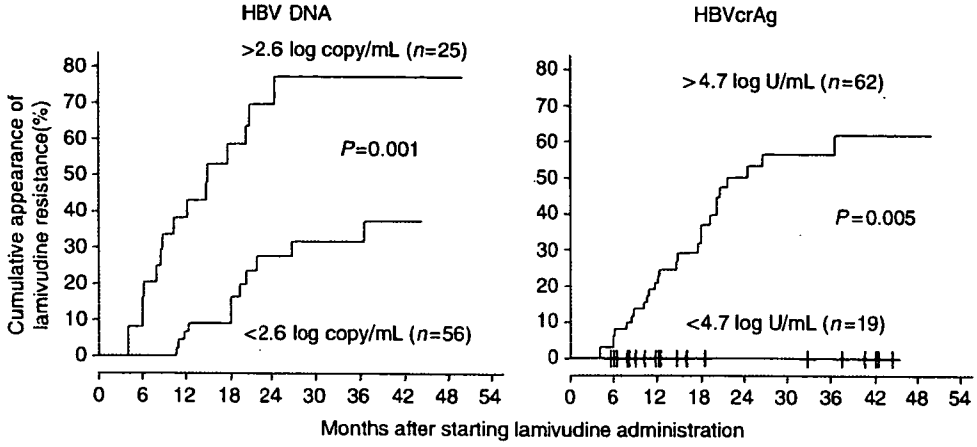

Fig. 3. Comparison of the cumulative occurrence of lamivudine resistance between patients who showed hepatitis B virus (HBV) DNA levels of less than the detection limit (2.6 log copy/ml) at 6 months after starting lamivudine administration and those who did not (left figure), and similarly between patients who showed HBV core-related antigen (HBVcrAg) levels of less than 4.7 log U/ml and those who did not (right figure).

coded by the core gene of the HBV genome with high sensitivity and a wide quantitative range. Serum HBVcrAg levels reflect the viral load in the natural course because these levels correlate linearly with those of HBV DNA (14, 15). On the other hand, the character of HBVcrAg is somewhat different from that of HBV DNA in patients undergoing anti-viral therapies such as lamivudine. That is, HBVcrAg levels decrease significantly more slowly than those of HBV DNA after the initiation of lamivudine administration.

HBV is an enveloped DNA virus containing a relaxed circular DNA genome, which is converted into a covalently closed circular DNA (cccDNA) episome in the nucleus of infected cells (18, 21–23). The cccDNA molecules serve as the transcriptional template for the production of viral RNAs that encode viral structural and non-structural proteins. Reverse transcription of the viral pregenomic RNA and second-strand DNA synthesis occur in the cytoplasm within viral capsids formed by the HBV core protein. Because lamivudine, a nucleoside analogue, inhibits reverse transcription of the pregenomic RNA, it directly suppresses the production of HBV virion. Thus, serum HBV DNA levels decrease rapidly after the initiation of lamivudine administration. On the other hand, the production of viral proteins is not suppressed by lamivudine because the production process does not include reverse transcription. Furthermore, it has been reported that the amount of cccDNA, which serves as a template for mRNA, decreases quite slowly after starting the administration of nucleoside analogues (24–26). Thus, it is reasonable that serum HBVcrAg levels decrease much more slowly than HBV DNA levels after the initiation of lamivudine therapy.

Significant markers that can predict the presence or absence of lamivudine resistance are clinically valuable because the emergence of this resistance and the subsequent recurrence of hepatitis are fundamental problems in lamivudine therapy. Serum markers that reflect the activity of HBV replication have been reported to be associated with the occurrence of lamivudine resistance (11, 12, 27, 28). However, neither the pretreatment existence of HBe antigen nor pretreatment levels of HBV DNA or HBVcrAg were found to be significant markers in the present study. These results may reflect a weak association between the pretreatment activity of HBV replication and the occurrence of lamivudine resistance (13, 29). Changes in HBV DNA and HBVcrAg levels after starting lamivudine administration clearly differed between patients with and without lamivudine resistance. Thus, HBV DNA and HBVcrAg levels at 6 months after starting lamivudine administration were analyzed to determine whether these levels might serve as predictive markers; both were found to be significantly lower in patients without lamivudine resistance at the tested point in time. Furthermore, patients who showed higher levels of HBV DNA and HBVcrAg at 6 months after the initiation of treatment were significantly more likely to develop lamivudine resistance than those who showed lower levels.

We believe that the measurement of HBV DNA levels is useful to identify patients who are at high risk for lamivudine resistance because as many as 70% of patients who were positive for HBV DNA at 6 months after starting lamivudine

administration developed lamivudine resistance within 2 years. However, a negative result of HBV DNA at 6 months does not necessarily guarantee the absence of lamivudine resistance because nearly 30% of such patients developed resistance within 2 years. On the other hand, HBVcrAg levels of less than 4.7 log U/ml at 6 months are a useful indicator of patients who are unlikely to develop lamivudine resistance, because no such patients developed resistance during the follow-up period in the present study. Lower serum HBVcrAg levels may reflect lower levels of cccDNA in hepatocytes because the mRNAs of HBVcrAg are transcribed from the cccDNA (18, 22, 23). This possibility may explain our finding that patients whose HBVcrAg levels decreased sufficiently were unlikely to develop lamivudine resistance, because cccDNA provides the templates for viral and pregenomic messenger RNA (18, 22, 23), which may be a source of lamivudine-resistant strains.

In conclusion, our results suggest that measurement not only of HBV DNA but also of HBVcrAg is useful for predicting the occurrence of lamivudine resistance. HBV DNA measurement is valuable for identifying patients who are at high risk of developing this resistance and HBcrAg measurement is valuable for identifying those who are at low risk.

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Increasing hepatitis C virus-associated hepatocellular carcinoma mortality and aging: Long term trends in Japan

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Abstract

Background: The incidence of hepatocellular carcinoma (HCC) in Japan has been increasing. The aim of the study was to determine the epidemiological trends in HCC mortality in Japan.
Methods: We reviewed the medical records of all patients whose death was caused by liver disease between 1981 and 2000 at two hospitals. The courses of death were separated based on presence or absence of HCC when death ensued. Additionally, cohorts of patients with HCC were analyzed in 5-year time periods.
Results: The number of deaths from hepatitis C virus (HCV)-associated HCC steadily increased 2.6 times from 49 to 128 during observation period. The mean age at death from HCV-associated HCC from 1996 to 2000 was significantly higher than that in the period from 1981 to 1985 ($p < 0.0001$).
Interpretation: Deaths from HCV-associated HCC increased from 1981 to 2000, consistent with the aging of the population in Japan.

Keywords: Hepatitis C virus; Hepatocellular carcinoma; Epidemiological

1. Introduction

Hepatocellular carcinoma (HCC) affects approximately half a million people each year worldwide, making it the fifth most common malignancy in men and the ninth most common in women [1–7]. Recently, a trend of increasing rates of HCC has been reported from several developed countries in North America, Europe and Asia [1–9], and the incidence of primary liver cancer in Japan has been increasing over the past four decades [10,11]. HCC often develops in patients with liver cirrhosis caused by hepatitis C virus (HCV), hepatitis B virus (HBV) or excessive alcohol consumption.

Of the hepatitis viruses that cause HCC, HCV is more common than HBV in Japan [12–15]. Although the age-adjusted incidence rates of HCC have been increasing during the period of rising HCC mortality, the temporal and demographic features of survival for HCC patients in Japan are unknown. Hence, we have analyzed these trends over time, using information from two independent databases that deal with HCC in Japan.

2. Patients and methods

We reviewed the medical records of all patients who died from liver disease and received medical care between 1981 and 2000 at the Liver Disease Center, National Nagasaki Medical Center and at The First Department of Internal

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Medicine, Nagasaki University School of Medicine. A total of 1001 patients were studied. All the patients were followed-up after diagnosis until death in one of the two hospitals and we were able to confirm their date of death and that death had occurred after severe liver disease.

All patients were entered into this study because sera were stored at −80 °C. These sera were used to assay HBV or HCV infection. A diagnosis of chronic HCV infection was based on the presence of anti-HCV antibody and HCV-RNA detected by polymerase chain reaction (PCR), whereas diagnosis of chronic HBV infection was based on the presence of hepatitis B surface antigen (HBsAg) or anti-hepatitis B core antigen (anti-HBc) reactivity. Diagnosis of HCC was based on histological findings or on characteristic images in dynamic computed tomography, dynamic magnetic resonance imaging and hepatic angiography. Demographic information, including age at death, sex and year of death, was collected from the patients' chart. Excessive alcohol consumers (an alcohol consumption of >50 g/day for 5 years) were not including in this study.

The courses of death were separated into those occurring with or without HCC when death ensued. Additionally, the patients with HCC were analyzed in 5 yearly intervals (1981–1985, 1986–1990, 1991–1995 and 1996–2000). Patients were classified according to 5-year age groups, and by HBV or HCV infection, and the number of patients in each age group with HBV- or HCV-associated HCC was calculated in each time period.

The SAS computer program for Windows was used to perform statistical analysis of the data, using analysis of variance (ANOVA).

3. Results

A total of 1001 patients died at the Liver Disease Center, National Nagasaki Medical Center and at The First Department of Internal Medicine, Nagasaki University School of Medicine from 1981 to 2000. The patients with HBV-associated HCC were 73.7% (210 of 285) in HBV-related disease and the patients with HCV-associated HCC were 84.5% (381 of 451) in HCV-related disease. There were 653 patients with HCC died. The mean time during followed-up were 2.5 years. The proportion of patients diagnosed with HBV-associated HCC was 32% (210 of 653), whereas 58% (381 of 653) had HCV-associated HCC, and an additional 2% (12 of 653) had HCC associated with both viruses (Table 1).

Table 1
Course of death from 1981 to 2000

	HBV	HCV	Overlap	Others	Total
HCC (%)	210 (32)	381 (58)	12 (2)	50 (8)	653 (100)
Chronic liver failure	47	35	1	36	119
GI bleeding	8	17	1	13	39
Other disease	3	5	0	16	24
Acute liver failure	10	1	3	19	33
Other cancer	7	12	0	114	133
Total (%)	285 (28)	451 (45)	17 (2)	248 (25)	1001 (100)

HCC, hepatocellular carcinoma; GI bleeding, gastrointestinal bleeding; HBV, hepatitis B virus; HCV, hepatitis C virus; overlap, both HBV and HCV positive; other, both HBV and HCV negative.

From 1981 to 2000, 210 patients died of HBV-associated HCC, whereas 381 died of HCV-associated HCC. Table 2 shows the number and the mean age at death from HBV- or HCV-associated HCC during the 5-year periods 1981–1985, 1986–1990, 1991–1995 and 1996–2000. The number of deaths from HBV-associated HCC was not changed within the range from 49 to 58 during the four 5-year periods: 54 (1981–1986), 49 (1986–1990), 49 (1991–1995) and 58 (1996–2000), and the mean age at death was not also statistically significantly different among the periods: 55.4 ± 9.9 (1981–1985), 55.6 ± 10.3 (1986–1990), 55.5 ± 10.6 (1991–1995) and 59.3 ± 10.2 (1996–2000). In contrast, the number of deaths from HCV-associated HCC steadily increased 2.6 times from 49 to 128 during same observation period: 49 (1981–1986), 90 (1986–1990), 114

Table 2
Mean age of KBV associated HCC deaths

Year	1981 - 1985	1986 - 1990	1991 - 1995	1996 - 2000	total
Number	54	49	49	58	210
Mean age (y.o.)	55.4	55.6	55.5	59.3	56.8
SD	9.9	10.3	10.6	10.2	10.3
	NS	NS	NS		
	NS	NS			
	NS				

Mean age of HCV-associated HCC deaths

Year	1981 - 1985	1986 - 1990	1991 - 1995	1996 - 2000	total
Number	49	90	114	128	381
Mean age (y.o.)	60.0	63.0	64.1	67.0	64.3
SD	8.1	7.0	7.2	7.9	7.8
	NS	NS	0.0267		
	0.0176	0.0016			
	< 0.0001				

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; S.D., standard deviation; NS, not significant.

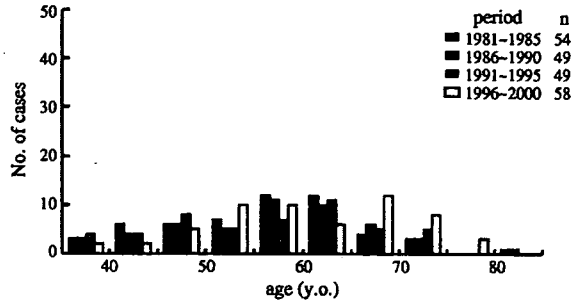

Fig. 1. Age distribution of the total number of deaths from hepatitis B virus-associated hepatocellular carcinoma from 1981 to 2000. There was no change of number of patients and age distribution of patients who died from hepatitis B virus-associated hepatocellular carcinoma during the four time periods.

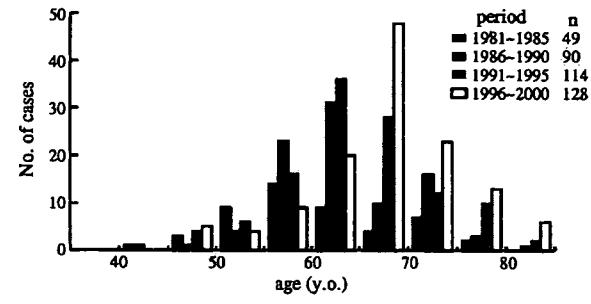

Fig. 2. Age distribution of the total number of deaths from hepatitis C virus-associated hepatocellular carcinoma from 1981 to 2000. The number of death from HCV associated HCC has increased 2.6 times during recent 20 years and this increase was provided by a close association with older shift of age distribution.

(1991–1995) and 128 (1996–2000). In addition, the mean age at death from HCV-associated HCC also increased over time. The mean age at death from 1996 to 2000 (67.0 ± 7.9 years old) was significantly higher than that from 1981 to 1985 (60.0 ± 8.1) ($p < 0.0001$), 1986 to 1990 (63.0 ± 7.0) ($p = 0.0016$) and 1991 to 1995 (64.1 ± 7.2) ($p = 0.0267$), respectively.

Fig. 1 shows the age distribution for deaths from HBV-associated HCC during the four 5-year periods. There was no change of number of patients and age distribution for deaths from HBV-associated HCC during these periods. In contrast, Fig. 2 shows the age distribution for deaths from HCV-associated HCC during the four 5-year periods. The number of patients with HCV-associated HCC aged more than 60 years in 1981–1985, 1986–1990, 1991–1995 and 1996–2000 were 22, 61, 88 and 110 patients, respectively. Fig. 2 indicated that the number of death from HCV associated HCC has increased during recent 20 years and this increase was provided by a close association with older shift of age distribution.

Table 3 shows the age distribution of HCC deaths in 5-year period (1981–1985, 1986–1990, 1991–1995 and 1996–2000). The number of patients with HCV-associated HCC obviously had an increase in the ratio of patients aged more than 60 years ($p < 0.0001$): 18.6% (1981–1985), 37.9% (1986–1990), 51.2% (1991–1995) and 54.4% (1996–2000). There was a significant difference of age distribution in the patients with HCV-associated HCC between aged more than and less than 60 years old in each 5-year period ($p < 0.0001$). In contrast, there was no difference in the age distribution of patients with other types of during these periods.

Fig. 3 shows the ratio between HCV-associated deaths and HBV-associated HCC deaths in 5-year period (1981–1985, 1986–1990, 1991–1995 and 1996–2000). The ratio between HCV-associated HCC and HBV-associated HCC increased and reached a plateau during the observation period: 0.9 (1981–1985), 1.8 (1986–1990), 2.3 (1991–1995) and 2.2 (1996–2000) (1981–1985 versus 1991–1995, $p = 0.0030$; 1981–1985 versus 1996–2000, $p = 0.0042$). Above all, the ratio of patients aged more than 60 years old increased during the observation period: 1.1 (1981–1985), 3.0 (1986–1990), 4.2 (1991–1995) and 3.8 (1996–2000) (1981–1985 versus

Table 3
Age distribution of HCC deaths in 5-year period

Age (y.o.)	1981–1985, no. (%)	1986–1990, no. (%)	1991–1995, no. (%)	1996–2000, no. (%)		p-Value
HBV						
<60	34 (28.8)	29 (18.0)	28 (16.3)	29 (14.4)	]	NS
>60	20 (17.0)	20 (12.5)	21 (12.2)	29 (14.4)		
HCV						
<60	27 (22.9)	29 (18.0)	26 (15.1)	18 (8.9)	]	<0.0001
>60	22 (18.6)	61 (37.9)	88 (51.2)	110 (54.4)		
Overlap						
<60	1 (0.9)	3 (1.9)	2 (1.2)	1 (0.5)	]	NS
>60	0	2 (1.2)	0	3 (1.5)		
Other						
<60	5 (4.2)	2 (1.2)	4 (2.3)	2 (1.0)	]	NS
>60	9 (7.6)	15 (9.3)	3 (1.7)	10 (4.9)		
Total	118 (100)	161 (100)	172 (100)	202 (100)		

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; overlap, both HBV and HCV positive; other, both HBV and HCV negative.

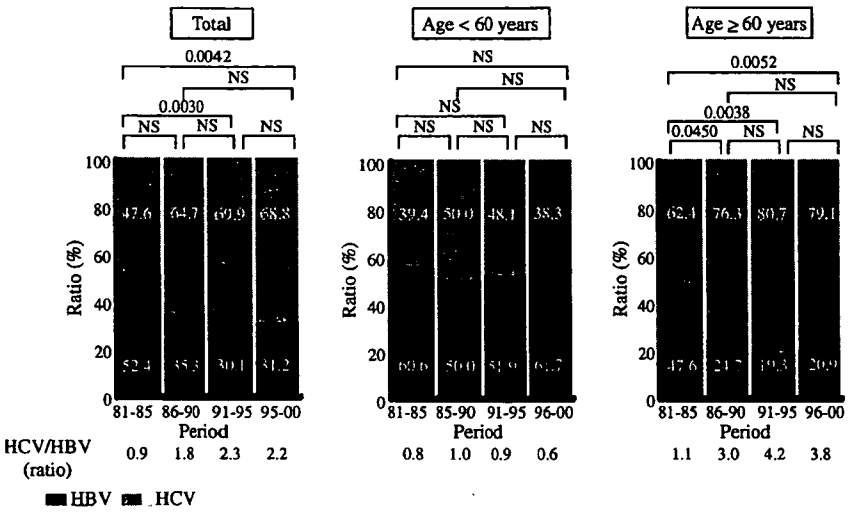

Fig. 3. Ratio between hepatitis C virus-associated hepatocellular carcinoma deaths and hepatitis B virus-associated hepatocellular carcinoma from 1981 to 2000. The ratio between HCV-associated HCC and HBV-associated HCC increased and reached a plateau during the observation period.

1986–1990, $p=0.0450$; 1981–1985 versus 1991–1995, $p=0.0038$; 1981–1985 versus 1996–2000, $p=0.0052$). In contrast, there was no difference in the ratio of patients aged more than 60 years old of during these periods.

4. Discussion

HCC accounts for approximately 6% of all human cancers. It is estimated that half a million cases occur annually worldwide, making HCC the fifth most common malignancy in men and the ninth in women [1–7,9]. The age-adjusted mortality rate from HCC has increased over the past decades in Japan [16], and in the current study more than 90% of deaths from HCC were HBV- and/or HCV-related and the number of deaths from HCV-associated HCC apparently increased 2.6 times from 1981 to 2000, and the mean age of deaths from HCV-associated HCC also significantly rose. During the same period, the number and the age distribution of deaths from HBV-associated HCC remained unchanged. The increase in the number of deaths from HCV-associated HCC seemed to be closely associated with the shift of age distribution of HCV infected population between 1981 and 2000. Although our data had the limitations of applying the findings from two hospitals to a general population, Kiyosawa described that deaths due to HCC in Japan have continued to increase in males, particularly in those older than 60 years of age between 1982 and 2003. This also suggests that the average age of diagnosis of HBV-related HCC was similar in all three time periods. In contrast, the average age of patients with HCV-related HCC rose from 61.6 years in 1982 to 63.1 years in 1990 and 67.8 years in 2003 [11]. The research group for population-based cancer registration in Japan described that incidence of HCC in Japan have continued to increase and reached a plateau in males and female from 1975 to 1999. Above all, the age distribution incidence and incident rate of HCC reached a peak older than 65 years old in males and female [17]. And, this study suggested that the ratio between HCV-associated HCC and HBV-associated HCC increased and reached a plateau from 1981 to 2000, especially more than 60 years old. Where did these findings and difference of HCC development between HCV and HBV, which were considered to be both oncogenic virus after long-term persistent infection with inflammation and fibrotic change in the liver but popular hepatitis virus infections in Japan, come from?

The simple reason may be explained as follows. From 1981 to 2000, mortality from a variceal hemorrhage in cirrhotic patients has declined [9,18]. Long term nutritional supplementation with oral branched-chain amino acids has been useful in the prevention of progressive hepatic failure, and improvement of surrogate markers and perceived health status in advanced cirrhosis has occurred [19,20]. Additionally, many new treatments and techniques have been introduced for HCC, including transcatheter arterial embolization, percutaneous ethanol injection therapy, microwave coagulation therapy, radiofrequency ablation, systemic chemotherapy and advance surgical techniques. However, these advances of medical treatment cannot explain the difference between HBV-associated HCC and HCV-associated HCC.

Alternatively, well considered reasons of the recent rapid increase of the number of patients who died from HCV-associated HCC in Japan, were shown in the current two studies. First, Hamada et al. recently reported that the majority of HCC patients develop HCC when they are aged over 60 years old, regardless of the timing of HCV infection. This result was obtained by the long-term observation of the patients infected by post-transfused HCV infection [21]. This also suggests that HCC has increased among patients over 60 years old with HCV infection and such phenomenon has never been observed nor reported till now in patients with HBV infection.

Second, the chronically HCV-infected population is aging in Japan. Yoshizawa et al. reported that age-specific prevalence rates for the presence of anti-HCV antibody among ~300,000 voluntary blood donors from Hiroshima in 1999 clearly increased with the age, reaching the highest rate of 7% in individuals who were more than 70 years old [11,22]. In a word, HCV infected people become older with years in Japan and they were regarded as a high risk for HCC. Then, the number of deaths from HCV-associated HCC has been increased recent 20 years in Japan.

El-Serag et al. reported that an increase in the number of cases of HCC affecting mainly younger age groups has occurred in the United States (U.S.) over the past two decades [23,24]. HCV infection accounts for most of the increase in the number of cases of primary liver cancer [4,6,7,9,25], while the rates of primary liver cancer associated with alcoholic cirrhosis and HBV infection have remained unchanged [4,6,9]. Tanaka et al. reported that HCV was introduced into the U.S. population around 100 years ago and was widely disseminated between 1954 and 1978 [26]. Most HCV-infected patients in the U.S. were born between 1940 and 1965 [27,28], and are therefore younger than HCV-infected Japanese patients. Hence, the burden of disease associated with HCV infection will probably increase in the U.S. during the next 10–20 years, as has occurred in Japan, as this cohort reaches an age at which complications of chronic liver disease typically occur [1–7,26]. The current study suggests that increased HCV-associated HCC will occur in the U.S. over the next two to three decades.

In conclusion, we found that the number of patients with HCV-associated HCC in Japan has increased, consistent with aging of the population, but the number of patients with HBV-associated HCC has remained unchanged over the last 20 years.

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ELSEVIER

Serum Levels of Interleukin-6 and Its Soluble Receptors in Patients with Hepatitis C Virus Infection

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ABSTRACT: Interleukin-6 (IL-6) is an important cytokine in liver regeneration, and elevated levels of IL-6 have been demonstrated in patients with chronic liver diseases (CLD). Many biological effects of IL-6 depend on naturally occurring soluble IL-6 receptors. In the present study we measured the concentrations of IL-6 and its soluble receptors in the sera of patients with CLD related to hepatitis C virus (HCV) infection. We studied 77 patients with varying degrees of HCV-related CLD. Serum levels of IL-6 and its soluble receptors (sIL-6R, sgp130) were measured by enzyme-linked immunosorbent assay. Serum IL-6 and sIL-6R were elevated in patients with CLD compared with healthy subjects. Serum levels of sgp130 did not differ between patients with chronic hepatitis and healthy subjects. However, in patients with liver cirrhosis, sgp130 was significantly elevated and was positively correlated with total bilirubin and negatively correlated with cholinesterase and prothrombin time. Our study demonstrated that in patients with HCV-related CLD, serum IL-6 and its soluble receptor levels are correlated with both liver function impairment and the degree of liver fibrosis. These observations suggest that the balance of IL-6 and its soluble receptors may correspond to the state of liver damage in patients with CLD. *Human Immunology 67, 27–32 (2006).* © American Society for Histocompatibility and Immunogenetics, 2006. Published by Elsevier Inc.

KEYWORDS: Hepatitis C virus; Interleukin-6; Liver cirrhosis; Soluble interleukin-6 receptor

ABBREVIATIONS

CLD	chronic liver disease
CH	chronic hepatitis
IL-6	interleukin-6
sIL-6R	soluble interleukin-6 receptor
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HGF	hepatocyte growth factor
LC	liver cirrhosis
TNF-α	tumor necrosis factor-α

INTRODUCTION

Interleukin-6 (IL-6) is a pleiotropic cytokine stimulating a variety of cell types, including hepatocytes [1–3]. IL-6 also modulates the hepatic expression of acute-phase proteins during inflammation [4,5]. Apart from its role in inflammation, IL-6 has been found to be essential for liver regeneration [6,7]. Results from IL-6 knockout mice have also indicated that IL-6 might be involved in triggering hepatocyte proliferation after hepatectomy [8]. The pathophysiological role of IL-6 in acute or chronic liver disease has been studied intensively [9]. Although IL-6 was consistently found to be elevated in liver diseases, such as chronic hepatitis and cirrhosis [10,11], the clinical relevance and molecular function of IL-6 in the pathogenesis of liver disease are only incompletely understood.

IL-6 mediates its diverse biological effects by interacting with a receptor complex consisting of a specific

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TABLE 1 Clinical and biochemical characteristics of patients with HCV infection

Characteristic	CH (n = 28)	LC (n = 17)	LC + HCC (n = 32)
Males (%)	12 (43)	4 (24)	19 (59)
Age	58.6 ± 11.2	65.8 ± 11.0	66.3 ± 7.3
ALP (normal range 98–251 U/l)	258 ± 65.3	404.4 ± 183.9	386.2 ± 117.7
AST (normal range 12–31 U/l)	66.1 ± 49.4	84.8 ± 28.0	86.4 ± 46.9
ALT (normal range 12–35 U/l)	90.3 ± 76.2	75.8 ± 38.2	73.8 ± 36.4
Bilirubin (mg/dl)	0.7 ± 0.2	1.5 ± 1.1	1.1 ± 0.7
Albumin (g/dl)	4.3 ± 1.0	3.6 ± 0.6	3.5 ± 0.5
Prothrombin time (%)	101.6 ± 18.2	73.6 ± 17.3	83.3 ± 20.3
Cholinesterase (normal range 160–400 IU/l)	257.4 ± 74.4	150.0 ± 59.0	141.9 ± 70.7
Total cholesterol (mg/dl)	174.0 ± 36.0	165.9 ± 36.9	142.2 ± 30.0
Platelets ($10^3/\mu l$)	187 ± 65	105 ± 37	91 ± 38
Fibrosis stage	F0:1/F1:14/F2:6/F3:3 (n = 24)	F4:9 (n = 9)	F3:1/F4:5 (n = 6)
HCV serotype	1:17/2:7 (n = 24)	1:10/2:2 (n = 12)	1:2/2:2 (n = 4)

ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Values expressed are means ± SD.

ligand-binding protein (IL-6R, gp80) and a signal transduction protein (gp130) [12]. When IL-6 binds to a cell through IL-6R (gp80), the complex facilitates its interaction with a second IL-6 receptor molecule, gp130, thereby triggering intracellular signal transduction [13]. In addition to their roles as membrane-bound proteins, IL-6R and gp130 also occur as receptors shed into the serum [14]. In several conditions, such as myeloma, chronic arthritis, and autoimmune diseases, elevated levels of soluble IL-6R (sIL-6R) have been observed [15]. sIL-6R and soluble gp130 (sgp130) have different functions. sIL-6R bound to IL-6 can interact with membrane-bound gp130 and thereby trigger activation of intracellular signaling pathways [16]. This can lead to an enhanced IL-6-mediated response.

The shed signal transducer, sgp130, itself can bind the IL-6/sIL-6R complex [17]. The resulting trimeric complex is no longer able to interact with membrane-bound gp130. In this case, sgp130 acts as an antagonist. Therefore, the biological activity of IL-6 depends on the balance of sIL-6R and sgp130. Previous studies have concentrated only on the behavior of IL-6 circulating levels in various liver diseases [18]. In this study, we evaluated serum levels of IL-6 and soluble forms of IL-6 receptors (sIL-6R, sgp130) in patients with hepatitis C virus (HCV) infections.

MATERIALS AND METHODS

Patients

We studied 77 patients with various degrees of chronic liver disease (CLD) related to HCV infection (Table 1). Of the patients, 28 had chronic hepatitis, 17 liver cirrhosis, and 32 had liver cirrhosis plus hepatocellular carcinoma (HCC). The diagnosis of HCC was made by several imaging modalities and confirmed histologically by sonography-guided fine-needle biopsy specimens in all patients. At the time of the study, none of the patients was receiving or had previously received interferon therapy. All patients were positive for anti-HCV antibodies detected by a third-generation enzyme immunoassay containing HCV antigens from the viral core and from areas of the nonstructural NS3, NS4, and NS5 regions (Ortho HCV SAVe 3.0; Ortho, Raritan, NJ) and were positive for HCV RNA in the serum as assessed by means of nested reverse transcription polymerase chain reaction. Viral serotyping was performed on 40 patients. Liver biopsy was performed on 39 patients; the degree of liver fibrosis was assessed using the METAVIR system [19]. All patients enrolled in this study were regularly followed with liver function tests every month and with ultrasonography or computed tomography of liver every 4 months. Of these, patients with marked fluctuations in these tests were excluded from this study. Patients with other concomitant causes of liver disease, such as autoimmunity or alcohol abuse (more than 40 g alcohol daily intake), or patients with metabolic disease, infections, or renal dysfunctions were not included in the study to avoid possible confounding factors. All patients were negative for hepatitis B surface antigen and had no symptom or sign related to HIV, cytomegalovirus, and *Toxoplasma gondii* infection.

None of the patients suffered from hemolytic anemia or renal failure or manifested features compatible with the presence of disseminated intravascular coagulation. In addition, sera from 23 healthy volunteers were used as controls (10 males and 13 females, mean age 45.5 ± 13.2 years). All healthy volunteers presented as normal in liver function tests, with negative serology for viral hepatitis and no history of liver disease.

Enzyme-Linked Immunosorbent Assay

For duplicate measurements solid-phase Quantikine Immunoassays (R&D Systems, Minneapolis, MN, USA)

were used to measure serum IL-6, sIL-6R, and sgp-130 according to the manufacturer's instructions.

Statistical Analysis

Data are presented as the means ± SD. The differences between quantitative variables were evaluated with the Mann–Whitney U test. A p value <0.05 for two-sided tests was considered statistically significant. The correlation between two variables was analyzed using the Spearman rank correlation test. Statistical analysis was performed with StatView software (SAS Institute, Inc., Cary, NC, USA). A p value <0.05 was required for statistical significance.

RESULTS

A total of 77 patients were studied. Of these, 35 were male and 42 female, with a mean age of 63.4 ± 10.3 years. According to the disease progression, the patients were divided into three groups (chronic hepatitis, liver cirrhosis, and liver cirrhosis plus HCC). The main clinical, biochemical, and functional characteristics of the patients are presented in Table 1.

Serum levels of IL-6 were measured in patients with HCV infections and were significantly higher in these patients than in healthy subject (Figure 1). Furthermore, this analysis revealed that circulating IL-6 levels were significantly higher in patients with liver cirrhosis (LC) than in patients with chronic hepatitis (CH).

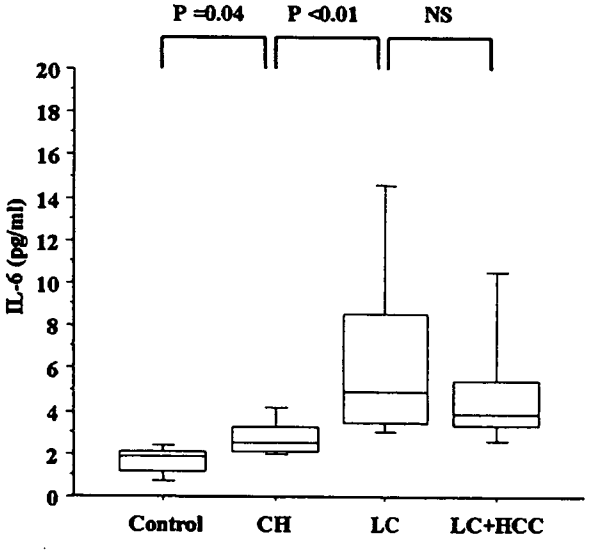

FIGURE 1 Serum IL-6 levels in patients with HCV infection and healthy controls. CH, chronic hepatitis; LC, liver cirrhosis; LC+HCC, liver cirrhosis plus hepatocellular carcinoma. The box contains the values between the 25th and the 75th percentiles and the horizontal line is the median. The error bars stretch to the 10th and the 90th percentiles.

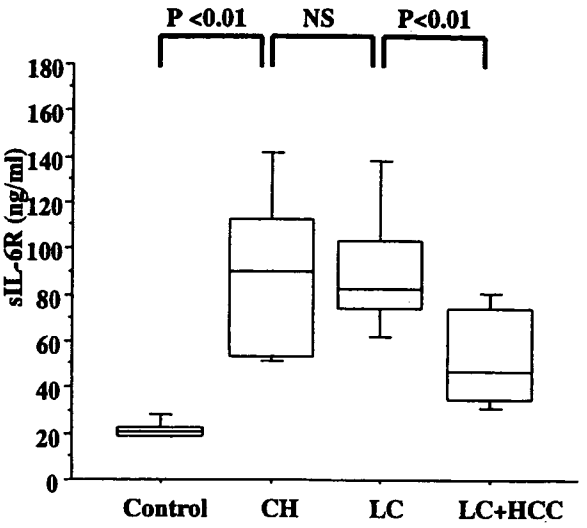

FIGURE 2 Serum sIL-6R levels in patients with HCV infection and healthy controls.

As presented in Figure 2, serum sIL-6R levels of patients with HCV infection were significantly higher than those in healthy subjects. No difference in sIL-6R level was detected between patients with CH and LC. However, sIL-6R levels varied significantly among patients with LC. That is, LC patients with HCC exhibited significantly lower levels of sIL-6R than did LC patients without HCC.

Serum levels of sgp130, which inhibits the action of IL-6, were also measured in patients with HCV infection (Figure 3). Patients with LC either with or without HCC exhibited elevated sgp130 values compared with healthy subjects. Conversely, no significant difference was observed in serum sgp130 levels between healthy subjects and patients with chronic hepatitis.

The relationships among IL-6, sIL-6R, sgp130, and clinicobiochemical parameters were also investigated. As presented in Figure 4, total bilirubin was significantly correlated with circulating sgp130 ($r = 0.49$, $p = 0.001$). In contrast, cholinesterase ($r = -0.48$, $p = 0.002$) and prothrombin time ($r = -0.39$, $p = 0.014$) were significantly inversely correlated with circulating sgp130 (Figure 5). Thus, we observed that in patients with HCV-related CLD, elevated levels of sgp130 were associated with impaired liver functions.

DISCUSSION

IL-6 plays a significant role in liver regeneration in conjunction with additional growth factors such as HGF or TNF-α [20,21]. Previous reports have demonstrated elevated IL-6 levels in patients with acute and chronic

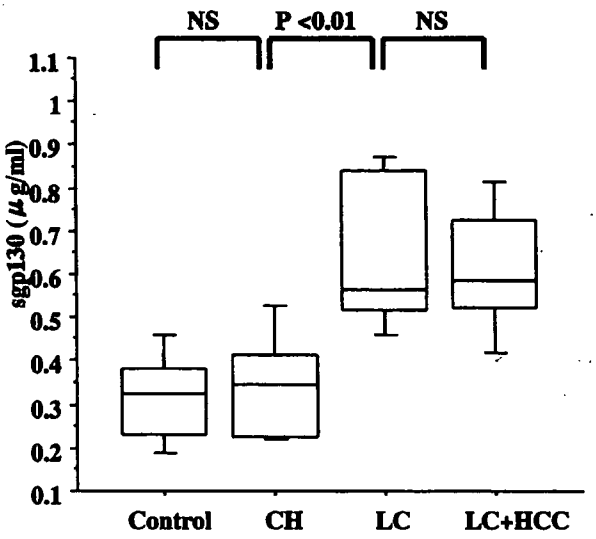

FIGURE 3 Serum sgp130 levels in patients with HCV infection and healthy controls.

liver disease [10,11,18]. The elevated levels of serum IL-6 were also demonstrated in HCV-infected patients [22,23]. However, it is unknown whether this cytokine activation represents only an epiphenomenon. To clarify the role of IL-6 in chronic liver disease, we studied the levels of soluble IL-6 receptors, which affect the biological effects of IL-6 in addition to IL-6 itself. The circulating levels of IL-6 and its soluble receptors have been demonstrated to be modulated by various diseases [24,25]. We therefore measured this cytokine and its soluble receptors in HCV-infected patients without any associated diseases or complications, such as infection, inflammation, or renal dysfunction.

One of the principle findings of this study was that both IL-6 and sIL-6R proteins were elevated in patients with HCV infection. In addition, the levels of IL-6 were significantly higher in patients with LC than in those with CH. Our data are consistent with those of a previous study demonstrating elevated circulating levels of sIL-6R in patients with liver cirrhosis [26].

sIL-6R is a ligand-binding protein that constitutes the extracellular part of the IL-6 receptor. sIL-6R markedly prolongs the IL-6 plasma half-life, and sIL-6R-bound IL-6 can interact with membrane-bound gp130 and thereby lead to activation of the intracellular signaling pathway [27]. This can lead to an enhanced IL-6-mediated response. Additionally, through this mechanism, primary unresponsive cells expressing only gp130 and no gp80 can be activated through the sIL-6R/IL-6 complex. This process has been called "*trans*-signaling" [28]. Considering this enhancing role of sIL-6R, we speculate that high levels of sIL-6R might potentiate the effects of IL-6 in HCV-induced chronic hepatitis or liver cirrhosis.

Results from studies using IL-6 knockout mice have indicated that IL-6 might be involved in hepatocyte proliferation [29]. These observations may suggest that the IL-6/sIL-6R system could be involved in liver regeneration after liver injury. Although the pathophysiological role of elevated IL-6/sIL-6R in HCV infection was not elucidated in this study, it could consist of a regenerative response against liver damage or inflammation.

Additional evidence supporting the idea that IL-6 plays a role in hepatocyte proliferation has come from experiments using double-transgenic mice expressing IL-6 and sIL-6R [30,31]. In these mice, hepatocyte proliferation was evident in the periportal area, and hyperplastic nodules were also observed. It is possible that elevated levels of IL-6/sIL-6R contribute to nodular regenerative changes in patients with HCV-induced liver cirrhosis. However, IL-6/sIL-6R may not be involved in the HCC association, because sIL-6R levels of LC patients with HCC were significantly lower than those without HCC.

Another main finding is that sgp130 levels were significantly higher in LC patients than in patients with chronic hepatitis. We evaluated the relationship between sgp130 levels and liver function tests, such as total bilirubin, cholinesterase, and prothrombin time. We observed that the increase in sgp130 is linked to impaired liver functions, including elevated bilirubin levels, prolonged prothrombin time, and reduced serum cholinesterase levels. Therefore, these findings may suggest that sgp130 elevation is related to progression of liver dysfunction as well as hepatic decompensation in patients with HCV-related CLD.

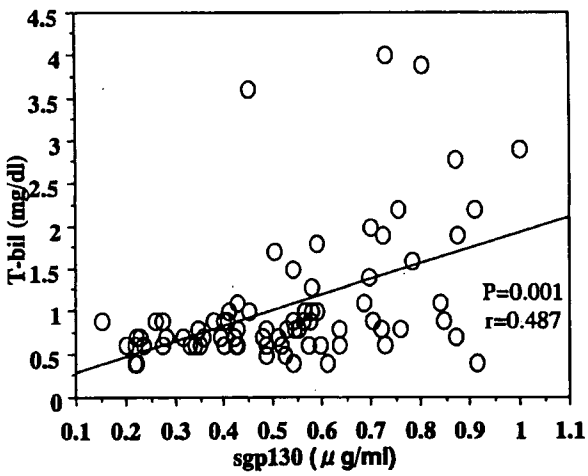

FIGURE 4 Correlation between individual values of total bilirubin and sgp130 in patients with HCV infection. T-bil, total bilirubin.

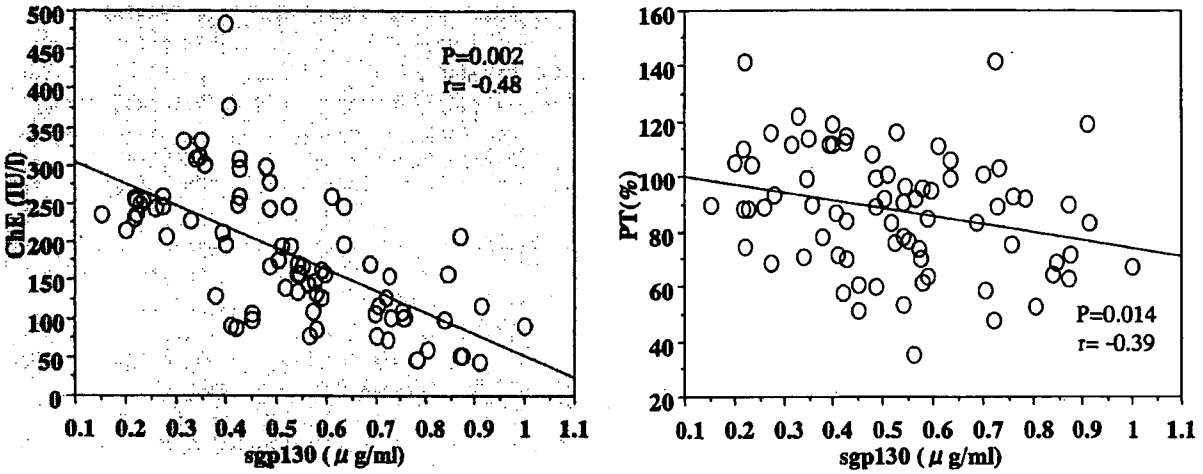

FIGURE 5 (A) Correlation between individual values of cholinesterase and sgp130 in patients with HCV infection. ChE, cholinesterase. (B) Correlation between individual values of prothrombin time and sgp130 in patients with HCV infection. PT, prothrombin time.

sIL-6R and sgp130 have different functions. The shed signal transducer sgp130 can itself bind the IL-6/sIL-6R complex and inhibit the action of IL-6. After injury, the liver has a remarkable capacity to restore major tissue loss through regeneration. IL-6 is an essential cytokine involved in liver regeneration. In fact, our data demonstrated that IL-6 and sIL-6R were significantly elevated in patients with chronic hepatitis and LC. However, under clinical conditions under which circulatory sgp130 is elevated, the IL-6/sIL-6R system may not maintain the liver regeneration. Although the exact mechanism by which sgp130 is elevated in LC patients was not elucidated in this study, this aberrant induction of sgp130 may lead to the abrogated IL-6/sIL-6R biological function and the development of liver dysfunctions and subsequent hepatic insufficiency in LC patients. Elevated levels of IL-6 and sIL-6R in HCV-related CLD likely contribute to compensatory hepatocyte growth during chronic liver injury. However, sgp130 levels in LC patients might antagonize the IL-6-mediated hepatotropic effect within the liver and could contribute to the impaired liver regeneration.

In conclusion, in this study, we have demonstrated that a progressive decline in liver function in patients with HCV-related CLD was paralleled by an increase in circulating sgp130 levels.

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HEPATOLOGY

Clinical features of hepatocellular carcinoma that occur after sustained virological response to interferon for chronic hepatitis C

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Abstract

Background and Aim: This study investigated the clinical features of hepatocellular carcinoma in patients with sustained virological response to interferon for hepatitis C viral (HCV) infection.

Methods: A total of 7715 patients with HCV infection were treated with interferon and followed up for more than 1 year after withdrawal of interferon in 64 Japanese hospitals and clinics between July 1988 and August 2001. Sustained virological response was obtained in 2515 (32.6%) patients. Of these 2515 patients, clinical data were collected for 38 patients in whom hepatocellular carcinoma developed. Sustained virological response was defined as HCV RNA negativity more than 6 months after the termination of interferon.

Results: All patients were HCV RNA negative at the time of diagnosis of hepatocellular carcinoma. The median period until the detection of hepatocellular carcinoma was 4.7 years (range 1.4–9.0 years). There were significant improvements in hepatic function including serum albumin, aspartate aminotransferase, alanine aminotransferase, indocyanine green test, platelet count and histological activity grade in comparison with those before interferon therapy and at the onset of hepatocellular carcinoma. The maximum tumor size in patients without medical follow-up for 1 year or more (median: 60 mm) was significantly larger than in patients who were periodically followed up for 6 months or less (median: 25 mm) ($P = 0.002$).

Conclusions: The present findings emphasize the importance of regular medical follow up of patients with HCV infection, as even patients showing a sustained virological response to interferon and in whom hepatic function has improved have the potential to develop hepatocellular carcinoma.

Key words: follow-up, hepatitis C virus, hepatocellular carcinoma, interferon, sustained virological response.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of most prevalent malignant tumors worldwide, and its incidence is increasing. Most cases are attributable to chronic hepatitis C virus (HCV) or hepatitis B virus (HBV) infection.[1,2] In Japan, epidemiological studies have shown that HCV is more common than HBV as the causative agent of HCC.[3,4] Because HCV infection is related to the development of cirrhosis and HCC, it was assumed that eradication of this infection would provide the most effective means of preventing HCV-related complications, including HCC.

Currently, interferon (IFN), peginterferon or combination therapy with ribavirin, are the available drugs that are effective for terminating HCV infection. IFN

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can induce a long-term favorable response and eradication of HCV RNA from serum after treatment cessation, although the response rate is not fully satisfactory.[5–8] Furthermore, patients with HCV appear to derive a definitive benefit in terms of prevention of progression to cirrhosis and the development of HCC.[7–15] However, even in some patients from whom HCV infection has been eliminated by IFN therapy, HCC can still be detected.[11–27] In these patients, the clinical features of developing HCC have not been fully investigated,[20] although they have been documented in individuals or small numbers of cases.[21–27]

The present study therefore attempted to elucidate the clinical features of HCC, especially the serial changes occurring in the period from before IFN therapy to the detection of HCC. A multicenter, collective study was conducted in the setting of hospitals and clinics belonging to the Japanese Society of Gastroenterology, Kyushu Division, in Japan, as it was felt that a study conducted in a single institution would provide inadequate numbers of sustained responders who developed HCC.

METHODS

Patients

This study was conducted at major hospitals and clinics belonging to the Japanese Society of Gastroenterology, Kyushu Division, Japan. A patient cohort in whom HCC had been detected among sustained responders to IFN therapy for chronic hepatitis C was collected by means of data collection instruments. All of the patients included had tested positive for HCV RNA before IFN therapy, and were followed up after withdrawal of IFN therapy for more than 1 year prior to the end of August 2001. Sustained virological response (SVR) was defined as HCV RNA negativity for more than 6 months after termination of IFN therapy. Diagnosis of HCC was based either on histological examination or on typical computed tomographic and/or angiographic findings at each institution. Patients were excluded if HCC was detected within 1 year after the termination of IFN therapy, because in such cases it was highly likely that the cancer had been present at the end of IFN treatment.

In Japan at the time of the study, the standard schedule was 6–10 MU IFN-α every day for the first 2–4 weeks and then three times a week for the following 20–22 weeks, or 6 MU IFN-β every day for 6–8 weeks. Patients treated with peginterferon or combination therapy with ribavirin were not included because these therapies had not been approved by the Ministry of Health, Labor and Welfare in Japan at the time of the study.

At the first data collection, hospitals were approached and information on the number of patients who had undergone IFN therapy for chronic hepatitis C and who had been followed up for more than 1 year after the termination of IFN therapy, the number of SVR patients among them, and the number of patients in whom HCC had developed among the SVR patients was requested; 64 hospitals responded, listed in Appendix I.

In the second data collection, carried out on SVR patients in whom HCC had developed, clinical data were requested for each patient from before IFN therapy and at detection of HCC.

Data collected

To elucidate the clinical features of HCC that developed in SVR patients, host-related, treatment-related and tumor-related variables before IFN therapy and at detection of HCC were investigated (Table 1). Assessments of the staging of liver fibrosis and the grade of inflammatory activity were based on the classification of Desmet *et al.*,[28] where staging is defined as: F0 (no fibrosis), F1 (fibrous portal expansion), F2 (bridging fibrosis), F3 (bridging fibrosis with architectural distortion), and F4 (cirrhosis); and grading is defined as: A0 (no activity), A1 (mild activity), A2 (moderate activity), and A3 (severe activity).

Follow-up ended with the last recorded visit before 31 August 2001. The period until the detection of HCC was measured from the day of termination of IFN therapy to the day when HCC was first diagnosed by imaging modalities such as ultrasonography or computed tomography. The medical follow-up period for the detection of HCC after SVR was defined as the interval

Table 1 Clinical features of 38 patients with chronic hepatitis C in whom hepatocellular carcinoma (HCC) developed after sustained response to interferon

Clinical feature	Before interferon	At detection of HCC	*P*-value
Host-related variables			
Age (years) [median (range)]	60 (36–71)	64 (38–77)	<0.0001
<60 [*n* (%)]	16 (42%)	4 (11%)	
>60 [*n* (%)]	22 (58%)	34 (89%)	
Sex [*n* (%)]			
Men	34 (89%)	—	—
Alcohol abuse [*n* (%)]†			
Positive	2 (5%)	—	—
Viral load before interferon (copies/mL) [*n* (%)]			
$>10^6$	2 (13%)	—	—

Table 1 *Continued*

Clinical feature	Before interferon	At detection of HCC	*P*-value
Serological group before interferon [*n* (%)]			
Group 1	6 (33%)	—	—
Group 2	12 (67%)	—	—
Hepatic function [median (range)]			
Platelet ($\times 10^4/mm^3$)	11.6 (6.6–31.0)	16.5 (7.3–31.0)	<0.0001
Total bilirubin (mg/dL)	0.7 (0.3–1.5)	0.7 (0.3–16.8)	0.32
Albumin (g/dL)	4.2 (3.3–5.0)	4.4 (3.2–5.2)	0.10
Aspartate aminotransferase (IU/L)	78 (29–288)	29 (14–159)	<0.0001
Alanine aminotransferase (IU/L)	109 (24–295)	23 (8–178)	<0.0001
Prothrombin time	81 (49–124)	89 (68–137)	0.03
Indocyanine green R_{15} (%)	15.0 (5.0–45.0)	10.6 (3.1–27.4)	0.0009
Histologic fibrosis staging [*n* (%)]			
F0	0 (0%)	1 (6%)	
F1	9 (26%)	3 (19%)	
F2	10 (29%)	8 (50%)	
F3	10 (29%)	2 (13%)	
F4	6 (17%)	2 (13%)	0.11
Histologic activity grade [*n* (%)]			
A0	0 (0%)	6 (38%)	
A1	7 (23%)	8 (50%)	
A2	17 (57%)	2 (13%)	
A3	6 (20%)	0 (0%)	0.001
Treatment-related variables			
Treatment periods (weeks) [median (range)]	24 (2–31)	—	—
Interferon type [*n* (%)]			
α	36 (95%)	—	—
β	2 (5%)	—	—
Total amount of interferon [median (range)]	480 (126–846)	—	—
Prior interferon therapy [*n* (%)]			
Positive	2 (5%)	—	—
Tumor-related variables			
Number of tumors [*n* (%)]			
Solitary	—	31 (82%)	—
Multiple (range)	—	7 (18%)	—
Maximum tumor size (mm)			
Median		30 (12–150)	
≤30 [*n* (%)]	—	21 (57%)	—
>30 [*n* (%)]	—	16 (43%)	—
Alpha-fetoprotein (ng/mL) [*n* (%)]			
>20	4 (16%)	15 (41%)	0.07
PIVKA-II (AU/mL) [*n* (%)]			
>0.063	0 (0%)	13 (43%)	0.01
Differentiation of HCC [*n* (%)]			
Well-differentiated	—	11 (44%)	—
Moderately differentiated	—	11 (44%)	—
Poorly differentiated	—	2 (8%)	—
Combined type	—	1 (4%)	
Period until development of HCC (years) [median (range)]	—	4.7 (1.4–9.0)	—
Period of medical follow-up (months) [median (range)]	—	3 (0.5–57)	—
First treatment for HCC[‡] [*n* (%)]			
Resection	—	16 (43%)	—
Local ablation	—	10 (27%)	—
Transarterial treatment	—	11 (30%)	—

PIVKA-II, protein induced by vitamin K absence or antagonist-II; R_{15}, indocyanine green retention rate at 15 min.
[†]Ethanol intake ≥80 g/day for ≥5 years. [‡]One patient has not yet undergone treatment for HCC.

during which checks for HCC were performed using tumor markers and/or imaging modalities.

Differences between data obtained before IFN therapy and at detection of HCC were evaluated using the Wilcoxon signed-rank test. All *P*-values presented in this report are of the two-tailed type. Differences at $P < 0.05$ were considered statistically significant. All analyses were conducted using SPSS 8.0 J (SPSS Inc. Chicago, IL, USA).

RESULTS

In the first data collection, a total of 7715 patients with chronic hepatitis C were identified who had been treated with IFN and followed up for more than 1 year after the termination of IFN therapy from July 1988 to August 2001 in 64 hospitals and clinics. A SVR was obtained in 2515 patients (32.6%), among whom HCC was detected in 42 (1.7%) from 24 hospitals (38%).

In the second data collection, clinical data were received for 41 patients from 23 hospitals. Of these patients, three were excluded from the analysis because of detection of HCC within 1 year after IFN therapy (one patient), concomitant hepatitis B virus infection (one patient), and a history of treatment for HCC before IFN therapy (one patient). Accordingly, the study subjects comprised 38 patients who had developed HCC after SVR to IFN therapy for chronic hepatitis C. The profiles of the patients are shown in Fig. 1.

Table 1 summarizes the clinical features of the 38 HCV patients in whom HCC developed after SVR to IFN therapy. All of the patients were HCV RNA negative at the time of HCC detection, when their median age was 64 (range 38–77) years, and 34 of the patients (89%) were ≥60 years of age. Thirty-four patients (89%) were men (sex ratio 8.5:1). When data from before IFN therapy and at the detection of HCC were compared, there were significant improvements in platelet count, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and indocyanine green retention rate at 15 min (ICG R_{15}). In the 16 patients who underwent liver biopsy before IFN therapy and at the time of HCC detection, serial changes in histological fibrosis staging and activity grade were observed (Fig. 2). Histological activity grade improved significantly after IFN therapy ($P = 0.004$). However, there was no significant improvement of histological fibrosis staging after IFN therapy ($P = 0.10$).

With regard to the HCC that developed, 31 patients (82%) had a solitary tumor and 22 patients (57%) had a tumor <3 cm in diameter. The median period until the detection of HCC was 4.7 years (range 1.4–9.0 years), and there were nine patients in whom HCC less than 3 cm in size developed more than 5 years after IFN therapy (Fig. 3). The median period of medical follow-up after the termination of IFN therapy was 3 months (range 0.5–57 months), and eight patients were not followed up for 1 year or more. The maximum tumor size in these patients (median 60 mm; range 40–150 mm) was significantly larger than in patients who were periodically followed up for 6 months or less (median 25 mm; range 12–51 mm) ($P = 0.002$). Of the 38 patients, 16 underwent hepatic resection for HCC.

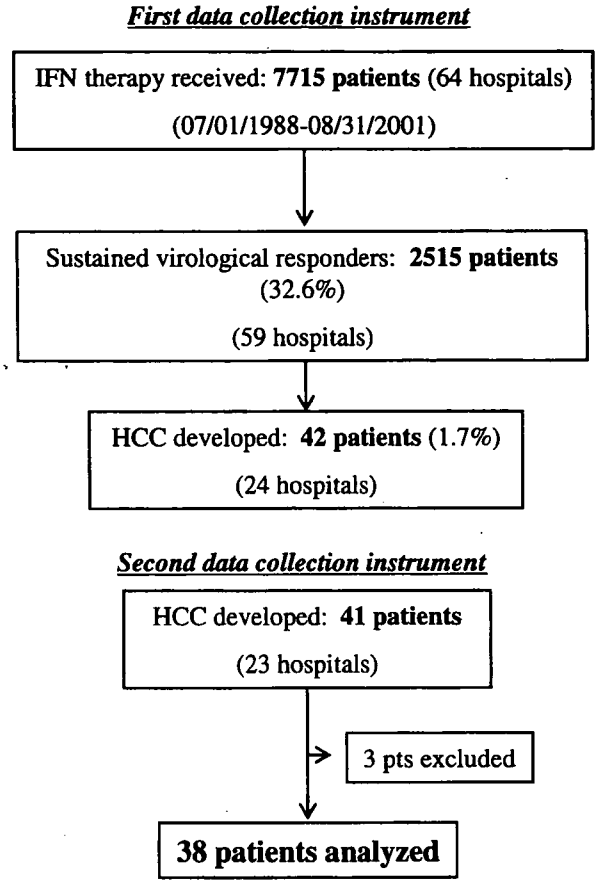

Figure 1 Profile of patients and data collection. One hospital did not respond to second data collection request. IFN, interferon; HCC, hepatocellular carcinoma.

DISCUSSION

Chronic hepatitis C is a progressive disease that is related to the development of cirrhosis and HCC. IFN, peginterferon, or combination therapy with ribavirin are widely used as standard treatments for chronic hepatitis C, the therapeutic scope being viral clearance and resolution of hepatic inflammation.[5–8] In theory, if successful in this respect, these treatments should have the additional effect of preventing HCC. Sustained eradication of HCV by IFN therapy has been shown to improve hepatic fibrosis as well as hepatic inflammation, and to suppress the occurrence of HCC.[5–15] However, there have been several reported cases of HCC that developed after successful IFN therapy.[11–27] The clinical features of HCC and the mechanisms of carcinogenesis have not yet been fully elucidated because development of HCC is very rare in sustained responders to IFN therapy.[20–27] Therefore, a multicenter study was set up to collect and analyze the clinical data for

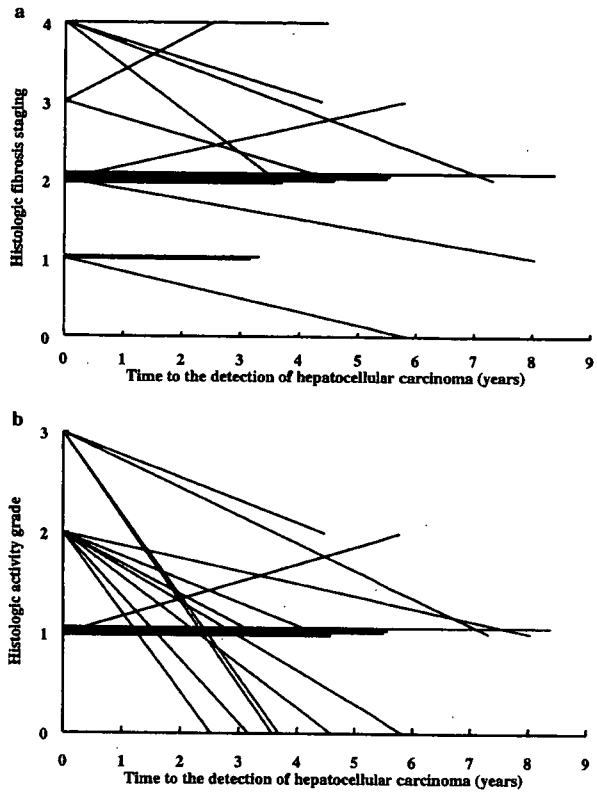

Figure 2 Serial changes in (a) histological fibrosis staging and (b) histological activity grading for each patient when compared before interferon therapy and at detection of hepatocellular carcinoma.

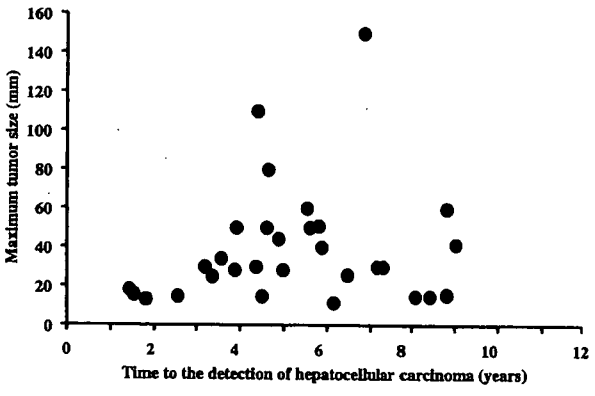

Figure 3 Maximum tumor size and time until detection of hepatocellular carcinoma.

patients who showed a SVR to IFN therapy for chronic hepatitis C and in whom HCC subsequently developed.

In this study, a total of 7715 patients with chronic hepatitis C received IFN therapy, and among them, a SVR was obtained in 2515 (32.6%). Among the patients with SVR who developed HCC, clinical data were collected for 38 patients. In regards to the clinical features of the HCC that developed in these patients, the percentage of those who were ≥60 years of age at the time of HCC detection (89%), and the percentage of men (89%) (sex ratio 8.5:1) were both high. In these patients, platelet count, albumin, AST, ALT, indocyanine green R_{15} and histological activity grade also improved significantly after IFN therapy ($P < 0.05$), although there was no significant improvement of histological fibrosis staging after IFN therapy ($P = 0.10$). Therefore, it was obvious that IFN therapy improved hepatic inflammation and hepatic function, as suggested by the results of other studies.[7–15] However, the other clinical features could not be clarified in this study, because we had no data from controls with which to compare the clinical variables of HCC that developed in patients showing SVR to IFN therapy. Potential control groups might include HCV patients with HCC who did not receive IFN therapy, or HCV patients with HCC who received IFN therapy but did not show a sustained response.[20–23] Additional comparative studies will be required in order to sufficiently elucidate the clinical features of HCC developing after SVR to IFN.

In the present study, there were 38 patients who developed HCC after successful IFN therapy, with a median period of 4.7 years (range 1.4–9.0 years) until detection of HCC. Moreover, the maximum tumor size in patients without medical follow-up for 1 year or more (median 60 mm) was significantly larger than in patients who were periodically followed up for 6 months or less (median 25 mm) ($P = 0.002$). As other studies have also indicated,[20,21] these findings suggest that the risk of HCC in sustained responders is not completely eliminated and that careful medical follow-up is important even after successful IFN therapy, which makes it difficult to determine the optimal follow-up period after SVR. If HCC had been detected at an earlier stage by regular follow-up, these patients could have been offered potentially curative treatment such as hepatic resection; such patients generally have good hepatic function after elimination of HCV. Moreover, it has also been reported that recurrence after curative treatment of HCC in SVR patients is less frequent than in non-SVR patients.[22,23] However, the enormous health care costs associated with screening all SVR patients for many years should be borne in mind. Therefore, it is also essential to identify the risk factors for development of HCC[20] and to establish the follow-up strategies in SVR patients.

Why does HCC develop even in patients showing a SVR to IFN therapy? HCV is a positive, single-stranded RNA virus without a DNA intermediate in its replicative cycle, so that integration of HCV nucleic acid sequences into the host genome, like that occurring in HBV infection, seems unlikely.[29] Therefore, HCV itself is probably not the causative factor of HCC after SVR. One assumption is that preexisting microscopic tumor foci that are not detected by diagnostic imaging are responsible for the appearance of HCC after SVR to IFN therapy, although in this study patients were excluded if HCC was detected within 1 year after the termination of IFN therapy. However, in the present series, there were nine patients in whom HCC less than 3 cm in size developed more than 5 years after IFN therapy. Although the rapidity of tumor growth may depend on individual tumor characteristics, considering

the late onset of small HCC in these patients, de novo HCC development after eradication of HCV should not be ignored. This has also been reported by Toyoda *et al.* on the basis of analysis that calculated the doubling time of HCC that occurred after SVR[24] and a long-term follow-up study of SVR patients.[21] It is conceivable that long-standing chronic liver inflammation and liver regeneration may provide the basis for tumor development. Carcinogenesis may not be a single-step event, but a complex, multi-step process, although the mechanisms are still unknown. Future studies should be aimed at defining the basic oncogenic mechanisms by which SVR patients develop HCC. Moreover, exploring the underlying mechanisms for the development of HCC in SVR patients may help identify new strategies for prevention of HCC.

In conclusion, even patients showing a SVR to IFN treatment of chronic hepatitis C and in whom hepatic function improves have the potential to develop HCC. The results of this study underline the importance of periodic medical follow-up for these patients.

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