Table 4 Risk of hepatocellular carcinoma (HCC) development

Factor	Relative risk (95% CI) SVR	Non-SVR
Male v female	1.66 (0.67–4.13)	1.97 (1.48–2.62)
Age (y)		
<39	1	1
40–49	1	7.61 (1.81–31.93)
50–59	7.67 (1.69–34.72)	17.84 (4.39–72.49)
60+	13.20 (2.93–59.53)	22.36 (5.48–91.26)
Fibrosis stage		
F0/1	1	1
F2	1.76 (0.47–6.67)	2.86 (1.59–5.13)
F3	3.10 (0.86–11.26)	6.19 (3.50–10.95)
F4	4.78 (1.13–20.18)	12.23 (6.81–21.95)

Relative risks were calculated by Cox proportional hazard regression separately in each group.

not have enough samples to calculate age stratified rates of fibrosis progression. The long term changes in HCC risk in interferon responders have not yet been clearly elucidated. Thus using the incidence rates observed in the seven year observation period was a compromise. All of the assumptions listed here may have underestimated, but not overestimated, the benefit of interferon therapy.

Lastly, we did not analyse the effect of alcohol consumption as there were very few heavy drinkers among the cohort. Alcohol is one of the major risk factors for HCC development and liver failure. The merit of successful interferon therapy may be greater in drinkers if they wish to continue alcohol. However, we recommend abstention to chronic hepatitis C patients whether or not they receive antiviral therapy.

DISCUSSION

To date, large scale cohort studies conducted in Japan, including ours, have unanimously indicated that by far the most important sequela of chronic hepatitis C is HCC development, and that interferon therapy significantly reduces its incidence. In contrast, several studies performed in Western countries found that HCC was less common, and interferon therapy did not have significant effects. The reason for this discrepancy has not been clarified. In this study, we showed that the risk of HCC substantially increased with age when patients of the same sex and fibrosis stage were compared (table 2). The prevalence of HCV infection in Japan is highly skewed to the older generations, and this may partly explain the high incidence of HCC in Japan. If this is the case, HCC incidence will increase substantially in Western countries in the near future, as it did in Japan in the 1980s.

The clinical importance of interferon therapy should be measured in terms of its efficacy in HCC prevention, at least in countries where HCC is the predominant complication of HCV infection. A popular indicator of efficacy of therapy in preventing a disease is the number (of patients) needed to treat (NNT), which is identical to the inverse of absolute risk reduction. Mathematically, NNT for one decrement in HCC development during a lifetime is equivalent to the life expectancy divided by the gain in HCC free survival. Supposing that the SVR rate is 100%, NNT is 3.92 (48.7/12.4; table 5) for a Japanese 30 year old male patient with fibrosis stage F3. This value should be divided by the expected SVR rate if the outcome is not known. As NNT is directly proportional to life expectancy, older patients have smaller values for NNT, indicating "higher efficacy", if the gain in HCC free survival is the same. This may not be intuitive for individualised consideration of indications for treatment.

Several authors have performed cost effective analyses of interferon therapy for chronic hepatitis C based on the Markov model.[31–35] In fact, our current model can be considered as a simplified Markov model where a chronic hepatitis C patient either achieves or does not achieve SVR with interferon therapy, and has the corresponding risk of HCC thereafter. Also, the HCC free survival is equivalent to the gain in quality adjusted life year, where a year before HCC development scores 1 and one after it scores 0. Owing to those simplifications, the model is not dependent on assumptive parameters but on observed data.

In conclusion, by using data obtained in a real cohort, we established an indicator of the benefit of interferon therapy—the gain in HCC free survival. This indicator may be useful in assessing the indications for interferon therapy and in selecting the best treatment protocol for individual patients.

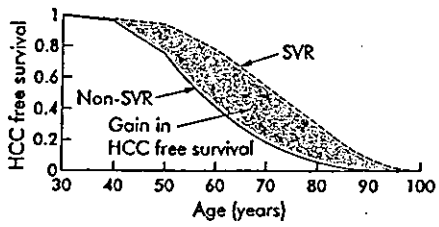

Figure 1 Gain in hepatocellular carcinoma (HCC) free survival by interferon therapy. The case of a 30 year old male patient with fibrosis stage F3. Cumulative HCC free survival curves were determined based on the patient specific HCC incidence rates and age and sex specific death rates in case of sustained virological response (SVR) and non-SVR. The area surrounded by the two curves indicates the gain in HCC free survival obtained by achieving SVR.

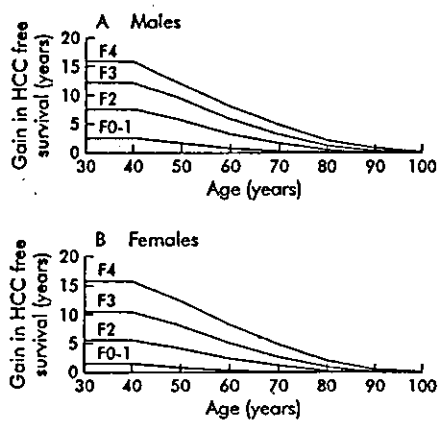

Figure 2 Gain in hepatocellular carcinoma (HCC) free survival by sustained virological response as a function of age and fibrosis stage.

Table 5 Gain in hepatocellular carcinoma (HCC) free survival by sustained virological response as a function of age and fibrosis stage

Age (y)	Life expectancy	F0/1	F2	F3	F4
Males					
30	48.7	2.48	7.66	12.40	15.98
40	39.1	2.52	7.68	12.41	15.96
50	29.9	1.68	5.75	9.45	12.14
60	21.4	0.84	3.38	5.95	8.14
70	14.0	0.40	1.70	3.26	4.98
80	8.0	0.15	0.67	1.40	2.38
Females					
30	55.3	1.45	5.60	10.52	15.73
40	45.5	1.46	5.61	10.51	15.69
50	36.0	0.93	4.24	8.17	12.44
60	26.9	0.44	2.52	5.17	8.39
70	18.2	0.22	1.30	2.81	4.95
80	10.6	0.08	0.52	1.18	2.24

Expressed in years, life expectancy was that in the Japanese general population in 2000. The gain in HCC free survival was the difference in expected cumulative HCC free survival with and without attaining a sustained virological response.

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Analysis of approach to therapy for chronic liver disease in an HCV hyperendemic area of Japan

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Abstract

In 1990, we conducted an epidemiological study of 509 residents in a hepatitis C virus (HCV) hyperendemic area in Japan. The purpose of the present study was to examine the approach to therapy for liver diseases accompanied by HCV and hepatitis B virus (HBV) infections among the surviving residents in the town after 12 years. Fifty-three with HCV or HBV infections among 385 people who resided in the town were clearly analyzed in 2002. The number of persons diagnosed with the liver diseases was as follows: HCV-related asymptomatic healthy carrier (1), past history of HCV infection (15), chronic hepatitis C (22), HCV-related liver cirrhosis (6), HCV-related hepatocellular carcinoma (HCC) (5), and HBV-related asymptomatic healthy carrier (4). HCC was detected in residents who did not have periodic regular hospital checkups. Only 19% of the 53 inhabitants consulted liver medical specialists, and 75% (3/4) who received interferon therapy received treatment from a liver medical specialist. It is necessary to provide continuous medical treatment to HCV carriers, minimizing difference in treatment quality in different medical institution. An efficient HCV medical checkup and a program of subsequence health management are important problems to be solved for improved health care.
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Keywords: Hepatitis C virus (HCV); Hyperendemic area; HCV carrier; Epidemiology; Hepatocellular carcinoma (HCC); Liver medical specialist

1. Introduction

Hepatitis C is a global health problem caused by infection with the hepatitis C virus (HCV). It is estimated that about 170 million people worldwide are infected with HCV [1]. HCV carriers in Japan are presumed to number 2 million people [2,3]. The growing incidence of HCC is expected to reach a plateau around the year 2015, and then to start to decrease according to the study of Yoshizawa [2]. However, there are many persons who are not aware that they are infected, some of whom will advance to liver cirrhosis or hepatocellular carcinoma (HCC) [4,5], and this has become an important social concern. Therefore, the following are now implemented as emergency measures by hepatitis C in the Ministry of Health, Labour and Welfare in Japan: (i) substantial dissemination of education and instructions for consulting; (ii) implementation of a hepatitis virus examination which utilizes the organization of the present health checkup; (iii) research and development of medical treatments, and maintenance of the medical-examination organization; and (iv) prevention and blocking the route of infection. The latter measure is urgent.

In our country, as part of a 5-year program from 2002 to 2007, candidates who reach certain turning points during every 5-year period from age 40–70, or those in whom abnormalities in liver function were pointed out during the basic health checkup according to the Health and Medical Service Law for the Aged will undergo examination for HCV and hepatitis B virus (HBV) infection.

Since 1990, we have conducted health screenings of the residents of "H town" (adult population: 7389), Fukuoka prefecture in northern Kyushu, Japan, which is known for

Abbreviations: HCV, hepatitis C virus; HBV, hepatitis B virus; anti-HCV, antibodies to HCV; HBsAg, hepatitis B virus surface antigen; HCC, hepatocellular carcinoma; IFN, interferon

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its high prevalence of liver diseases. We previously reported that the town had a high prevalence of HCV carriers, and that HCV infection was the principal cause of liver disorders [6–12]. The prevalence of anti-HCV antibodies (anti-HCV) among the local residents of "H town" in 1990 was 23.6% (120/509) [6].

In 1990, 509 randomly selected subjects participated in the study for examination of liver diseases accompanied by HCV or HBV infections. In the present study, we analyzed the approach therapy for HCV-or HBV-infected liver disease among the surviving residents after 12 years in the same town.

2. Subjects and methods

2.1. Subjects

As shown in Fig. 1, of 509 subjects, 69 people had died, and 55 people had moved out to other regions. Thus, just 385 of the original 509 people resided in H town in May 2002. Of these 139 inhabitants agreed to participate in the medical follow-up survey, 26 did not agree to participate, and the remaining 220 inhabitants did not declare their intention either way in 2002. Of the surviving 139 inhabitants, 35 were infected with HCV and 4 with HBV. In these 39 inhabitants with HCV or HBV infection, the following items were questioned: present health condition, continuous regular hospital checkups, medical treatment received in the past 12 years in the hospital, the name of the family doctor, and the kind of medicine taken. The 39 subjects were examined in liver function tests, and for antibodies to anti-HCV, serum HCV RNA, hepatitis B virus surface antigen (HBsAg), and ultrasonographic examination. Only 14 residents of the surviving 220 inhabitants who did not declare their intention to participate in 2002 stated by telephone the current condition of their liver disease and their primary physician treating this disease. We then asked the primary physicians about the treatment for each of these resident's liver disease, the diagnosis of liver disease, and the latest data. Consequently, we analyzed the details of liver disease in 53 inhabitants positive for anti-HCV or HBsAg in 2002.

Informed consent was obtained from all residents after the purpose and methods of the study were explained.

2.2. Serological and abdominal ultrasonic echo examination

Sera from 39 residents were provided for the following liver function tests: serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gammaglutamyl transpeptidase (γ-GTP), lactate dehydrogenase (LDH), total bilirubin (T.Bil), total protein (TP), albumin (Alb) and gamma-globulin (γ-glob.). Sera were also examined for the presence or absence of HCV or HBV infection. Anti-HCV was measured by a chemiluminescent enzyme immunoassay (CLEIA) kit (Lumipulse II HCV, Fujirebio Inc., Tokyo, Japan). HCV RNA in the sera was detected using the

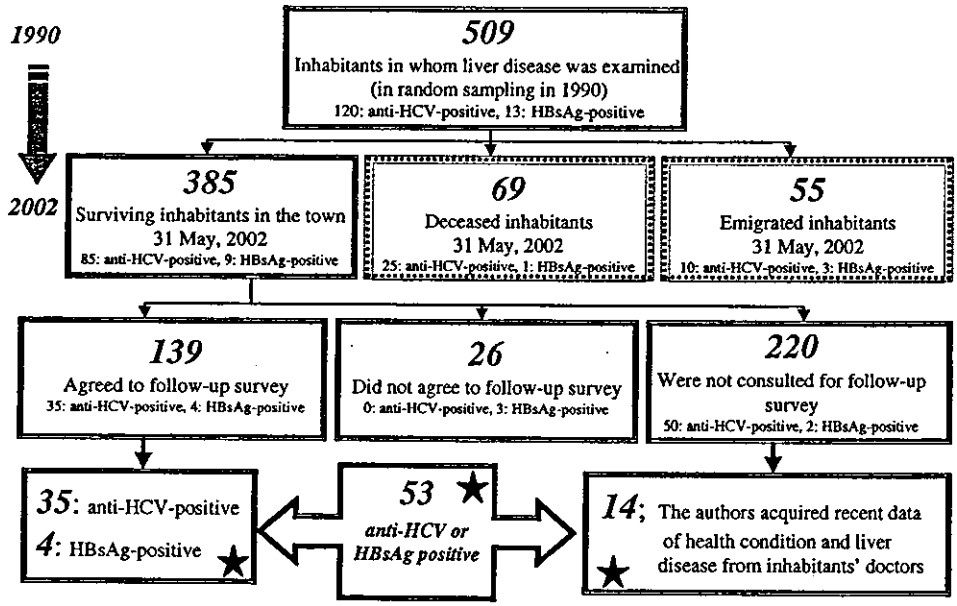

Fig. 1. Diagram for pursuing the prognostic investigation of 509 inhabitants. Of the 509 subjects, 69 people had died, and 55 people had moved out to other regions by 31 May 2002. Thus, just 385 of the original persons investigated in 1990 resided in H town in May 2002. Of these, 139 persons agreed to participate in the medical follow-up survey, but 26 did not agree. The remaining 220 inhabitants did not declare their intention either way. These 139 subjects were interviewed in person by two trained interviewers, and were their liver disease examined. Among the 220, we could ask their primary physicians about treatment for 14 of them liver disease, diagnosis of liver disease, and the latest data. Consequently, we analyzed the details of liver disease in 53 inhabitants positive for anti-HCV or HBsAg in 2002.

Amplicor HCV test (Nippon Roche, Tokyo, Japan). HCV RNA was quantified by Roche Amplicor Monitor assay. HBsAg was assayed by a chemiluminescent immunoassay (CLIA) kit (ARCHITECT™, HBsAg QT, Dainabot Co. Ltd., Tokyo, Japan). Ultrasonographic examination in subjects with abnormalities of liver function tests and who were anti-HCV- or HBsAg-positive was performed in all subjects in order to investigate the shape of the liver and lesions occupying the hepatic space. Computed tomography and liver biopsy were performed in some subjects.

3. Results

We clearly analyzed the treatment of liver diseases accompanied by HCV or HBV infection in 53 surviving inhabitants in 2002. As shown in Table 1, there were 49 inhabitants with HCV infection and 4 with HBV. The diagnoses of HCV-related liver diseases were: asymptomatic healthy carrier (1 case), past history of HCV infection (15), chronic hepatitis C (CH-C) (22), HCV-related liver cirrhosis (LC) (6), HCC with CH-C (2) and HCC with LC (3). The diagnoses of HBV-related liver disease were: asymptomatic healthy carrier (4). Three of 22 with CH-C were treated with interferon (IFN). Effect of IFN was sustained virologic response (1 case), non responder (1), and currently undergoing IFN therapy (1). One of six with HCV-related LC was treated with IFN. IFN effect was judged as non responder. Table 1 shows the treatment of liver disease in the 53 inhabitants. Ten subjects (18.9%, 10/53) visited the hospital regularly to consult the liver medical specialist. Here a "liver medical specialist" means a doctor authorized by The Japan Society of Hepatology. The treatments were distributed as follows: regular follow-up at least every 3 or 6 months by blood test or abdomen ultrasonographic examination (32 subjects), medication for liver disease other than IFN treatment, such as glycyrrhizin and ursodeoxycholic acid (10), IFN treatment (4), treatment of HCC (3), and untreated (4). Of four residents who did not visit the hospital periodically, one HCC was observed in a 76-year-old woman, in spite of having been advised that they were HCV carriers in 1990. Moreover, the other resident with liver disease accompanied by HCV infection who did not consult with the liver medical specialist also developed HCC (a 65-year-old man).

On the other hand, there were three residents who were undergoing medical treatment of HCC. One of these is a 68-year-old woman, who developed HCC in 1996, and underwent percutaneous ethanol injection therapy (PEIT) for a total of 12 times as well as therapy. The other is a 76-year-old woman, who received surgical treatment for HCC in 1993, underwent PEIT and percutaneous radiofrequency ablation (PRF) in 1999, and had received chemotherapy by transcatheter arterial embolization (TAE) in 2002. One person of the last group is an 82-year-old woman, who had received PEIT treatment in 2000.

Table 2 shows the characteristics of 22 persons with chronic hepatitis C and 6 with HCV-related liver cirrhosis. This table gives more details of the residents with hepatitis C and liver cirrhosis in Table 1. Age, sex, AST and ALT values, the level of HCV RNA (KIU/ml), and other complications are shown for each resident. Three of the four inhabitants (75%) treated with IFN consulted liver medical specialists. These results reaffirm the significance of cooperation in the medical treatment of liver disease between the primary physician and the liver medical specialist.

Fig. 2 shows the diagnosis of liver diseases in 1990 in the 49 inhabitants with accompanied by HCV infection in 2002. Most HCV carriers showed an advanced stage of disease. Just one of 27 inhabitants with chronic hepatitis C recovered from sustained HCV infection by IFN treatment. Two of the inhabitants surveyed became infected with HCV between 1990 and 2002.

Table 1
Treatment of liver diseases in 53 inhabitants positive for anti-HCV or HBsAg

Case (number of patients)	Follow-up (32)	Treatment of liver disease without IFN (10)	History of IFN (4)	History of treatment for HCC (3)	No treatment (4)
HCV-related ASC (1)					
Past history of HCV infection (15)					
CH-C (22)					
LC-C (6)					
CH-C + HCC (2)					
LC-C + HCC (3)					
HBV-related ASC (4)					

ASC: asymptomatic healthy carrier; CH-C: chronic hepatitis C; LC-C: HCV-related liver cirrhosis; HCC: hepatocellular carcinoma; (✱) hepatologist; (◉) no hepatologist involved.

Table 2
Characteristics of 22 persons with chronic hepatitis C and 6 HCV-infected persons with live cirrhosis

	Follow-up (32)	Treatment of liver disease without IFN (10)	History of IFN (4)	No treatment (4)
	Age (sex, AST/ALT [IU/l], HCV RNA level [KIU/ml], the other)	Age (sex, AST/ALT [IU/l], HCV RNA level [KIU/ml])	Age (sex, AST/ALT [IU/l], HCV RNA level [KIU/ml], IFN effect)	Age (sex, AST/ALT [IU/l], HCV RNA level [KIU/ml])
CH-C (22)	(1) 67 (F, 33/32, 930) #[a] (2) 66 (F, 24/31, 194) #[a] (3) 72 (M, 40/40, ND, liver meta. For rectal cancer (4) 81 (M, 15/12, ND (5) 84 (M, 38/22, >850) (6) 35 (F, 25/30, 48) (7) 66 (F, 27/66, ND) (8) 47 (F, 27/43, 520) (9) 89 (F, 29/17, 733) (10) 77 (F, 38/35, 270) (11) 51 (F, 22/20, >850) (12) 56 (F, 30/26, 166)	(1) 50 (F, 30/28, 573) (2) 75 (F, 27/13, 260) (3) 74 (M, 45/31, 379) (4) 67 (F, 37/37, 114) (5) 66 (F, 27/30, 696)	(1) 70 (M, 48/22, 352, NR) #[a] (2) 65 (M, 41/34, HCV RNA negative, SVR) #[a] (3) 57 (M, 67/59, undergoing IFN + ribavirin treatment)	(1) 65 (F, 71/99, 423) (2) 52 (F, 90/115, >850)
LC-C (6)	1. 75 (F, 37/15, ND, terminal bladder cancer)	(1) 86 (M, 79/70, ND) (2) 69 (F, 23/27, 366) (3) 57 (F, 49/55, 201) (4) 68 (M, 101/78, 558)	(1) 53 (M, 55/57, >850, NR) #[a]	

CH-C: chronic hepatitis C; LC-C: HCV-related liver cirrhosis; ND: not done; SVR: sustained virologic response; NR: non responder.
[a] Hepatologist.

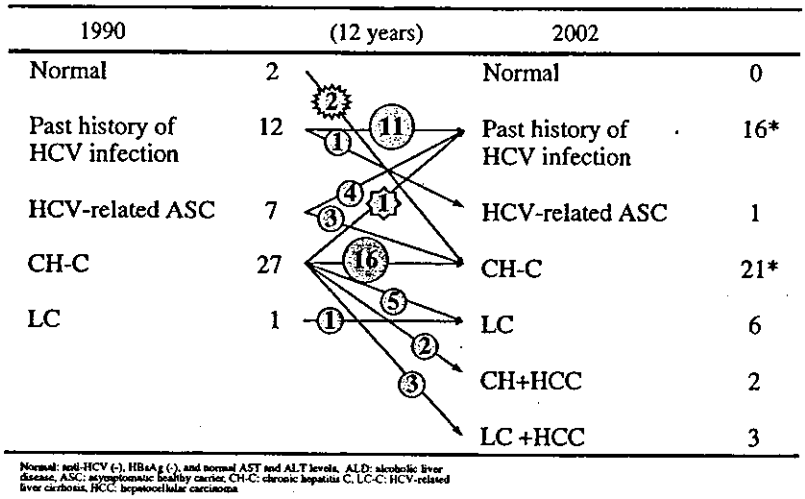

Fig. 2. The diagnosis off liver disease 12 years ago in 49 inhabitants with liver diseases accompanied by HCV infection in 2002. Among the 22 with chronic hepatitis C indicated in Table 1, one person had recovered from HCV infection due to IFN treatment. Therefore, he was counted under "past history of HCV infection". Most HCV carriers had an advanced stage of disease. A newly infected person is denoted by O, and IFN therapy with eradication of HCV is denoted by O.

4. Discussion

We previously reported studies of "H town", a hyperendemic area of HCV infection [6–12]. In the present report, we have analyzed the details of liver disease of 53 inhabitants positive for anti-HCV or HBsAg in 2002 through a follow-up survey after 12 years of the original 509 inhabitants in whom liver disease examined in an HCV hyperendemic area in 1990. HCC was detected in residents who did not visit the hospital regularly for 1 year or more, in spite of being advised of their HCV infection in 1990. Only 19% who did visit the hospital regularly consulted liver medical specialists, and 75% of residents with IFN treatment history received medical treatment by the liver medical

specialist. Though some of the inhabitants who underwent a treatment of liver disease without IFN treatment and a follow-up shown in Table 2 had indication to undergo an IFN treatment, few of them received explanations about an IFN treatment. Some of residents suffered from the medical institution as hepatitis C or liver cirrhosis might be able to cure HCV infection, if they are treated with IFN treatment at the younger age. The present study suggests that the cooperation is essential in the medical treatment of liver disease between the primary physician and the liver medical specialist.

IFN treatment is administered as the standard therapy for chronic hepatitis accompanied by HCV infection. Recently, the anti-viral drug ribavirin has been used in combination with IFN and has resulted in significantly improved responses: current studies show a 28–66% sustained response after 48 weeks of treatment [13,14]. The side-effects of the combination therapy are, however, "universal, significant, and possibly serious." Ribavirin frequently causes hemolytic anemia leading to necessary dose reductions and is a known teratogen [15]. For the remaining patients who cannot be treated with IFN, glycyrrhizin is often used. In Japan, glycyrrhizin has been an accepted treatment of chronic hepatitis for over 20 years [16]. Glycyrrhizin is known to prevent the development of HCC [17]. The purpose of the medical checkup in the high-risk group infected with HCV is the followings three items, as Yoshizawa has advocated [2]: (i) IFN therapy for eradication of HCV so that the patient is no longer at high risk for HCC; (ii) conservative (anti-inflammatory) therapies, such as glycyrrhizin, ursodeoxycholic acid, and phlebotomy, for postponing the development of HCC; and (iii) diagnosis of HCC at an early stage for early treatment and prolonged lifespan.

By telephone interview, we were able to acquire the reasons why some of the residents did not have medical checkups and did not visit the hospital regularly, in spite of being recognized as infected with HCV in 1990. These reasons were: the high cost of medical care, the absence of liver disease, to keep the condition a secret, "too busyness", etc. While we emphasize consultation and medical examinations for HCV carriers, we must not forget efforts to provide accurate information of liver diseases to the general population or to trained medical workers. Since 2003 an HCV carrier discovered newly by an examination in the H town has been obliged to undergo a precision examination at a designated hospital holding a full-time hepatologist. Moreover, the letter of introductions and the report of health guidance from the municipality to a medical institution, the result report and the request letter of health guidance from a medical institution to that municipality were made. Now, based on the flow chart of HCV examination, hospitals of a primary medical institution and a second medical institution cooperate together.

In conclusion, it is necessary to continuously provide medical treatment to recognized cases of HCV carriers, minimizing difference in treatment quality in different medical institution. An efficient HCV medical checkup and a program of subsequent health management are important problems to be solved for improved health care.

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Hepatitis B virus genotype G is an extremely rare genotype in Japan

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Abstract

Background: Hepatitis B virus (HBV) has been classified into seven genotypes (A–G). HBV genotypes have a geographically characteristic distribution. Since HBV genotype G (HBV/G) was identified recently, little is known about the distribution of HBV/G in Japan. The aim of this study was to clarify this issue.
Patients and methods: Seven hundred and twenty-one serum samples obtained from patients with HBV in Japan were investigated. The patients included 149 asymptomatic carriers, 325 with chronic hepatitis, 129 with liver cirrhosis, and 118 with hepatocellular carcinoma. Six HBV genotypes (A–F) were determined by restriction fragment length polymorphim targeting to the S region of the HBV genome. Furthermore, HBV/G was investigated by polymerase chain reaction with hemi-nested primers derived from an HBV/G-specific nucleotide sequence.
Results: Of the 721 serum samples investigated, 12 subjects were classified as having HBV/A, 88 HBV/B, 610 HBV/C, 3 HBV/D, and 1 HBV/F. Seven subjects had a mixed infection with distinct genotypes, two with HBV/A and HBV/D, and five with HBV/B and HBV/C. HBV/G was not identified among the 721 samples.
Conclusion: HBV/G was not identified in a large cohort of patients with HBV, either single or dual infection. HBV/G seems to be an extremely rare genotype in Japan.
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Keywords: Distribution; Genotypes; Hepatitis B virus; Japan; Polymerase chain reaction; Restriction fragment length polymorphism

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1. Introduction

Hepatitis B virus (HBV) infects approximately 350 million individuals worldwide and can cause a wide spectrum of liver disease [1]. HBV has been classified into seven genotypes based on an entire genome difference of more than 8% [2–4]. HBV genotypes have a geographically characteristic distribution [5]. HBV genotype A (HBV/A) and HBV/D are the most common genotypes worldwide, and account for the majority of cases in Europe and Africa. HBV/B and HBV/C are found in East Asia. HBV/E is confined to Africa, and HBV/F has been identified in indigenous populations of Central and South America. In 2000, a unique strain harboring a 36-base pair (bp) insertion into the core region was identified in France and was phylogenetically classified into the seventh genotype, G [4]. Thereafter, HBV/G was revealed to be distributed in San Francisco [6,7], Germany [8], Mexico [9], and Canada [10], and accounted for 1–5% in these areas. Although little is known about the virological and clinical characteristics of HBV/G, one of its unique characteristics is frequent coinfection with the other genotypes. In San Francisco, eight of the eight HBV/G patients were coinfected with HBV/A [6,7], and all of the HBV/G isolates from Canada were also coinfected with HBV/A, or HBV/A and HBV/C [10].

In Japan, HBV/C is the most common genotype, accounting for approximately 85% of all genotypes, and HBV/B follows with 12% [11–13]. However, little is known about the distribution of HBV/G in Japan. We have formerly investigated the 540 sera from patients with hepatitis B collected in and around Nagoya, and found that there were no HBV/G among them [14]. However, the serum samples in the study was obtained from a restricted area, a central part of Japan, therefore, further studies including serum samples collected from the other part of Japan had been required to conclude how often HBV/G distributed in Japan. Moreover, since HBV/G is frequently coinfected with the other genotypes, there is a possibility that HBV/G might exist as a minor population in the sera classified into the other six genotypes (A–F). At this time, to elucidate this issue, we conducted nationwide study of the distribution of HBV/G by analyzing sera obtained from patients with hepatitis B, including those whose genotypes were already known, using hemi-nested polymerase chain reaction (PCR) with HBV/G-specific primers. We also discussed the issues of HBV/G to date.

2. Materials and methods

2.1. Patients

Seven hundred and twenty-one serum samples were collected from patients with HBV in Japan. The patients resided in Hokkaido, Iwate, Yamagata, Niigata, Tokyo, Kanagawa, Nagano, Nagoya, Kyoto, Fukuoka, and Okinawa. The patients in this study were overlapped with some of the previous report [11]. They included 470 (65.1%) males and 251 (34.8%) females. The mean ± S.D. age was 43.6 ± 14.9 years (Table 1).

Table 1
Demographics of the 721 patients in this study

Sample	721
Gender (M:F)	470:251
Age (year)	43.6 ± 14.9
ALT (IU)	78.8 ± 115.8
ALP (IU)	240.8 ± 155.2
γ-GTP (IU)	52.2 ± 96.2
T. bil (mg/dl)	0.99 ± 1.60
HBeAg (%)	45.2
HBV DNA[a] (LGE/ml)	5.69 ± 1.84
Diagnosis	
Asymptomatic carrier	149
Chronic hepatitis	325
Liver cirrhosis	129
Hepatocellular carcinoma	118

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GTP, gamma-glutamyl transpeptidase; LGE, log genome equivalents; T. bil, total bilirubin; TMA, transcription-mediated amplification.

[a] Value was calculated using available data of transcription-mediated amplification of 255 subjects.

2.2. Detection of hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg) and HBV DNA level

HBsAg was detected by a particle-agglutination test using a commercial kit (Serodia: Fujirebio, Tokyo, Japan), and HBeAg was detected by ELISA using a commercial kit (Serodia: Kokusai-shiyaku, Tokyo, Japan), following the manufacturer's recommendations. Levels of HBV DNA were determined by the transcription-mediated amplification (TMA) method (Chugai Industry, Tokyo, Japan), and the results were expressed as log genome equivalents (LGE) per millilitre.

2.3. Determination of six HBV genotypes (A–F) by restriction fragment length polymorphism (RFLP)

DNA was extracted from 100 μl of serum samples using commercial kits (Smitest EX R&D: Genome Science, Fukushima, Japan) under manufacturer's recommendation. The extracted DNA was amplified in a 50-μl reaction mixture containing 0.5 μM of a sense primer MF1 (5′-YCC TGC TGG TGG CTC CAG TTC-3′: nt. 55–75), 0.5 μM of an antisense primer MR2 (5′-AAG CCA NAC ART GGG GGA AAG C-3′: nt. 730–709), 2.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems Japan Co. Ltd., Tokyo, Japan), 0.2 mM each dNTPs, 3 mM $MgCl_2$, and 1× AmpliTaq Gold Buffer. The reactions were performed in a GeneAmp PCR system 9600 thermocycler. The sample was denatured at 96 °C for 9 min, and subjected to 40 cycles of PCR (95 °C for 1 min; 60 °C for 1 min; 72 °C for 1 min) followed by 72 °C for 5 min at final extension in a 96-well cycler (GeneAmp 9600; Perkin-Elmer, Norwalk, CT, USA). The amplified product

was subjected to the second round PCR with a sense primer MF2 (5′-GTC TAG ACT CGT GGT GGA CTT CTC TC-3′: nt. 246–271) and MR2 under the same condition as the first round PCR. The second round PCR product with the length of 485 bp was subjected to the digestion with five kinds of restriction enzymes. Genotype B could be distinguished by digestion with *Ear*I because of no recognition site of it was existed. Similarly, genotype C also could be distinguished by digestion with *Alw*I, as no recognition site of it was found within the amplified product. Only genotype E had a recognition site of *Nci*I, and only genotype F had no recognition site of *Hph*I. Finally, the distinction between genotypes A and D were done by digestion with *Nla*IV. Genotype A has a recognition site of *Nla*IV, result in the generation of fragments of 220 and 265 bp. While genotype D had two recognition site of *Nla*IV, result in generation of fragments of 34, 186, and 265 bp. Therefore, genotypes A and D were distinguished by if each of 220 and 186 bp were observed, respectively. The digested amplicon were run on 3% agarose gel stained with ethidium bromide and observed under UV light [15].

2.4. Identification of HBV/G

Nucleic acids extracted from serum were subjected to PCR with hemi-nested primers designed on the 36-bp insertion in the C gene of HBV/G genomes. In brief, the DNA was amplified by the first round of PCR for 40 cycles with HBHKF1 (sense: 5′-ACG GGG CGC ACC TCT CTT TAC-3′ [nt. 1519–1539]) and HBHKR2 that involved the 36-bp insertion characteristic of HBV/G (antisense: 5′-AGC CAA AAA GGC CAT ATG GCA-3′ [nt. 17–37 in the core gene of HBV/G]) in the presence of AmpliTaq Gold (Applied Biosystems, Foster City, CA). The second round of PCR was performed for 40 cycles on the product of the first-round PCR with HBHKF2 (sense: 5′-GCA CTT CGT TTC ACC TCT GCA-3′ [nt. 1581–1601]) and HBHKR2. Then, the products were examined for fragments of 357 bp [15].

3. Results

3.1. Demographics, laboratory findings, and diagnosis of the patients

The mean value of alanine aminotransferase (ALT), alkaline phosphatase, gamma-glutamyl transpeptidase, and total bilirubin in the sera was 78.8 ± 115.8 IU, 240.8 ± 155.2 IU, 52.2 ± 96.2 IU, 0.99 ± 1.60 mg/dl, respectively (Table 1). Three hundred and twenty-six patients (45.2%) were positive for HBeAg. The mean value of HBV DNA measured by TMA was 5.69 ± 1.84 LGE per millilitre. One hundred and forty-nine patients (20.1%) were diagnosed as asymptomatic carriers, 325 (45.1%) with chronic hepatitis, 129 (17.9%) with liver cirrhosis, and 118 (16.4%) with hepatocellular carcinoma.

Table 2
Six genotypes (A–F) and HBV genotype G in 721 subjects from Japan

Genotype	No.	No. of HBV genotype G
A	12	0
A+D	2	0
B	88	0
B+C	5	0
C	610	0
D	3	0
F	1	0

3.2. HBV/G among 721 serum samples

Of the 721 serum samples investigated, 12 subjects were classified as having HBV/A, 88 HBV/B, 610 HBV/C, 3 HBV/D, and 1 HBV/F (Table 2). Seven subjects had a mixed infection with distinct genotypes, two with HBV/A and HBV/D, and five with HBV/B and HBV/C. HBV/G was not identified among the 721 samples.

4. Discussion

Several lines of evidence about the clinical significance of HBV genotypes have been accumulated in recent years. HBV/C causes more severe liver diseases than HBV/B by prolonging active hepatitis accompanying HBeAg production [16,17]. In a Western study, the rate of sustained remission after seroconversion was higher in genotype A than in genotype D hepatitis in patients who seroconverted to anti-HBe, and mortality related to liver disease was more frequent in genotype F than in genotype A or genotype D hepatitis [18]. Clinical data concerning HBV/G are very limited. One previous study analyzed 165 patients living in San Francisco and showed that the ALT level was higher in HBV/G than in HBV/C, and HBeAg was more prevalent in HBV/G than in HBV/C or HBV/D [7]. Further studies with a large sample size are warranted to confirm these findings.

Coinfection with distinct genotypes was seen also in other than HBV/G. In this study, coinfections with HBV/A and HBV/D as well as HBV/B and HBV/C were observed. In the previous study, analyzed 256 sera from the USA, Japan, Uzbekistan, Bangladesh, South Africa, and Cameroon, coinfection with distinct genotypes was identified in 28 subjects (10.9%) [19]. The occurrence of coinfection with distinct genotypes is important in virological aspects. It is reported that genomic recombination between distinct genotypes resulted in hybrid HBV strains, which causes distinct degree of liver diseases [20,21]. In such cases, genomic recombination never occurs without coinfection with distinct genotypes. However, clinical implication of coinfection with distinct genotypes per se still remains unanswered.

Ten years before the classification of HBV/G by Stuyver et al. [4], a unique strain with a 36-nucleotide insertion into the core region, which is known to a characteristic of HBV/G nowadays [22], was isolated from a homosexual man with hu-

man immunodeficiency virus infection [23]. Laboratory findings of his serum showed a few curious values. One was that HBeAg was detected in his serum in spite of a stop codon existing in the precore region of its genome, generally aborting the production of HBeAg at the stage of translation. Stuyver et al. also observed the same phenomenon, detection of HBeAg despite the stop codon in the precore region, and speculated that HBV/G might harbor another mechanism for producing HBeAg. Two years later, the mystery was solved by demonstration of coinfection with HBV/A in four of four sera with HBV/G [6]. It was explained that the HBeAg in the sera was produced by the coinfected precore wild type HBV/A. Furthermore, it was revealed that eight of the eight HBV/G patients from San Francisco were coinfected with HBV/A [7], and three of the three HBV/G patients were coinfected with HBV/A, or HBV/A and HBV/C in Canada [10]. These findings of the high frequency of coinfection of HBV/G with other genotypes give rise to another question, of whether HBV/G is competent to replicate by itself. An inoculation experiment in chimpanzees or an expression study in cultured cells would be required to answer this question.

The entire genome sequence of HBV/G has been reported from France [4,24], the USA [22], and Germany [8] so far. Interestingly, the sequence homology of these strains was surprisingly high. In one study in the USA, 10 HBV/G isolates, including 8 from San Francisco as well as 2 from France (FR1 [4] and B1-89 [24]), had a sequence homology of 99.3–99.8% among themselves [22]. Furthermore, another report from Germany showed that the HBV/G isolate (235/01) was nearly identical (sequence homology of the entire length was 99.7%) to both B1-89 and FR1 [8]. There are a few possible explanations for this finding. One possibility is that there are epidemiological links among French, German, and American HBV/G. A patient with HBV/G from Germany [8] and a homosexual male patient with HBV/G from San Francisco [23] were both positive for human immunodeficiency virus type-1. Thus, HBV/G might spread among a specific population, such as homosexual men or intravenous drug users. This would be also associated with the fact that HBV/G was not found among the patients in the current study, in which homosexual and intravenous drug were not included. The other possibilities are that HBV/G has a high genetic stability or was introduced into humans very recently. The mutation rate of HBV has been estimated to be 4.57×10^{-5} per site per year [25]. Thus, HBV/G might have an exceptionally low mutation rate under specific conditions, or the time since its introduction into humans might not have been long enough to gain a genetic diversity like that of the other six genotypes. To elucidate this issue, more HBV/G isolates from a wide variety of areas should be investigated.

In conclusion, HBV/G was investigated in a large cohort of patients with HBV from various areas in Japan, but no HBV/G isolate was identified, in either single or dual infection. The finding of the current nationwide study, the same as that of the previous study investigated the patients in a restricted area, indicates that HBV/G is extremely rare in Japan. Further studies with a large sample size from various areas in the world are required to further reveal the virological and clinical characteristics of HBV/G.

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A cohort study of chronic liver disease in an HCV hyperendemic area of Japan: a prospective analysis for 12 years

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Abstract. A mass screening in 1990 of H town in Japan demonstrated a high prevalence of hepatitis C virus (HCV) infection in our previous studies. The purpose of the present study was to evaluate the prognosis and natural history of liver disease among the same residents after 12 years. Of 509 residents, 69 people had died, and 55 people had moved to other regions. In all, 139 persons of the remaining 385 residing in H town were examined for liver function tests, antibodies to HCV (anti-HCV), serum HCV RNA, and hepatitis B virus surface antigen (HBsAg). The data of 14 of these 385 people were collected from medical records. The cause of death of the 69 individuals was investigated. The prognosis of liver disease could be clarified after 12 years in 222 of the 509 residents. Most of the residents with liver disease had an advanced stage of disease. Of the 69 persons who died, the mortality rate caused by liver cirrhosis or hepatocellular carcinoma (HCC) was 44 and 53%, respectively, among 25 persons with positive anti-HCV, and 19 with positive HCV RNA. One person with positive HBsAg died of HCC. Persons with chronic HCV or HBV infection had significantly higher mortality rates from liver cirrhosis and HCC than those without infection ($P<0.00001$). The present study suggests that early detection and treatment for HCC should be carried out as HCV carriers age. Furthermore, persistent HCV carriers should receive therapy for suppression of the development of HCC. The eradication of HCC should be considered a national goal.

Introduction

Hepatitis C virus (HCV) is recognized as a major threat to global public health. Although representative prevalence data are not available from many countries, the available data indicate that ~3% of the world's population is infected with HCV. It is estimated that ~170 million people worldwide are infected with HCV (1), of whom some 2 million (1%) reside in Japan (2,3). HCV leads to serious consequences such as liver cirrhosis and hepatocellular carcinoma (HCC) (4,5). Of the HCC cases in Japan, ~16% is caused by hepatitis B virus (HBV) infection and ~80% by HCV infection. The growing incidence of HCC is expected to reach a plateau around the year 2015, and then to start to decrease according to the study of Yoshizawa (2).

Up to now we have continued carrying out health screenings of the residents of H town (adult population: 7,389) (Fig. 1), Fukuoka prefecture in northern Kyushu, Japan where the prevalence of HCV infection is the highest in the country (6). We previously reported that the town had a high prevalence of HCV carriers, and that HCV infection was the principal cause of liver disorders (7-13). In 1990, 10% (739 people) of the 7,389 inhabitants were randomly selected, and as a result, 509 subjects participated in the study for examination of liver diseases accompanying HCV or HBV infections. In the study, the positive findings of antibodies to HCV (anti-HCV), HCV RNA, and hepatitis B surface antigen (HBsAg) were 23.6 (120/509), 17.9 (91/509), and 2.6% (13/509), respectively (7).

In the present study, we conducted cohort studies from August 1990 to May 2002 on the long-term prognosis of HCV carriers with the same subjects in the town and investigated risk factors for deaths due to HCC and liver cirrhosis.

Materials and methods

Subjects. Of the 509 subjects, 69 people had died, and 55 people had moved to other regions. Thus, just 385 people of the original subjects resided in H town in May 2002. Of these 139 persons (51 men/88 women; mean age ± SD, 66.6±13.1) agreed to participate in the follow-up survey (Fig. 2). These 139 subjects were interviewed in person by 2 trained interviewers. The following items were questioned: present health condition, regular hospital visits, medical treatment received in the past 12 years in the hospital, the name of the family doctor, and the kind of medicine taken. The data of 14 of the 385 people were collected from their medical records. Informed consent was obtained from all residents after the purpose and methods of the study were explained.

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Abbreviations: HCV, hepatitis C virus; HBV, hepatitis B virus; anti-HCV, antibodies to HCV; HBsAg, hepatitis B virus surface antigen; HCC, hepatocellular carcinoma; IFN, interferon

Key words: hepatitis C virus (HCV), hepatocellular carcinoma (HCC), hyperendemic area, epidemiology, cohort prospective study

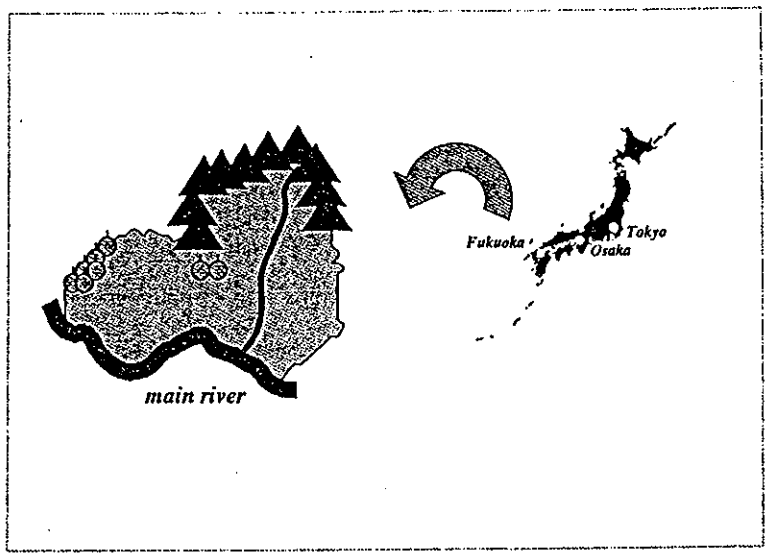

Figure 1. Location of H town of Fukuoka prefecture in northern Kyushu, Japan. The total area of the town is 44.98 km², and there are 7,389 adults in the population. Centering on the hot springs of the Chikugo River, production of fruit trees is a prosperous endeavor.

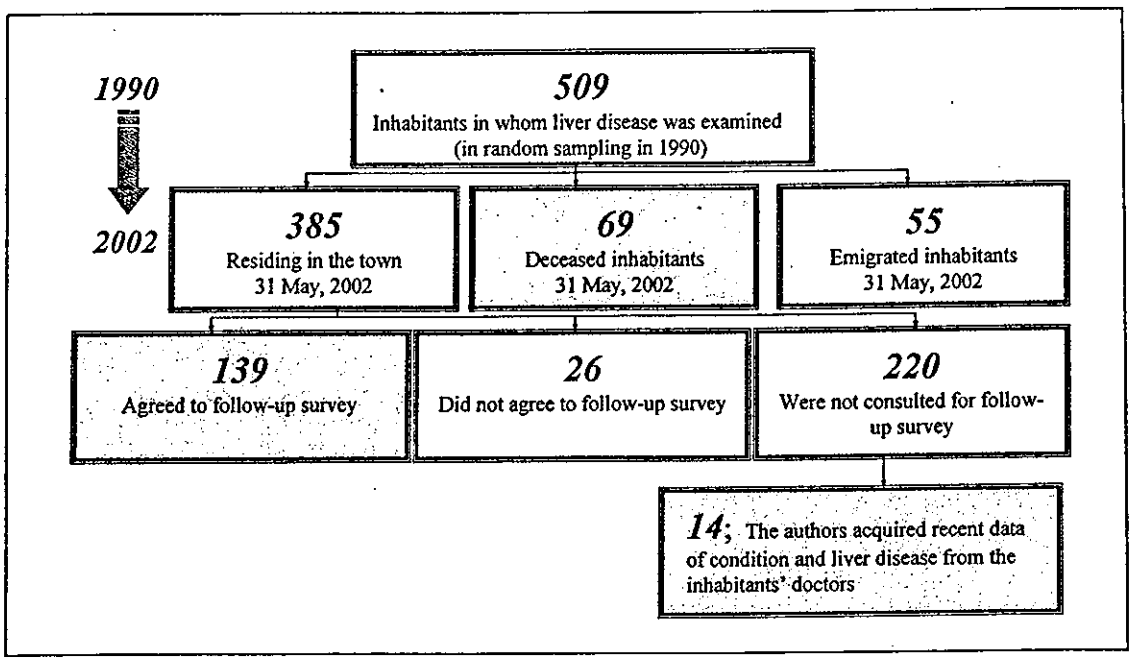

Figure 2. Diagram for pursuing the prognostic investigation of 509 inhabitants. Of the 509 subjects, 69 people had died, and 55 had moved to other regions by May 31, 2002. Thus, just 385 people of the original inhabitants investigated in 1990 resided in H town in May 2002. Of these 139 persons agreed to participate in the medical follow-up survey, but 26 did not agree. The remaining 220 inhabitants did not declare their intention either way. Among these 220 persons, data of 14 people were collected from their medical records. The cause of death of the 69 persons was investigated. Consequently, we were able to follow-up the outcome of liver diseases in 222 of the 509 original residents with or without liver diseases.

Examination for liver diseases. Sera from 139 residents were provided for the following liver function tests: serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), lactate dehydrogenase (LDH), total bilirubin (T.Bil), total protein (TP), albumin (Alb) and γ-globulin (γ-glob). Sera were also examined for the presence or absence of HCV or HBV infection. Anti-HCV was measured by a chemiluminescent enzyme immunoassay (CLEIA) kit (Lumipulse II HCV, Fujirebio Inc., Tokyo, Japan). HCV RNA in the sera was detected using the Amplicore HCV

Table I. Progression in 35 inhabitants with positive anti-HCV in 2002.

1990	1995	1999	2002	No.
+	ND	+	+	1
+	+	ND	+	11
+	+	+	+	22
-	+	+	+	1

+, positive; -, negative; ND, not done.

test (Nippon Roche, Tokyo, Japan). Hepatitis B virus surface antigen (HBsAg) was assayed by a chemiluminescent immunoassay (CLIA) kit (Architect™, HBsAg QT, Dainabot Co. Ltd., Tokyo, Japan). Ultrasonographic examination in subjects with abnormalities in the liver function tests and positive for anti-HCV or HBsAg was performed in order to investigate the shape of the liver and lesions occupying the hepatic space.

Data of 14 of the 385 persons who resided in H town were collected from their medical records.

Documentary notices to the residents. The detailed results of the liver disease tests and a letter of introduction to the medial office were mailed in written form to all the subjects who received the medical check-up.

Statistical analysis. The χ^2 test and the unpaired Student's t-test were used for statistical analyses. Differences were judged significant when $p<0.05$ (two-tailed).

Results

The prevalence of anti-HCV in 2002. Of the 139 subjects examined, those positive for anti-HCV, HCV RNA, and HBsAg were 25.2 (35/139), 15.1 (21/139), and 2.9% (4/139), respectively. When the data over time of 35 subjects with positive anti-HCV were compared, it was found that 34 persons continued being positive from 1990 (Table I). One person who was negative in 1990 was newly infected by 1995. Of the 35 subjects with positive anti-HCV (Table II), 26 persons (26/35, 74.3%) continued being positive or negative for HCV RNA, but 9 persons (9/35, 25.7%) showed changes in these indicators.

When HCV RNA changes between 1990 and 2002 were examined, it was found that of the 139 subjects (Table III), 3 of 24 persons with positive HCV RNA had received interferon (IFN) treatment. HCV RNA had become negative in only 1 of these 3 subjects due to IFN treatment.

The prevalence of the HCV infection classified by age. Fig. 3 shows the prevalence of HCV infection according to age, which compares the findings for 1990 and 2002. The highest rate of positive anti-HCV in both 1990 and 2002 was found in subjects aged 70-79 years. However, the highest rate of positive HCV RNA in 1990 was found in the group aged 70-79 years, while it was found in subjects >80 years, in 2002. Therefore, it appeared that the HCV carrier was aging.

Table II. Progression of HCV RNA in 35 inhabitants with positive anti-HCV.

	1990	1995	1999	2002	n	Total
+→+	+	ND	+	+	1	
	+	+	ND	+	6	19
	+	+	+	+	12	
+→-	+	ND	+	-	1	
	+	-	ND	-	1	
	+	-	-	-	2	5
	+	+	-	-	1	
-→+→-	-	+	-	-	2	2
-→+	-	+	ND	+	1	
	-	+	+	+	1	2
-→-	-	-	ND	-	2	
	-	-	-	-	5	7

+, positive; -, negative; ND, not done.

Table III. Comparison of HCV RNA in 1990 and 2002 for 139 inhabitants examined in 2002 in the town.

1990		2002
+/24[a]	→	+/19 (79.2%)
	→	-/5 (20.8%)
-/115	→	+/2 (1.7%)
	→	-/113 (98.3%)

+, positive; -, negative. [a]Three inhabitants of 24 HCV RNA positive inhabitants were treated by interferon (IFN) for chronic liver disease. The therapeutic response of one of them was judged as CR after IFN therapy. However, the others were judged as NR after therapy. The therapeutic response after IFN therapy was judged as: complete responder (CR), normal AST and ALT levels and HCV RNA-negative × ≥24 weeks; partial responder (PR), normal AST and ALT levels but HCV RNA-positive × ≥24 weeks; non-responder (NR), neither normal nor negative results × ≥24 weeks.

Moreover, there were no HCV carriers in subjects aged 30-39 years and 40-49 years of the subjects who participated in the study in 2002.

Analysis of cause of death of the 69 persons who died by May 31, 2002. Of the 509 inhabitants in whom liver diseases were examined in 1990, 69 (34 men/35 women; mean age at death, 76.6 years) had died by May 31, 2002 during the follow-up period (Fig. 2, Table IV). Of these 69, 36.2% (25/69) were positive for anti-HCV, 27.5% (19/69) for HCV RNA, and 1.4 % (1/69) for HBsAg. Anti-HCV, HCV RNA,

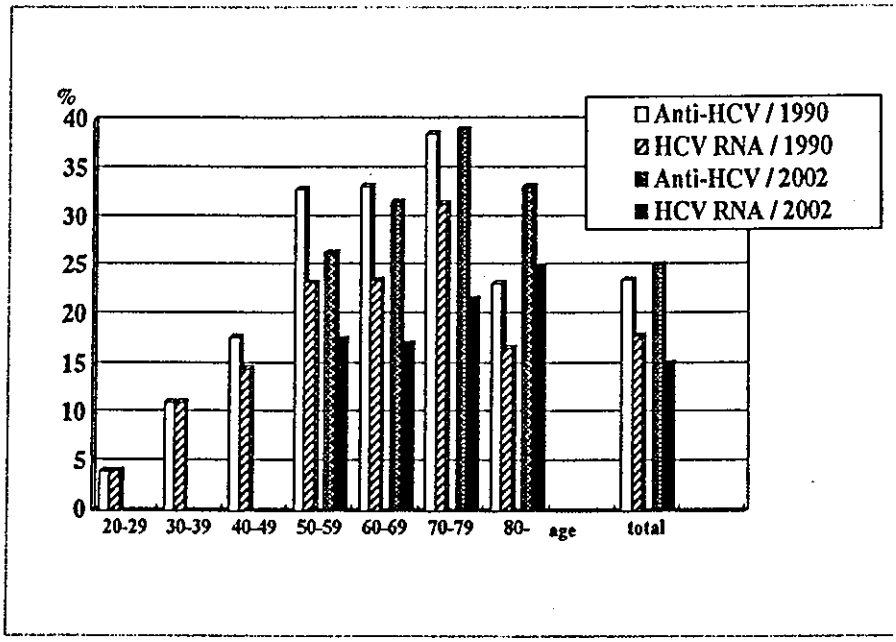

Figure 3. The rate of HCV infection according to age in the inhabitants in H town in 1990 and 2002. The highest rate of positive HCV RNA in 1990 was found in the group aged 70-79 years, while in 2002, it was in persons >80 years. Thus, the HCV carrier was aging. There were no 20-29-year-old among the residents who were consulted in the medical follow-up in 2002, because residents who were 20 years old in 1990 were 32 years old in 2002. Although 8 residents in the 30-39-year-old group and 21 residents in the 40-49-year-old group participated in the medical follow-up in 2002, all of these persons were negative for anti-HCV and HCV RNA.

Table IV. Characteristics of persons who died due to HCC or HCV-related liver cirrhosis of 69[a] inhabitants who died from 1990 to 2002.

	Total	HCC	LC	HCC or LC
n (%)	69	9 (13)	4 (5.8)	13 (18.9)
Average age	76.6	74	69.8	72.7
Sex (male/female)	34/35	6/3	3/1	9/4
Anti-HCV (+) (%)	25 (36.2)	8 (32.0)	3 (12.0)	11 (44.0)
HCV RNA (+) (%)	19 (27.5)	8 (42.1)	2 (10.5)	10 (52.6)
HBsAg (+) (%)	1 (1.4)	1 (100)	0 (0)	1 (100)
Anti-HCV(-) + HCV RNA(-) + HBsAg(-)(%)	43 (62.3)	0 (0)	1 (2.3)	1 (2.3)

[a]As shown in Fig. 3, of the 509 inhabitants examined for liver diseases in 1990, 69 had died by May 31, 2002 as found during the follow-up. [b]$p<0.05$, [c]$p<0.0001$, [d]$p<0.00001$.

and HBsAg were negative in 62.3% (43/69). Nine of these 69 people had died of HCC (6 men/3 women, mean age at death: 74 years) and 4 had died of liver cirrhosis (3 men/1 woman, mean age at death: 69.8 years).

Of the 25 people with positive anti-HCV, 11 (44.0%) had died of HCC or liver cirrhosis. Of the 19 people with positive HCV RNA, 10 (52.6%) had died of HCC or liver cirrhosis during the 12-year observation period. One person with positive HBsAg had died of HCC. Of the people with negative findings for anti-HCV, HCV RNA, and HBsAg, 1 had died of alcoholic liver cirrhosis (1/43, 2.3%). Persons with chronic HCV or HBV infection had significantly higher mortality rates from HCC and liver cirrhosis than persons who were not infected with HCV or HBV ($P<0.00001$).

Change of individuals with liver disease for 12 years. Of the 509 persons examined in 1990, the outcome of liver disease could be shown clearly in 222 residents in 2002 (Figs. 2 and 4).

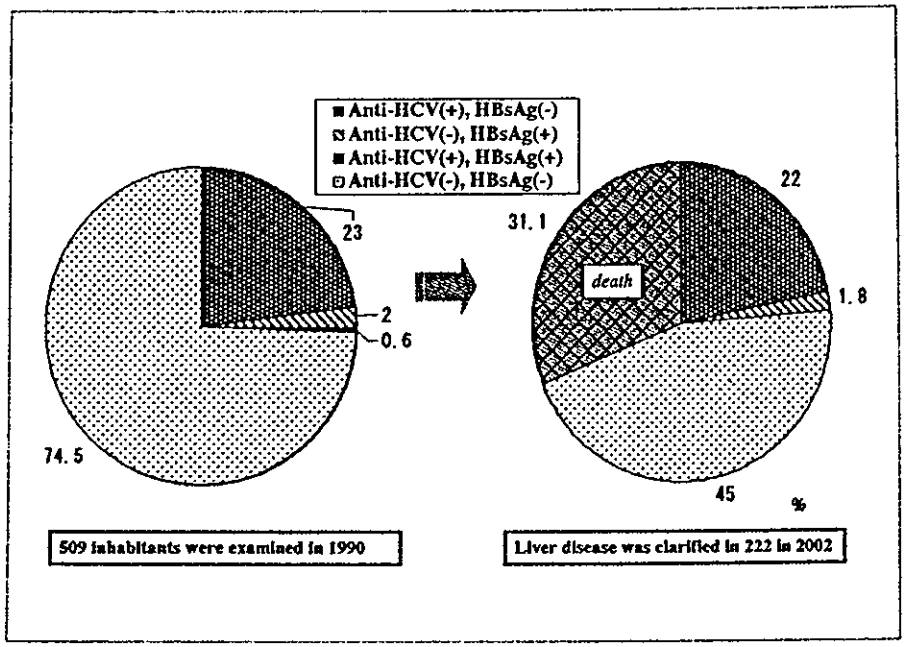

Figure 4. Patterns of liver disease in residents who received medical checkups in 1990. Of 509 persons examined in 1990, there were 222 residents in whom the outcome of liver disease could be clearly shown in 2002.

Table V. Twelve years after residents were diagnosed with past history of HCV infection in 1990.

1990	n		2002	n
Past history of HCV infection	31	→	Past history of HCV infection	11
		→	HCV-related ASC	1
		→	Normal	1
		→	Deceased	7
Anti-HCV (+), HCV RNA (-), HBsAg (-)			Cause of death Alcoholic LC (1) Other than liver disease (6)	
		→	Unknown liver disease due to non-participation in the follow-up survey	11

ASC, asymptomatic healthy carrier; LC-C, HCV-related liver cirrhosis; HCC, hepatocellular carcinoma.

These include the 139 persons who agreed to the follow-up survey, the 69 persons in whom the cause of death was clear, and 14 persons for whom we were able to collect their medical records from the family doctors. Of the 222 people, the rate of positive anti-HCV and negative HBsAg was 22%. On the other hand, the rate of positive HBsAg and negative anti-HCV was 1.8% (Fig. 4).

Meanwhile, how did the liver disease progress in the 12 years? Table V shows the distribution of the diagnosis of liver disease in 2002 among the persons diagnosed with past history of HCV infection (positive anti-HCV, negative HCV RNA, negative HBsAg, and normal liver function data) in 1990. Of 31 people diagnosed with a past history of HCV infection in 1990, the diagnosis of liver disease in 2002 was: those just with past history of HCV infection (n=11), asymptomatic healthy carrier (n=1), normal (n=1), deceased (n=7), and unknown liver disease due to non-participation in the follow-up survey (n=11). Among the 7 deceased, 1 died from alcoholic liver cirrhosis, and 6 persons died from causes other than liver disease. Of 18 persons diagnosed as HCV-related asymptomatic healthy carriers (positive anti-HCV, positive HCV RNA, negative HBsAg, and normal liver function data) in 1990, the diagnosis of liver diseases in 2002 was: past history of HCV infection only (n=4), chronic hepatitis C (n=3), death by causes other than liver disease (n=1), and unknown liver disease due to non-participation in the follow-up survey (n=10). Table VI shows the distribution of the diagnosis of liver disease in 2002 among the persons

Table VI. Twelve years after residents were diagnosed with chronic hepatitis C in 1990.

1990	n		2002	n
CH-C	61	→	CH-C	16
		→	CH-C post IFN (CR)	1
		→	LC-C	5
Anti-HCV (+), HCV RNA (+), HBsAg (-)		→	LC-C+HCC	3
		→	CH-C+HCC	2
		→	Deceased	12
			Cause of death	
			LC (2)	
			HCC (3)	
			Other than liver disease (7)	
		→	Unknown liver disease due to non-participation in the follow-up survey	22

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

Table VII. Twelve years after residents were diagnosed with HCV-related liver cirrhosis in 1990.

1990	n		2002	n
LC-C	6	→	LC-C	1
		→	Deceased (cause of death: HCC)	4
Anti-HCV (+), HCV RNA (+), HBsAg (-)		→	Unknown liver disease due to non-participation in the follow-up survey	1
LC-C+HCC	1	→	Deceased (cause of death: HCC)	1

LC-C, HCV-related liver cirrhosis; HCC, hepatocellular carcinoma.

diagnosed with chronic hepatitis C (positive anti-HCV, positive HCV RNA, negative HBsAg, and abnormal liver function data) in 1990. Of the 61 persons diagnosed with chronic hepatitis C in 1990, the diagnosis of liver disease in 2002 was: chronic hepatitis C (n=16), chronic hepatitis C post-IFN (n=1), liver cirrhosis (n=5), HCC (5), deceased (n=12), and unknown liver disease due to non-participation in the follow-up survey (n=22). Among the 12 deceased, 2 died from HCV-related liver cirrhosis, and 3 died from HCC. Table VII shows the distribution of the diagnosis of liver disease in 2002 among the persons diagnosed with HCV-related liver cirrhosis or HCC (positive anti-HCV, positive HCV RNA, negative HBsAg, and abnormal liver function data) in 1990. The person diagnosed with HCV-related liver cirrhosis with HCC in 1990 died of HCC.

Figs. 5 and 6 show the outcome of 216 persons with liver diseases (excludes HBV carriers) and 6 persons with persistent HBV infection, respectively, identified among the inhabitants in the 12-year follow-up. The residents for whom we could not analyze the long-term prognosis of liver disease were deleted from these numbers. Approximately half of sustained HCV carriers, such as asymptomatic healthy carrier, chronic hepatitis, liver cirrhosis, and HCC, had an advanced stage of disease in the 12-year follow-up. Just 1 (2.6%) out of 39 inhabitants with chronic hepatitis C recovered from sustained HCV infection due to IFN treatment. Two (1.3%) of the 143 inhabitants without HCV infection (alcoholic liver disease or fatty liver, 16 inhabitants; normal, 127 inhabitants) became infected with HCV infection (alcoholic liver disease or fatty liver, 16 inhabitants; normal, 127 inhabitants).

In 1990, 10 persons had negative anti-HCV and positive HBsAg. Three persons diagnosed as HBV-related asymptomatic healthy carriers in 1990 remained the same in 2002. The remaining person with HBV-related liver cirrhosis had died from HCC.

Three persons had both positive anti-HCV and HBsAg in 1990. One who was an HBV-related asymptomatic healthy carrier and had a past history of HCV infection in 1990 was