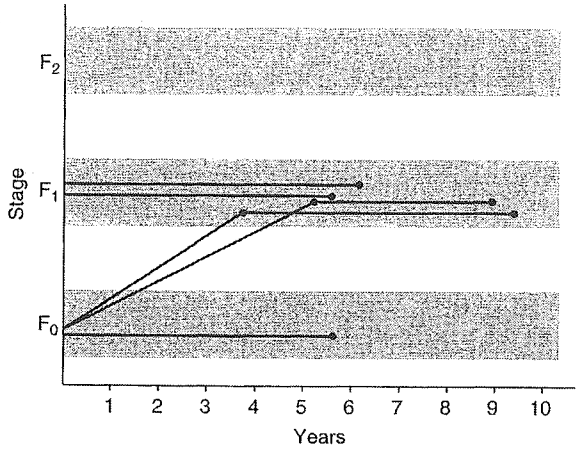

Fig. 1. Follow-up study of liver histology on asymptomatic hepatitis C virus carriers whose alanine aminotransferase levels remained normal during the follow-up period. Five patients with persistently normal serum aminotransferase levels submitted to repeat biopsies and the stage of liver fibrosis progressed from F0 to F1 in two patients after 3.4 and 5 years.

3.5. Follow-up study of AFP and ultrasonography

Three patients in group C showed transient elevation of AFP over 20 ng/ml. No patients in groups A or B had elevations of serum AFP during their follow-up periods. HCC was not detected in any patients by ultrasonography and/or computed tomography. AFP titers in those three patients did not increase further.

4. Discussion

The present study demonstrated several characteristics of HCV carriers with persistently normal ALT levels (PNAL).

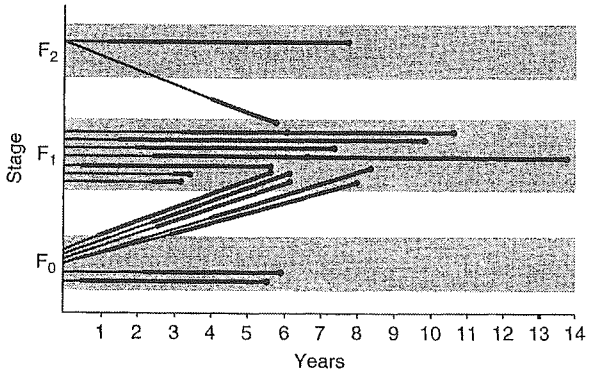

Fig. 2. Follow-up study of liver histology on asymptomatic hepatitis C virus carriers whose alanine aminotransferase levels were transiently elevated during the follow-up period. Sixteen patients with transient elevation of serum aminotransferase levels submitted to repeat biopsies and the stage of liver fibrosis progressed from F0 to F1 after 5.3–8.1 years in five patients. One patient showed the regression of the stage of liver fibrosis from F2 to F1 after 5.5 years. The left side edge of the large bar indicates the initial recording of abnormal serum aminotransferase during follow-up period.

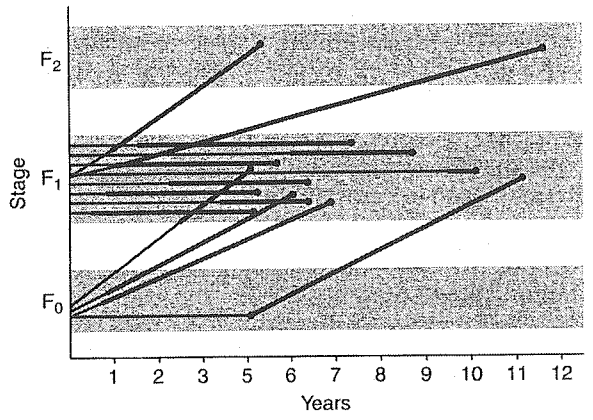

Fig. 3. Follow-up study of liver histology on asymptomatic hepatitis C virus carriers whose alanine aminotransferase levels became persistently abnormal during the follow-up period. Fourteen patients who developed continuously abnormal serum aminotransferase levels submitted to repeat liver biopsies after 4.0–10.3 years. Progression of the stage of liver disease was noted in six patients, of whom four progressed from F0 to F1 and two from F1 to F2 after 4.0–10.3 years. The left side edge of the large bar indicates the initial recording of abnormal serum aminotransferase during follow-up period.

Serum HCV RNA levels were similar to patients with symptomatic chronic hepatitis, however, the frequency of HCV genotype 2 was significantly higher in HCV carriers with PNAL than those with chronic hepatitis C (data not shown here, of 123 patients with chronic hepatitis C in our clinic 75% had genotype 1 and 22% were genotype 2). Females were predominant among the HCV carriers with PNAL compared with chronic hepatitis [4] which is similar to other reports [5,33–35]. Female HCV carriers with continuously normal ALT had significantly lower ALT levels at entry as shown in Table 6. Of the 105 female patients, 44 had ALT levels $\leqq$19 U/L and showed mild liver injury compared with carriers with whose ALT levels were 20–30 U/L. However, the progression rate of fibrosis was not significantly different.

The serum ferritin and serum thioredoxin (TRX) levels in HCV carriers with PNAL showed normal ranges and were significantly lower than in chronic hepatitis C patients, as we have reported previously [26]. The frequency and grade of fatty liver and iron loading were quite low compared with chronic hepatitis C patients, also as reported previously [26]. Liver histology was minimal to mild and moderate chronic hepatitis was noted in only around 8% of subjects. Long-term follow-up study demonstrated that 29% of HCV carriers with PNAL developed chronic hepatitis with persistently high serum ALT within 5 years, 57% showed transient elevation of serum ALT, and 14% had continuously normal ALT. There are many reports concerning the natural course of liver fibrosis in chronic hepatitis C patients including patients with normal serum ALT level [5,33–41]. More than half of chronic hepatitis C patients show progression of stage of liver fibrosis from F1 to F2-4 within 10 years and it was previously reported that progression of liver fibrosis in HCV carriers with PNAL was more rapid compared with the present result [5]. The main reason for this discrepancy between the previous reports and the present result might be due to the difference in the definition of the normal range of serum ALT. Poynard et al. [37] reported that the median rate of progression of fibrosis per year was 0.1333 fibrosis unit, which was 1.5–3 times faster than the present results in HCV carriers with PNAL.

These results indicate that HCV carriers with PNAL are in a condition with less oxidative stress [26] and they have a lower risk of cirrhosis and hepatocarcinogenesis compared to chronic hepatitis patients [13,22].

It is well known that the rate of the development of hepatocellular carcinoma (HCC) is correlated with the progression of liver fibrosis; the stage of liver disease [9,11,13]. Sustained low serum ALT also lowers the rate of the development of HCC [9,13,42]. No HCC was detected during the follow-up period in any of the HCV carriers in this study, reflecting the results of previous clinical studies.

Peginterferon and ribavirin administration for 48 weeks resulted in sustained virological response in around 40% of patients with genotype 1 [15], however, this therapy is expensive and induces various side effects.

The present results indicate that most HCV carriers with persistently normal serum ALT have a good prognosis with a low risk of developing hepatocellular carcinoma. Antiviral treatment for these patients should take into consideration the follow-up results of blood chemistry and liver histology.

Table 6
Characteristics of 61 female patients in groups A–C followed over 5 years

	Group A (N=8)	Group B (N=34)	Group C (N=19)
Age (y.o.)	49.6±12.9	44.9±12.5	48.2±8.9
BMI (kg/m^2)	20.8±2.9	20.6±2.1	21.8±2.5
Ferritin (ng/ml)	73.4±33.7	59.3±56.8	76.8±47.1
ALT (U/L)*	15.8±3.2	22.4±4.6	23.9±4.9
HCV RNA (KIU/ml)**	186.5±141.8	474.6±486.0	454.0±575.2

Values were expressed as mean±SD. There were no significant differences in their age, BMI, ferritin, and HCV RNA levels in three groups. *Serum ALT level was significantly lower in group A compared with group B (group A vs group B; P=0.0045) and with group C (group A vs group C; P=0.0003), however, no significant difference was noted between group B and C (P=0.0758). **There were no significant differences in serum amount of HCV RNA between group A and B (P=0.3529) and group A and C (P=0.8676).

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Natural Course of Asymptomatic Hepatitis C Virus–Infected Patients and Hepatocellular Carcinoma After Interferon Therapy

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A long-term follow-up study was performed to identify the natural course of chronic HCV carriers with persistently normal serum ALT level (PNAL; ≤30 U/L) and to clarify the effect of interferon therapy on the inhibition of the development of hepatocellular carcinoma (HCC) in chronic hepatitis C patients with elevated ALT levels. One hundred twenty-nine HCV carriers with PNAL underwent liver biopsy, 69 were followed for more than 5 years, and 35 underwent serial liver biopsies. We included 1246 chronic hepatitis C patients (stage F1: 231, F2: 638, F3: 336, F4: 41) who received interferon therapy and were followed for more than 2 years (mean, 7.7 years). Approximately 90% of HCV carriers with PNAL had normal to mild liver histology, and 30% developed symptomatic chronic hepatitis C within 5 years. The frequency of steatosis and iron loading was significantly lower in these patients than in symptomatic chronic hepatitis C patients. The progression rate of fibrosis was slower than in chronic hepatitis C patients with elevated serum ALT levels. HCC was noted in 157 chronic hepatitis C patients after interferon therapy, and the development of HCC was significantly reduced in both sustained responders and transient biochemical responders compared with nonresponders. HCC in sustained responders mainly developed in male patients older than 55 years with advanced stage liver histology at entry. Approximately 30% of HCV-infected patients with PNAL become candidates for antiviral therapy within 5 years. Interferon therapy lowers the rate of the development of HCC in both sustained responders and transient biochemical responders.

Hepatocellular carcinoma (HCC) occurs at a high rate in the patients with advanced stage chronic hepatitis C,[1–3] and 20%–30% of HCV-infected patients change to patients with persistently normal serum ALT levels (PNAL; ≤40 U/L).[4,5] These asymptomatic HCV carriers are predominantly female. There are a few reports that HCV carriers with PNAL showed slower progression of liver fibrosis[6–8] compared with patients with chronic hepatitis C with elevated serum ALT levels; however, Puoti et al[9] reported that HCV-infected patients with normal serum ALT (≤40 U/L) had a more progressed stage of liver fibrosis. This discrepancy might be mainly derived from the difference in the definition of asymptomatic HCV carriers and the range of normal serum ALT in each institution. Recently, an Italian group[10] demonstrated that normal range of serum ALT level was ≤30 U/L in men and ≤19 U/L in women.

Thus, it is important to clarify the pathophysiology and natural course of HCV carriers with PNAL and to clarify whether they are candidates for antiviral treatment. We also aimed to clarify the effect of interferon (IFN) therapy on the development of HCC in chronic hepatitis C patients in each stage of hepatic fibrosis including sustained responders (SRs) to IFN therapy.

Materials and Methods

HCV carriers with PNAL were defined as follows: serum ALT ≤30 U/L more than 1 year and at least 3 different occasions and platelet count of more than 150,000/μL. Patients with diabetes mellitus and obesity (body mass index, more than 30 kg/m^2) were excluded from this study. One hundred twenty-nine (male, 24; female, 105) HCV carriers with PNAL underwent liver biopsy, 69 were followed for more than 5 years (mean follow-up period, 8.5 years; Table 1), and 35 underwent serial liver biopsy during 3.4–13.4 years after the first biopsy. Formalin-fixed liver specimens were stained with hematoxylin-eosin, Masson's trichrome stain, and Perls' Prussian blue stain. Steatosis was defined as having fat droplets in more than 10% of hepatocytes. Serum ferritin and thioredoxin (oxidative stress marker)[10] levels were determined. Quantification and determination of HCV RNA and genotyping were established.

Abbreviations used in this paper: HCC, hepatocellular carcinoma; IFN, interferon; NR, nonresponder; PNAL, persistently normal serum ALT levels; SR, sustained responder; SVR, sustained viral responder; TR, transient biochemical responder.

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Table 1. Characteristics of 129 HCV Carriers With PNAL Who Underwent Liver Biopsy and 69 HCV Carriers With PNAL Followed > 5 Years

	HCV carriers with PNAL (N = 129)	Those followed > 5 y (N = 69)
Follow-up period (*y*)	5.7 ± 3.6	8.5 ± 2.4
Sex (male/female)	24/105	8/61
Age (*y*)	48 (21–77)	45 (29–71)
Men	49.8 ± 16.4	42.3 ± 14.9
Women	47.2 ± 12.5	46.63 ± 11.6
ALT (*U/L*)	8–30	9–30
Men	22.5 ± 5.7	21.1 ± 5.4
Women	21.6 ± 4.8	22.3 ± 5.1
PLT ($\times 10^4$/*mL*)	15–31	15–31
Men	20.3 ± 4.4	20.9 ± 5.3
Women	21.8 ± 4.4	21.2 ± 4.0
Ferritin (*ng/mL*)	5–225	5–225
Men	76.2 ± 53.5	84.6 ± 59.2
Women	60.0 ± 43.3	66.6 ± 52.5
HCV RNA (*KIU/mL*)	6–3350	22–2100
G1 (n = 58)	648.9 ± 622.57[a]	595.1 ± 561.1[b] (N = 32)
G2 (n = 45)	356.2 ± 628.8	211.0 ± 219.2 (N = 27)
Mixed & unclassified	6–1994	

NOTE. Values were expressed as mean ± standard deviation. *P* values were calculated by Mann-Whitney *U* analysis with correction for tie.
PLT, platelet count; KIU, kilo international unit.
[a]*P* = .0012 (G1 vs G2).
[b]*P* = .0006 (G1 vs G2).

One thousand two hundred forty-six chronic hepatitis C patients (stage F1: 231, F2: 638, F3: 336, F4: 41) received 24 weeks of IFN therapy within 6 months after liver biopsy and were followed for more than 2 years (mean follow-up period, 7.7 years). All of these patients underwent liver biopsy before IFN therapy, and their stages of fibrosis were evaluated.

Another study included 3626 patients (2344 men, 1282 women) with chronic hepatitis C who had received IFN monotherapy. Patients with positive HBsAG and/or with high titer of anti-HBcAG were excluded in these 2 studies. Cox proportional hazards analysis was used to compare SRs (N = 1197; 776 men, 421 women) who developed HCC in a multicenter, retrospective cohort study. Mean follow-up was 5.9 ± 1.9 years.

Data values are expressed as medians with interquartile ranges. We compared continuous variables by using the Mann-Whitney *U* test. The Kruskal-Wallis test was used for multiple group comparisons, and Spearman correction coefficient was used to examine the relationship between groups. Frequency analysis was performed with the χ^2 test and Fisher exact test. *P* values of less than .05 were considered significant.

Results

Of the 129 patients with PNAL, 17 had normal liver histology, 102 exhibited minimal to mild chronic hepatitis, and only 10 patients exhibited F2 and/or A2 liver histology. Steatosis (9 of 129, 7%) and iron loading (6 of 50, 12%) were at significantly lower rate in these patients compared with chronic hepatitis C patients with abnormal serum ALT. Of the 104 patients who were followed for more than 2 years (mean, 6.8 years), 90 (86.5%) exhibited abnormal ALT (≥31 U/L) at least once during the follow-up period. Approximately 30% of these 102 changed to chronic hepatitis with elevated serum ALT levels within 5 years. Of the 69 patients who were followed for more than 5 years, 10 (14%) had persistently normal ALT levels (group A) during the follow-up period, 39 (57%) had transient elevation of ALT (group B), and 20 (29%) changed to chronic hepatitis (group C). The rate of progression of fibrosis in groups A, B, and C were 0.05, 0.04, and 0.08 per year, respectively. Serum ferritin and thioredoxin levels were within the normal range; however, serum thioredoxin levels were significantly higher in chronic hepatitis and cirrhotic patients.[11] No HCC was noted in any of the 129 HCV carriers with PNAL during the follow-up period.

Of the 1246 patients who received IFN therapy, 397 showed sustained biochemical response (SR; 352 were sustained virologic responders [SVRs]), 317 were transient biochemical responders (TRs; relapsers), and 532 were nonresponders (NRs). SR rate in each stage was as follows: F1: 45%, F2: 34%, F3: 22%, and F4: 10%. HCC developed in 4 of F1 patients, in 43 in F2, in 92 in F3, and in 19 in F4. The annual incidences of HCC in NRs in each stage were 0.5% in F1, 1.8% in F2, 5.2% in F3, and 8.6% in F4. The annual incidences of HCC in SRs, TRs, and NRs were 0.3%, 1.1%, and 3.3%, respectively (Table 2). There were significant differences in the development of HCC between SRs versus TRs, TRs versus NRs, and SRs versus NRs.

Among 1197 SR patients, HCC was detected in 27 patients (25 men, 2 women) (Figure 1) who were mainly male patients older than 55 years with advanced stage liver histology at entry of IFN therapy. Of the 27 HCC patients, 25 were SVRs and 2 were SRs.

Table 2. Annual Incidence of HCC After IFN Therapy (Follow-up Period, 7.7 Years)

Stage	SR (n = 397)	TR (n = 317)	NR (n = 532)
F1 (n = 231)	1/104 (0.2%)	1/72 (0.2%)	2/55 (0.5%)
F2 (n = 638)	1/216 (0.1%)	8/170 (0.6%)	34/252 (1.8%)
F3 (n = 336)	6/73 (1.1%)	16/68 (3.4%)	69/195 (5.2%)
F4 (n = 41)	0/4 (0%)	2/7 (4.9%)	17/30 (8.5%)
Total 1246	8/397 (0.3%)	23/317 (1.1%)	96/532 (3.3%)

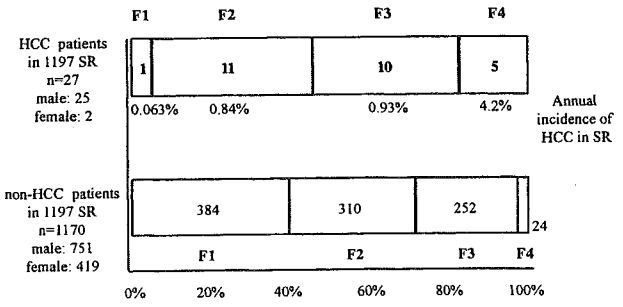

Figure 1. Fibrosis (F) stage of patients who developed HCC in 1197 SRs compared with those without HCC.

Discussion

Most HCV-infected patients with PNAL exhibited minimal to mild chronic hepatitis, had normal serum ferritin and thioredoxin levels, and exhibited slow progression of liver fibrosis when normal range of serum ALT was defined as ≤30 U/L. No HCC was detected in any of the patients. However, approximately 30% of them changed to chronic hepatitis with abnormal serum ALT within 5 years.

IFN therapy lowered the rate of progression to HCC in both SRs and TRs. The development of HCC increased in proportion to the progress of hepatic fibrosis in both TRs and SRs. The inhibition of the development of HCC was almost the same in TRs with SR for 4–5 years after IFN therapy[2]; however, after that the occurrence of HCC gradually increased in TRs.

HCC was detected in 27 patients among 1197 SRs; 25 were men and 2 were women. SRs among male patients older than 55 years with advanced stage liver fibrosis have a risk of development of HCC after IFN therapy.[12]

The present results indicate that most HCV carriers with PNAL have a good prognosis with a low risk of developing HCC. Antiviral therapy for these patients should take into consideration the follow-up results of blood chemistry and liver histology.[13]

The results of IFN therapy demonstrate that a transient biochemical response in chronic hepatitis C patients suppressed the progression of liver disease, resulting in the delay in the development of HCC; however, these effects were within 5 years. Longer therapy might be needed for the longer inhibition of the development of HCC.

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ORIGINAL ARTICLE

Navigator-Echo-Based MR Provides High-Resolution Images and Precise Volumetry of Swine Livers Without Breath Holding or Injection of Contrast Media

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The accurate calculation of hepatic volume by computed tomography (CT) or magnetic resonance (MR) is complicated by the need for breath holding and the injection of contrast media. These are often contraindicated in patients with liver failure, and we examined the ability of unenhanced 3-dimensional (3-D) navigator-echo-based MR (NE-MR) to accurately image livers and measure volumes without breath holding compared to unenhanced (plain) or gadolinium-diethylene triamine pentaacetic acid enhanced MR (Gd-MR) in miniature swine (n = 8). Without breath holding, diaphragm movement monitoring with NE-MR reduced motion artifacts in hepatic images compared with the other modalities. Without the injection of contrast media, the signal-to-noise ratios of the images obtained using NE-MR were significantly higher than those from plain MR; Gd-MR was superior to NE-MR, however (79.5 ± 7.5 vs. 63.2 ± 6.0 or 97.8 ± 8.1, respectively; $P < 0.01$ for each). Overall, NE-MR produced improved high-resolution liver images. Consequently, liver volumes calculated based on NE-MR images were more highly correlated with actual liver weights compared to plain or Gd-MR in the whole livers (n = 8; r = 0.937 vs. 0.835 or 0.904, respectively). Also, NE-MR demonstrated significantly strong correlation between actual weights and volumetry-calculated volumes in regenerative livers 7 days after massive hepatectomy (n = 10, r = 0.989, $P < 0.01$). In conclusion, our results indicate that without breath holding or the injection of contrast media, 3-D NE-MR can provide both high-resolution liver images and precise hepatic volumes in patients with liver failure due to liver surgery (massive hepatectomy and living donor liver transplantation) or fulminant hepatic failure. *Liver Transpl 12:72–77, 2006.* © 2005 AASLD.

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Both computed tomography (CT)[1] and magnetic resonance (MR)[2] are frequently used to evaluate graft size preoperatively in living donor liver transplantation (LDLT). These techniques are also able to assess the functional hepatic reserve and degree of liver regeneration in patients undergoing hepatectomy (including donor operation of LDLT),[3,4] ablation,[5] and LDLT.[2] Additionally, CT volumetry can evaluate disease severity and prognosis in patients with fulminant hepatic failure (FHF).[6] The precise measurement of hepatic volume using CT or MR requires both the minimization of motion artifacts and high-resolution images to obtain dis-

Abbreviations: CT, computed tomography; MR, magnetic resonance; LDLT, living donor liver transplantation; FHF, fulminant hepatic failure; 3-D, 3-dimensional; NE-MR, navigator-echo-based MR; Gd-DTPA, gadolinium-diethylene triamine pentaacetic acid; GD-MR, Gd-DTPA-enhanced MR; SNR, signal-to-noise ratio.

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tinct boundaries between the liver and the surrounding extrahepatic organs or abdominal wall. Therefore, breath holding and the bolus injection of contrast media are indispensable for the precise measurement of hepatic volume.[1,2] However, nephrotoxicity and the possibility of allergic reactions including anaphylaxis limit the use of contrast media.[7]

Patients with liver injury secondary to surgery (massive hepatectomy and LDLT) or FHF frequently exhibit decreased consciousness as well as comorbid conditions including renal and respiratory dysfunction. In these cases, because breath holding and the administration of contrast media are often contraindicated, the measurement of precise hepatic volumes by conventional CT or MR is difficult. These patients, however, would benefit most from the information gathered by these techniques in the development of prognosis and treatment plans. Thomsen et al. reported that respiratory gating by monitoring the movement of the abdominal wall reduces motion artifacts in MR images of human livers.[8] However, abdominal wall movement may not directly correlate with respiratory movement. Three-dimensional (3-D) navigator-echo-based MR (NE-MR) imaging was initially developed to examine moving structures.[9,10] NE-MR reduces motion artifacts without breath holding and is primarily used in imaging cardiovascular and cerebrovascular disease.[11,12] Recently, several studies demonstrated a possible role for NE-MR in monitoring liver disease.[13–15] However, it is not known whether unenhanced NE-MR is capable of providing high-resolution images and precise liver volumetry without breath holding.

In the present study, we examined the quality of 3-D NE-MR images of whole livers of miniature swine without breath holding or contrast media injection. We also assessed the accuracy of NE-MR-based hepatic volumetry in whole livers as well as in the regenerative liver after massive hepatectomy.

MATERIALS AND METHODS

Animals

Female Crown miniature swine, 4 to 6 months of age and weighing 11.7-16.2 kg, were obtained from Japan Farm (Kagoshima, Japan). The animals were maintained under constant room temperature (25°C) and given free access to water and the indicated diet throughout the study. The protocol for animal studies was approved by the ethics committee of the Graduate School of Medicine, Kyoto University (Kyoto, Japan). All animal experiments were performed after 1 to 3 weeks acclimation on a standard diet.

MR Imaging

After being sedated by intramuscular injection of 5 mg/kg ketamine hydrochloride, 0.2 mg/kg butorphanol tartrate, and 0.08 mg/kg medetomidine chloride, 8 animals underwent abdominal MR imaging with a 1.5-T Magnetom Sonata (Siemens, Erlangen, Germany) with a maximum gradient strength of 40 mT/m and a slew rate of 200 mT/m/second. A circular polarized head coil was used in all cases.

Three types of MR sequences were performed per animal sequentially as follows: (1) unenhanced T1-weighted fat-suppressed fast low angle shot 3D with NE (NE-MR), (2) unenhanced T1-weighted fat-suppressed fast low angle shot 3-D without navigator echo (plain MR), and (3) gadolinium-diethylene triamine pentaacetic acid (Gd-DTPA) enhanced T1-weighted fat-suppressed fast low angle shot 3-D. The scan parameters were as follows: TR = 161.5 milliseconds; TE = 2.01 milliseconds; image matrix = 175 × 320; FOV = 258 × 330 mm; pixel size, 1.5 × 1.0 mm; slice thickness, 3.0 mm; flip angle, 12 ˜ 30°; band width = 200 Hz/pix. The NE scanning respiration control was as follows: Scout TR = 100 milliseconds, Accept window = ± 3.0 mm, and Search window = ± 30.5 mm. Gd-DTPA was used as a contrast agent. Gd-DPTA-enhanced MR (Gd-MR) imaging was scanned 1 minute after bolus intravenous injection of 0.2 mL/kg Gd-DTPA. NE-MR imaging was performed with prospective k-space filling. 3-D images of the livers, synchronized with breathing, were reconstituted by 3-D motion correlation technique using navigator echo.[9,10] Selective excitation pulses were projected through the left diaphragm at 2 vertical directions for tracking its craniocaudal motions, which were synchronized with the motions of the viscera (Fig.1A). While tracking the diaphragmatic movements (Fig.1B), 3-D liver images were reconstituted repeating the real-time scanning and acquisition of 2-dimensional axial images of the liver with fast low angle shot 3-D at 1 fixed point during diaphragmatic movement (Fig.1C).

Whole-Liver Signal-to-Noise Ratios

Whole-liver signal-to-noise ratio (SNR) was measured in 10 axial slices of NE-, plain, and Gd-MR for each pig. The average values from 8 animals were compared between the 3 different modalities.

Whole-Liver Volumetry

After MR imaging animals were anesthetized by inhalation of sevoflurane, nitric dioxide, and oxygen. Animals were sacrificed and actual liver weights were measured. Liver volumes were estimated from the 3-D images by the volume rendering method, and the hepatic volumes were calculated with image analysis software VG Studio MAX 1.2 (Volume Graphics, Heidelberg, Germany).

NE-MR Volumetry of Regenerative Livers After Massive Hepatectomy

After being anesthetized by inhalation of sevoflurane, nitric dioxide, and oxygen, massive hepatectomy was performed on 10 pigs as described previously with minor modifications.[16] Briefly, the left lateral, left median, and right median lobes of the liver were removed using the finger-fracture method without Pringle's maneuver after the branches of hepatic arteries, portal veins, and

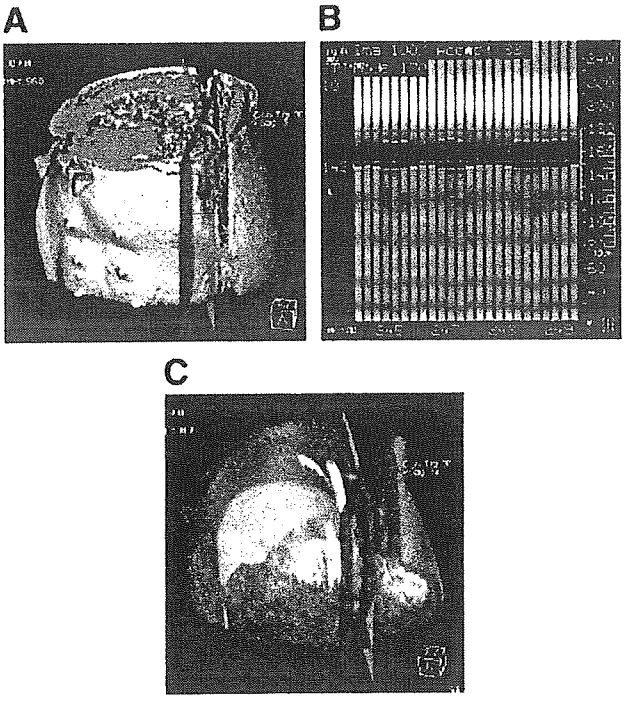

Figure 1. MR in combination with navigator-echo monitoring respiratory movement. (A) Selective-excitation pulses (green and red planes) are projected through the left diaphragm at 2 vertical directions for tracking craniocaudal motions of the membrane. (B) These pulses enable the scanning and acquisition of axial images of the liver with fast low angle shot 3-D at 1 fixed point in real time. Red and green boxes indicate Search and Accept windows, respectively. (C) A 3-D image of the liver reconstituted by navigator echo technique.

bile ducts feeding these lobes were ligated. The animals were subjected to NE-MR at postoperative day 7, and the actual weights of the remnant livers were measured after sacrifice. The hepatic volumes were calculated as described above.

Statistical Analysis

Values are presented as mean value ± SD. StatView statistical software (Version5.0, SAS Institute Inc., Cary, NC) was used for data analysis. Statistical differences between groups were assessed by 1-way analysis of variance and by Scheffe's F test as a post-hoc test. Linear regression analysis was used to generate correlation equations. $P < 0.01$ was considered statistically significant.

RESULTS

NE-MR Reduces Motion Artifacts and Increases SNR, Resulting in High-Resolution Images of the Liver

All animals safely underwent the 3 MR imaging modalities during sedation. The imaging duration of NE-MR was 122.1 ± 14.3 seconds, while that of plain or Gd-MR took 65.8 ± 3.1 seconds.

We first examined the effect of NE-MR on motion artifacts in axial images of whole livers compared to plain and Gd-MR in eight miniature swine. NE-MR reduced the motion artifacts in all images from the cranial to the caudal side, while considerable motion artifacts arose in images generated with the other 2 modalities, presumably due to diaphragmatic movement during respiration, resulting in a blurred liver edge (Fig. 2).

We next measured SNR values in 10 axial slices of the whole liver at the same axial level with NE-MR, plain, and Gd-MR for each pig (n = 8). NE-MR produced significantly higher SNR values compared to plain MR, but it was significantly worse than Gd-MR (79.5 ± 7.5 vs. 63.2 ± 6.0 or 97.8 ± 8.1, respectively; $P < 0.01$ for each) (Fig. 3).

Because of the reduced motion artifacts and increased SNRs, images generated using NE-MR were substantially clearer than those obtained using plain or Gd-MR (Fig. 2). Additionally, the hepatic boundaries with the heart were especially poorly resolved using Gd-MR compared to NE-MR, likely due to the latter's enhanced cardiac imaging as well as decreased motion artifacts (Figs. 2A, 2G).

Overall, images generated by NE-MR without breath holding or the injection of contrast media exhibited more distinct hepatic boundaries compared to the other 2 modalities.

Hepatic Volumes Determined by NE-MR Correlate Significantly Liver Weights

Liver volumes determined using volumetry (n = 8) were 311.0 ± 33.1 mL (range, 254.4-366.1), 306 ± 25.2 mL (range, 264.6-348.5), and 302.1 ± 28.4 mL (range, 259.0-351.3) in NE-, plain, and Gd-MR samples, respectively; their actual weights were 306.9 ± 42.1 g (range, 254.4-374.0). The calculated volumes for all imaging methods significantly correlated with actual liver weights, ($P < 0.01$ in all), but NE-MR generated the highest correlation coefficient among them (r = 0.937 vs. 0.835 and 0.904 in NE-, plain, and Gd-MR, respectively) (Fig. 4A-4C).

We next evaluated the accuracy of hepatic volumetry determined with NE-MR at postoperative day 7 in animals following massive hepatectomy. The actual weights of the regenerative livers were 217.5 ± 24.2 g (range, 193-260), and the hepatic volumes measured by NE-MR volumetry were 261.4 ± 30.1 mL (range, 234.6-308.0). The relationship between the actual weights of the regenerative livers and the calculated hepatic volumes were linear with statistical significance (r = 0.989, $P < 0.01$) (Fig. 4D).

DISCUSSION

The accurate measurement of hepatic volumes is required for donors of LDLT as well as patients with liver failure due to surgery (massive hepatectomy and LDLT) or FHF. Both conventional CT and MR can accomplish this, but they require breath holding and/or the injection of contrast media. These are frequently contrain-

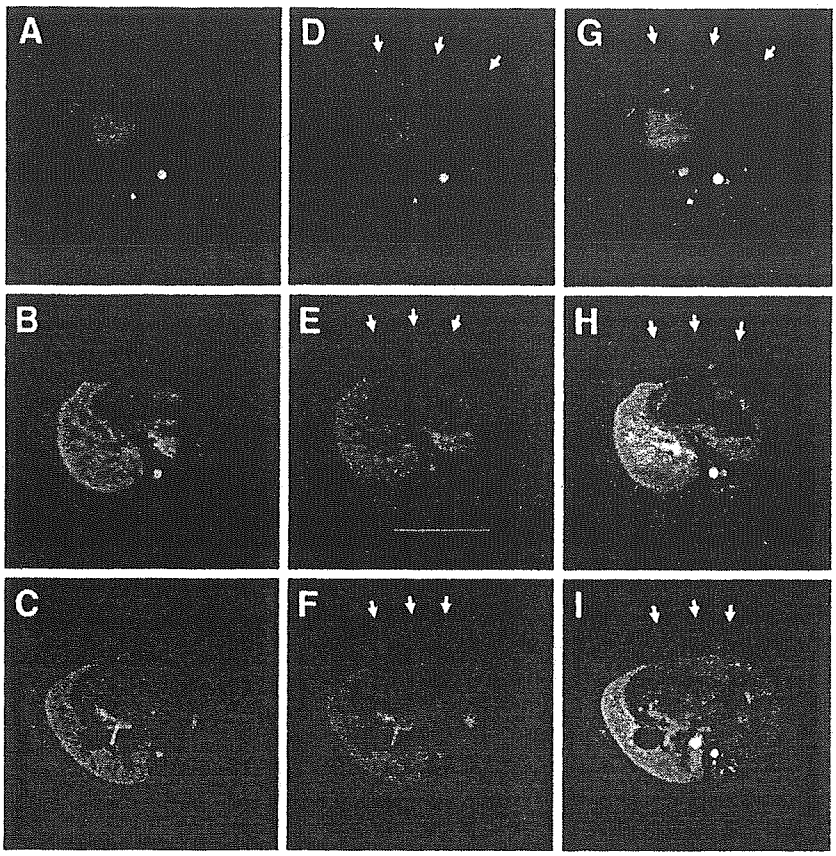

Figure 2. Representative axial images of the liver. Axial images of miniature swine livers were obtained by (A-C) NE-, (D-F) plain, and (G-I) Gd-MR as described in Materials and Methods. Despite continuing spontaneous breathing, NE-MR reduced motion artifacts in the all axial images of the whole liver from the (A, D, G) cranial side to the (C, F, I) caudal side, while both plain and Gd-MR demonstrated considerable motion artifacts, resulting in a lack of sharpness of the liver edge (arrows). Interestingly, NE-MR provided clear images of the liver from gastrointestinal tract without contrast medium compared to plain MR (C, F). Additionally, images obtained by Gd-MR exhibited obscure hepatic/cardiac boundaries compared to NE-MR (A,G).

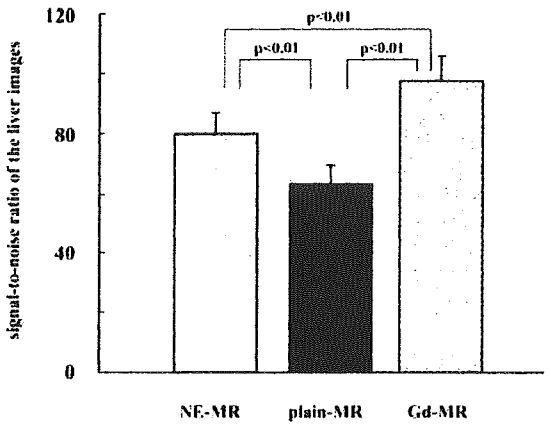

Figure 3. Comparison of SNRs among NE-, plain, and Gd-MR. The SNRs of 10 axial slices of the whole liver, each of which was scanned at the same axial level by NE-, plain, and Gd-MR, were measured in each pig (n = 8). SNRs of the livers using NE-MR were significantly higher than that of plain MR but significantly lower than that of Gd-MR (79.5 ± 7.5 vs. 63.2 ± 6.0 or 97.8 ± 8.1, respectively; $P < 0.01$ for each).

dicated in hepatic patients, however, due to comorbid conditions such as renal and respiratory dysfunction and decreased awareness. Therefore, the development of a novel imaging modality that requires neither breath holding nor contrast media is needed.

In the present study, we scanned swine livers using 3-D NE-MR that monitored the diaphragmatic movement in real time. Respiratory gating based on abdominal wall movement reduces motion artifacts,[8] but we did not compare hepatic images obtained with NE-MR or MR with respiratory gating in human subjects in this study. However, diaphragmatic motion correlates better with respiratory movement than abdominal wall motion, and this is especially true in females who exhibit increased thoracic respiration. Compared with conventional MR without navigator echo, breathing associated motion artifacts were dramatically reduced using NE-MR in the present study (Fig. 2). Furthermore, conventional MR requires breath holding and provides only 2-dimensional abdominal images with 10-mm slice thicknesses, but 3-D NE-MR generates 3-D abdominal images with 3 mm of slice thickness. Additionally, the hepatic boundaries were clearly distinguished from the surrounding extrahepatic organs, including the heart and gastrointestinal tract, even without breath holding or injection of contrast media using NE-MR. Interestingly, the calculated values for SNR for NE-MR and Gd-MR were significantly higher than that of plain-MR (Fig. 3). Conversely, although the interval from the injection of contrast media to the start of imaging may have decreased the SNR values obtained with Gd-MR, this value was significantly higher than that using NE-MR (Fig. 3). However, despite the high SNR, the liver images generated using Gd-MR exhibited obscure hepatic/cardiac boundaries compared to

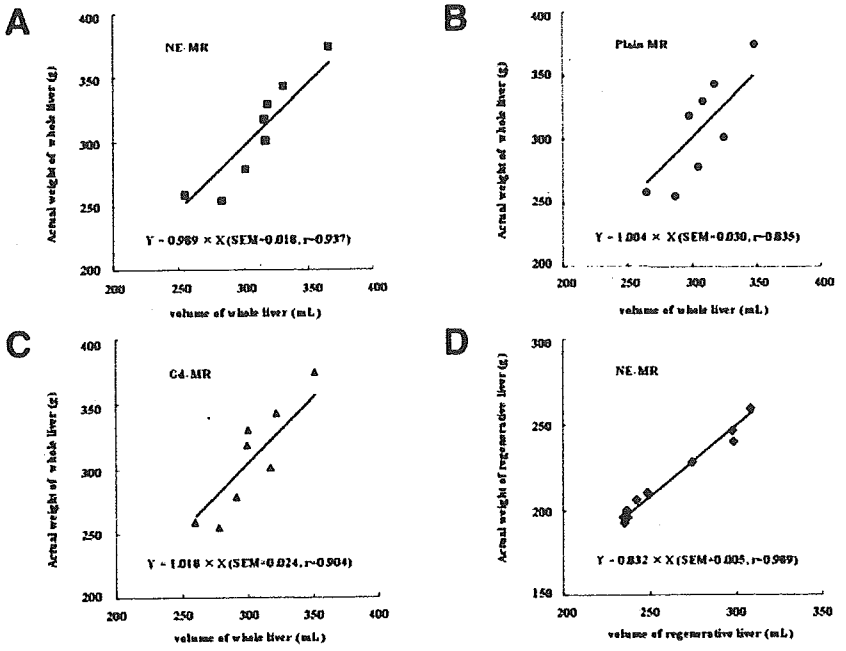

Figure 4. Liver volumes determined by MR-measured volumetry using NE-, plain, and Gd-MR. In whole livers of miniature swine (n = 8), the relationship among the actual weights and the volumetry-calculated volumes was linear with statistical significance in all 3 modalities ($P < 0.01$ in all). However, (A) NE-MR showed a higher correlation coefficient compared to (B) plain MR and (C) Gd-MR (r = 0.937 vs. 0.835 and 0.904 in NE-, plain, and Gd-MR, respectively). (D) In the regenerative swine livers 7 days after hepatectomy (n = 10), NE-MR also showed a high correlation coefficient between the actual weights and the volumetry-calculated volumes with statistical significance (r = 0.989, $P < 0.01$)

NE-MR (Fig. 2A, 2G). Taken together, these results indicate that even with continued breathing, unenhanced 3-D NE-MR increases the SNR of the liver images and provides higher-resolution images of the liver, compared with the 2 modalities.

NE-MR was initially developed to reduce motion artifacts during the imaging of moving structures. Several investigators have examined the feasibility of this technique for proton MR spectroscopy,[13] temperature monitoring,[14] and projection profiling matching[15] for MR-guided interventional procedures in the liver. However, these studies focused only on the real-time motion tracking of certain lesions in the liver. In this study, we demonstrated that without breath holding or contrast media, real-time 3-D NE-MR generated high-resolution hepatic images and allowed for accurate liver volumetry. Also, NE-MR with contrast media can provide more detailed vascular information (data not shown), which is indispensable for preoperative assessment of LDLT donors.

Although we did not verify the advantages of NE-MR compared to enhanced CT, the findings presented here suggest that in patients in whom either breath holding or the administration of contrast media is not possible, NE-MR has great promise for the precise calculation of hepatic volumes. From an ethics standpoint, NE-MR involves no radiation exposure, and this is particularly important for healthy donors in LDLT who require repeated scanning for postoperative sequential assessment of liver regeneration. Furthermore, the absence of contrast media is particularly important because the hepatic excretion of contrast media, normally 1-2% of the total, is increased in patients with renal dysfunction[17] and delayed in those with liver dysfunction.[18] Finally, contrast media can adversely affect liver function directly.[19]

Shadow artifacts derived from the selective excitation pulses are projected as 2 vertical lines through the left diaphragm in NE-MR (Fig. 2A-2C) and may interfere with the integral reconstitution of the image of the whole liver. In the present study, counterclockwise rotation of porcine torsos up to approximately 25°, right shoulder down, moved the left lateral lobes to the right side, resulting in the avoidance of these artifacts. Because simple maneuvers were able to minimize these shadow artifacts, they will not likely be problematic in the evaluation of hepatic volumes in most cases of humans with a remnant liver after massive hepatectomy, a partial liver graft transplanted in LDLT, or an atrophic liver caused by FHF.

Under the sedation conditions used in this study, both normal and massively hepatectomized swine safely underwent NE-MR. Calculated liver volumes were approximately 120% of their actual weight in the regenerative livers after massive hepatectomy, while calculated volumes were virtually equal to the actual weights in the whole livers (Fig. 4A, 4D). The reasons for this discrepancy are unclear but may arise from increased blood drainage from regenerative livers at sacrifice. Alternatively, the composition of regenerative livers may differ somewhat leading to reduced specific gravity.

In conclusion, we present evidence that 3-D NE-MR provides high-resolution images and precise volume measurements of swine livers without breath holding or the injection of contrast media. Although the utility of NE-MR in humans remains unresolved, our results suggest that this novel imaging modality can be applied to patients with liver failure due to surgery or FHF for whom conventional techniques are contraindicated.

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Spontaneous elimination of hepatitis C virus RNA in individuals with persistent infection in a hyperendemic area of Japan

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Abstract

The natural course of hepatitis C virus (HCV) carriers is not well understood. We examined the clinical characteristics of individuals exhibiting spontaneous elimination of HCV as part of a cohort study of residents of a HCV hyperendemic area in Japan. In individuals who were judged to have persistent HCV infection in 1995, 302 had at least 4 annual ALT measurements between 1993 and 2000, and had not been treated with IFN. They were tested for the presence of HCV RNA in 2001 and/or 2002 and HCV RNA could not be detected in 20 of the 302 individuals. In these 20 individuals, 7 were confirmed to have detectable HCV RNA and 13 were not until 2000. Thus, 2.4% (7/289) were judged to have spontaneously eliminated the HCV infection during that 6-year period. Although there were no differences in age, sex, ALT levels, or serologically defined HCV genotype between individuals with and without exhibiting spontaneous elimination, there was a significant relationship between the elimination of HCV RNA and a low level of HCVcAg (<20 pg/mL) ($P < 0.001$) upon testing in 1995. These results suggest that spontaneous elimination of HCV RNA following persistent infection is rare and appears to be related to viral load.

Keywords: HCV; HCV core antigen; ALT; Community-based population

1. Introduction

Hepatitis C virus (HCV) infection is one of the most common causes of acute or chronic liver diseases, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [1,2]. Persistent viral infection is estimated to occur in approximately 40–60% of patients with symptomatic acute hepatitis C [3,4]. The clinical features and progression of HCV in these carriers, however, have not been fully characterized.

Although the spontaneous elimination of HCV is thought to be rare in individuals with persistent viral infection in comparison to those with acute disease, the reported frequencies have varied considerably [5–9]. Furthermore, spontaneous elimination during chronic infection has been reported to be related to several factors, such as age, sex, parturition, additional surgical procedure, or stages of HCC [6–10]. The occurrence of elimination and the associated predisposing factors in the general population, however, have not been examined sufficiently.

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In this study, we sought to elucidate the clinical and virological features of individuals with persistent HCV infection in a hyperendemic population of Japan. We also evaluated the frequency of spontaneous elimination of chronic HCV infection in this population.

2. Materials and methods

2.1. Study population

Town C is a small town in midwestern Miyazaki Prefecture, Japan, located in a rural area isolated by densely forested mountains. Farming is the principal occupation. A local government-sponsored general health examination program, begun in 1993, has been conducted annually for residents over 20 years of age. Collected blood samples were sent to a commercial laboratory in Miyazaki City for routine blood chemistry analyses. Additional blood samples were stored at or below −30 °C until testing. As part of a collaborative effort between the University of Miyazaki, Faculty of Medicine and the local government and public health service, an ultrasonography screening program began in 1994 to detect HCC in Town C residents who have been identified as positive for anti-HCV antibodies. A research study was initiated in 2001, in which additional virologic and epidemiologic data were collected in addition to the ultrasonography liver disease screenings.

2.2. Serologic studies and viral markers

Study individuals were comprised of Town C residents, who had received a government-sponsored general health examination between 1993 and 1995 that included testing for antibodies against HCV (anti-HCV) using an enzyme immunoassay (EIA) kit (Immunocheck F-HCV Ab, International Reagents Co., Kobe, Japan). This kit was a second-generation assay in which HCV-derived recombinant polypeptides c11 (a structural core protein) and c7 (a nonstructural (NS) protein covering NS3) were used. Anti-HCV titers were determined from the ratio of response intensity of the sample determined by EIA to that determined for a negative control [(intensity of sample − intensity of negative control)/(cut-off − intensity of negative control)]. Ratio values lower than 1.0 were considered to be negative for anti-HCV antibody. Since 2001, anti-HCV was measured in serum samples by a third generation chemiluminescent enzyme immunoassay (CLEIA), in accordance with the manufacturers' instructions (Ortho-Clinical Diagnostics, Raritan, NJ). This assay uses HCV-derived recombinant polypeptides c25 (a structural and a NS protein), c33c (a protein covering NS3) and NS5 (a protein covering NS5 region). Assay results (cut-off index) are calculated as a normalized signal relative to the cut-off value (signal/cut-off [S/C] ratio). The cut-off value was calculated using the formula; luminescence of standard HCV positive serum × 0.28. A sample with an S/C ratio of ≥1.00 was considered to test positive.

We also evaluated the results of biochemical tests measuring ALT [normal value (nl): <35 IU/L], aspartate aminotransferase (AST; nl: <40 IU/L), and γ-glutamyltranspeptidase (γ-GTP; nl: male <70 IU/L, female <30 IU/L) measured from 1993 to 2000.

Serum levels of HCV core antigen (HCVcAg) were tested in all individuals who were anti-HCV antibody-positive using a fluorescence enzyme immunoassay (FEIA) (Immunocheck F-HCVAg Core, International Reagents Co., Kobe) [11,12]. The use of two high-affinity monoclonal antibodies directed against amino acids 21–40 or 41–60 of the HCVcAg in the FEIA gave a lower limit of detection of 8 pg/mL HCVcAg. Using the detection of HCV RNA as the gold standard, we determined the sensitivity and specificity of the FEIA to be 84.5 and 99.4%, respectively [13].

In 1995, we assessed the presence of HCV RNA by qualitative reverse transcription polymerase chain reaction (RT-PCR) (Amplicore HCV, Nippon Roche, Tokyo, Japan) among those individuals whose HCVcAg levels were below the 8 pg/mL limit of detection of the FEIA. In 2001–2002, we also tested for the presence of HCV RNA in all available samples, both stored and newly acquired, by RT-PCR (Amplicore HCV v2.0, Nippon Roche).

Serologically defined genotype (serotype) of HCV was determined using a serological genotyping kit (Immunocheck F-HCV Grouping, International Reagents Co., Kobe).

2.3. Statistical analysis

All statistical analyses were performed using STATVIEW 4.5 software (Abacus Concepts, Berkeley, CA). Gender, prevalence of HCV serotype, and frequency of HCV RNA elimination were compared using one-factor ANOVA, χ^2-test or Fisher's exact test, as appropriate. Additional parameters were compared using Scheffe's test or the Mann–Whitney U test, as appropriate. A p value less than 0.05 was considered to be statistically significant.

3. Results

3.1. Prevalence of anti-HCV positivity

Initial testing of Town C demonstrated an overall prevalence of anti-HCV antibody positivity of 20.6% (1151/5577), which gradually increased with age (Table 1). There were no differences in the prevalence of anti-HCV antibody positivity between males and females. Eight hundred thirty-six of the 1151 anti-HCV antibody-positive individuals were tested for HCVcAg levels in 1995, at least 6 months after the measurement of anti-HCV antibody titers. Five hundred twenty-eight (63.2%) of those tested were HCVcAg-positive by FEIA, but were not examined for HCV RNA by RT-PCR; 63 (7.5%) were HCVcAg-negative, but tested positive for HCV RNA