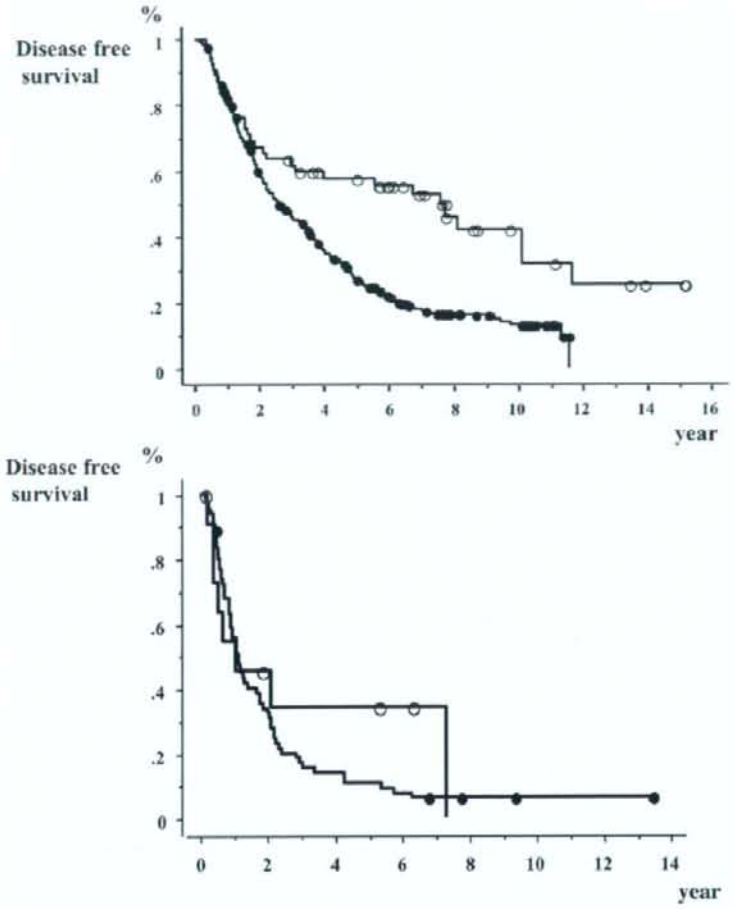

FIGURE 3. Comparison of disease-free survivals between patients with hepatitis-B-related hepatocellular carcinoma (HCC) and patients with hepatitis-C-related HCC in TNM Stages by Liver Cancer Study Group of Japan. Top, stages I and II. Open circles, hepatitis-B-related HCC patients (n = 54). Closed circles, hepatitis-C-related HCC patients (n = 285). Log-rank test: $P = 0.0002$. Bottom, stages III and IV. Open circles, hepatitis-B-related HCC patients (n = 12). Closed circles, hepatitis-C-related HCC patients (n = 66). Log-rank test: $P = 0.42$.

stages I and II, the DFS of the HC-HCC group was significantly lower than that of the HB-HCC group, and the DFS of the two groups were remarkably different 2 years after surgery and later. In contrast, there were no significant differences between the DFS of the two groups in stages III and IV. In the comparison of Child's classification, both in Child's A and in Child's B + C, the DFS of the HC-HCC group was lower than that of the HB-HCC group, especially 2 years after surgery and later.

Univariate and Multivariate Analysis for DFS of HCC Patients Who Underwent Hepatic Resection

Univariate analysis and unadjusted hazard ratios for DFS were calculated on the HCC patients who underwent hepatic resection (Table 3). The risk of recurrence from HCC was 1.92 times greater in HC-HCC patients than in HB-HCC patients ($P = 0.002$, 95% confidential interval [CI], 1.37–2.70). In the other factors, ALT and ICG-R15 were selected from the clinical factors for DFS. From tumor factors: tumor size, number of tumors, and TNM stage by LCSGJ; from surgical factors: operation time, blood loss, perioperative transfusion, and surgical margin; and from pathologic factors: histologic grade (Edmondson's grade), extracapsular invasion, satellite nodules, and venous invasion were significantly strong predicators of risk for recurrence.

Multivariate analysis was performed for DFS using the selected variables of which the P values were less than 0.05 in univariate analysis (Table 4). Viral hepatitis was chosen for a serologic factor, and ALT was chosen from hepatic inflammatory activity factors, ICG-R15 from hepatic functional reserve factors, and from tumor factors TNM stage by LCSGJ, which includes the factors of both tumor size and tumor number. Blood loss and surgical margin were selected from operative factors, and Edmondson's grade, extracapsular invasion, and venous invasion from pathologic factors. As a result, viral hepatitis was one of the independent prognostic factors for DFS together with

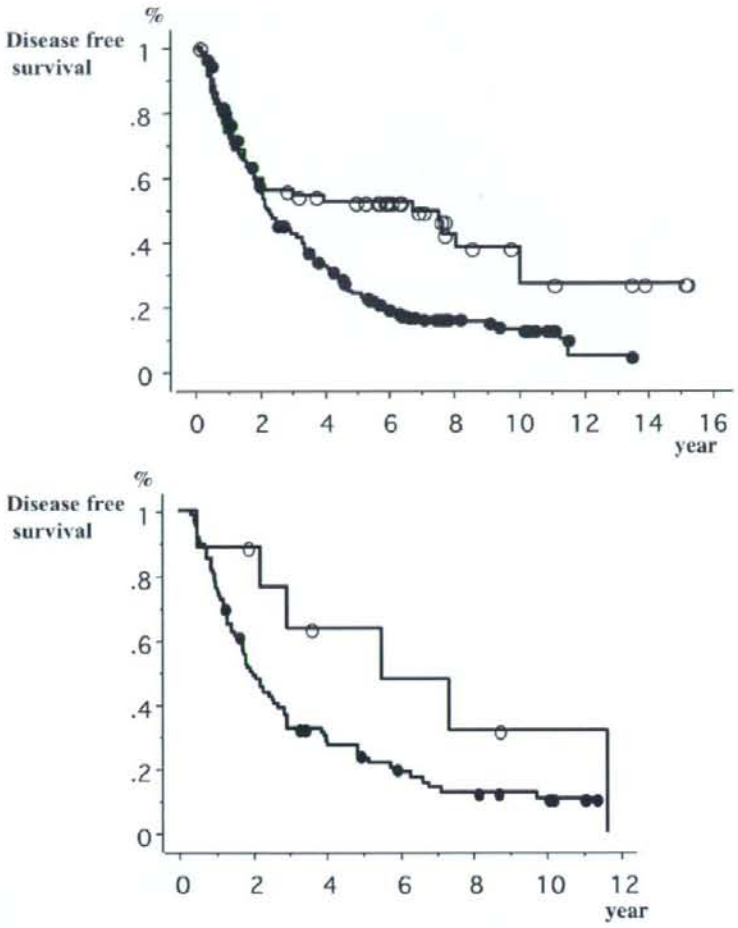

FIGURE 4. Comparison of disease-free survivals between patients with hepatitis-B-related hepatocellular carcinoma (HCC), and hepatitis-C-related HCC using Child's classification. Top, Child's A. Open circles, hepatitis-B-related HCC patients (n = 57). Closed circles, hepatitis-C-related HCC patients (n = 261). Log-rank test: $P = 0.001$. Bottom, Child's B + C; Open circles, hepatitis-B-related HCC patients (n = 9). Closed circles, hepatitis-C-related HCC patients (n = 90). Log-rank test: $P = 0.06$.

TNM stage, surgical margin, and Edmondson's grade ($P = 0.002$). The risk of recurrence from HCC increased to 1.93 times greater in HC-HCC patients than in HB-HCC patients after adjustment and the 95% CI became 1.27 to 2.93. When the patients whose first recurrence was distant metastasis were accounted for censored cases at the time of discovering the metastasis, the adjusted hazard ratio for the viral types was not changed (hazard ratio, 2.11; 95% CI, 1.36–3.28).

DISCUSSION

The findings about the differences of various kinds of clinicopathologic characters such as age, hepatic inflammatory activity, liver functional reserve, and tumor and operative factors, etc., between HB-HCC and HC-HCC patients, which we indicated in this study, were consistent with those in previous reports for the most part[17,22] (Table 2). On the other hand, the opinions about the differences of long-term clinical course after hepatic resection of the two groups were controversial. Takenaka et al[17] reported the comparison of the outcome of a total of 126 patients with HB- or HC-HCC who underwent hepatic resection, and described that the survival and DFS at 5 years were similar in both groups. Similarly, some other reports described that long-term prognosis was not influenced by hepatitis virus types.[23–26] On the other hand, Wu et al[7] reported that the DFS rates between the two groups were not significantly different, but that the survival of HB-HCC patients was significantly lower than that of HC-HCC patients. They described that the poorer prognosis in HB-HCC patients was caused by a higher incidence of poor prognostic characters in the HB-HCC group. Haratake et al[27] similarly reported that the prognosis of HB-HCC patients was worse than that of HC-HCC patients. These inconsistent findings possibly resulted from insufficient number of patients and follow-up periods for satisfactory analysis. We

TABLE 3. Univariate Analysis and Unadjusted Hazard Ratios of Prognostic Factors for Disease-Free Survival on HCC Patients Who Underwent Hepatic Resection

			DFS Rate (%)				
	No. of Patients	50% DFS (yr)	5 yr	10 yr	*P* Value	Hazard Ratio	95% CI
Viral hepatitis							
B type	66†	6.73†	54†	38†		1.00 (Reference)†	
C type	351†	2.21†	24†	12†	0.002†	1.92†	1.37–2.70†
Age (yr)							
≤65	298	2.43	30	17		1.00 (Reference)	
>65	119	2.10	27	13	0.28	1.14	0.90–1.46
Gender							
Male	317	2.30	29	15		1.00 (Reference)	
Female	100	2.23	26	20	0.92	1.01	0.78–1.31
ALT (U/l)							
<80	227†	2.43†	32†	21†		1.00 (Reference)†	
≥80	190†	2.21†	24†	9†	0.03†	1.28†	1.02–1.59†
Alb (g/dL)							
≥3.5	320	2.37	30	18		1.00 (Reference)	
<3.5	97	2.22	26	10	0.20	1.18	0.92–1.52
T bil (mg/dL)							
<1.0	291	2.45	31	17		1.00 (Reference)	
≥1.0	126	2.09	25	14	0.24	1.16	0.91–1.47
ICG-R15 (%)*							
<20	249†	2.55†	33†	20†		1.00 (Reference)†	
≥20	161†	2.10†	25†	11†	0.03†	1.29†	1.03–1.62†
Prothrombin time (%)							
>80	327	2.28	30	17		1.00 (Reference)	
≤80	90	2.43	24	13	0.74	1.05	0.80–1.38
Child's classification							
A	317	2.37	30	18		1.00 (Reference)	
B + C	98	2.21	27	12	0.26	1.16	0.90–1.49
Preoperative TAE							
No	272	2.36	32	20		1.00 (Reference)	
Yes	145	2.37	25	12	0.30	1.13	0.89–1.44
AFP (ng/mL)							
≤100	245	2.45	29	9		1.00 (Reference)	
>100	172	1.82	27	20	0.47	1.10	0.85–1.41
Tumor size (cm)							
≤3.0	256†	2.89†	33†	19†		1.00 (Reference)†	
>3.0	161†	1.63†	21†	9†	0.0002	1.52†	1.22–1.90†
No. of tumors							
1	318†	2.97†	33†	20†		1.00 (Reference)†	
2 or more	99†	1.42†	15†	6†	<0.0001†	1.92†	1.50–2.46†
TNM stage by LCSGI							
I + II	339†	2.95†	32†	19†		1.00 (Reference)†	
III + IV	88†	1.13†	14†	7†	<0.0001†	2.05†	1.58–2.66†
Operation time (mm)							
≤180	135†	3.00†	34†	22†		1.00 (Reference)†	
>180†	282†	2.11†	26†	13†	0.01†	1.35†	1.06–1.72†
Blood loss (ml)							
≤1000	210†	3.00†	33†	19†		1.00 (Reference)†	
>1000	207†	2.08†	24†	13†	0.02†	1.31†	1.05–1.63†
Surgical margin (mm)							
≥10	176†	3.46†	34†	19†		1.00 (Reference)†	
<10	241†	2.01†	25†	14†	0.002†	1.42†	1.13–1.77†

(*Continued*)

TABLE 3. *(Continued)*

	No. of Patients	50% DFS (yr)	DFS Rate (%)		*P* Value	Hazard Ratio	95% CI
			5 yr	10 yr			
Transfusion							
No	233†	2.85†	33†	20†		1.00 (Reference)†	
Yes	184†	1.93†	23†	11†	0.01†	1.32†	1.06–1.64†
Resection range							
Hr0 + Hr5	262	2.37	29	16		1.00 (Reference)	
Hr1 + Hr2 + Hr3	155	2.13	28	16	0.88	1.03	0.82–1.29
Edmondson's grade							
I	79†	3.98†	40†	19†		1.00 (Reference)†	
II + III	262†	1.93†	24†	15†	0.005†	1.53†	1.14–2.06†
Extracapsular invasion							
No	161†	3.09†	33†	18†		1.00 (Reference)†	
Yes	186†	1.80†	23†	15†	0.005†	1.41†	1.11–1.79†
Satellite nodule							
No	242†	2.66†	29†	17†		1.00 (Reference)†	
Yes	94†	1.33†	24†	14†	0.02†	1.44†	1.10–1.87†
Venous invasion							
No	210†	2.57†	30†	17†		1.00 (Reference)†	
Yes	130†	1.42†	23†	14†	0.02†	1.33†	1.04–1.70†
Noncancer tissue							
Normal or fibrosis	145	2.36	33	26		1.00 (Reference)	
Corrhosis	272	2.21	28	11	0.08	1.28	0.97–1.68

HCC, hepatocellular carcinoma; DFS, disease-free survival; ICG R 15, indocyanine green 15 min retention test (normal ≤10%); AFP, alpha fetoprotein; TAE, transarterial embolization; Hr0, partial resection; HrS, subsegmentectomy; Hr1, one segmentectomy; Hr2, two segmentectomy; Hr3, three segmentectomy; LCSGJ, Liver Cancer Study Groups of Japan.

*Three patients were excluded because of ICG excretion abnormalities, and four were not measured. There are several deficits in microscopic factors because of necrosis by preoperative TAE.

†Factor with significant differences.

have shown the difference of hepatitis viral type had a dramatic impact on long-term DFS after surgery by a long follow-up period and a large number of patients (Fig. 2). If the follow-up period of our study was short and the comparative study was only about survivals, as the survivals of the two groups were almost the same until 7 years after surgery even in our study, our conclusions might be similar to previous reports,[23–26] which stated hepatitis virus type was not influential in long-term prognosis.

In both the HB-HCC and HC-HCC groups, the recurrence curves were steep for the first 2 years. Afterward, though the curve of the HB-HCC group became very gentle, ie, the recurrence rate decreased dramatically, that of the HC-HCC group kept going down until 7 years after surgery, ie, the recurrence rate remained high during the later years (Fig. 2). The recurrences in the early period after surgery were considered to be mostly attributed to intrahepatic metastasis in both the HB-HCC and HC-HCC groups,[28] whereas the recurrences occurring in the later follow-up years after surgery in the HC-HCC group can be presumed to have been caused by the higher frequency of metachronous carcinogenesis.[29] We showed HC-HCC had the stronger hepatic inflammatory activity shown by the higher level of ALT, and the poorer liver functional reserve shown by the worse grade of Child's classification (Table 2). These findings are regarded to be the high-risk factors of developing HCC. However, even in the same liver function subgroup, which was separated based on Child's classification categorizing the degree of liver function, the potential of recurrence in the later follow-up years was stronger in the HC-HCC group than in the HB-HCC group regardless of the grade of liver functional reserve (Fig. 4). In addition, multivariate analysis showed that the difference of viral hepatitis itself was one of the independent prognostic factors for risk of recurrence (Table 4). These data may suggest that the high frequency of recurrence due to metachronous carcinogenesis in HC-HCC patients is caused not only by the high inflammatory activity or poor functional reserve but also by the other etiologic pathway induced by chronic HCV infection. Takano et al[30] reported that the incidence of HCC in chronic hepatitis C patients was 2.7 times higher than that in chronic hepatitis B patients. Ikeda et al[31] described that the incidence rates of HCC in HC patients and HB patients were 4.8% and 2.1% at the 5th year, 13.6% and 4.9% at the 10th year, respectively, and that the rates showed significant differences.

Even in earlier stages, such as TNM stages I and II, the recurrence rates of HC-HCC continued increasing until the later follow-up years, although the rates of HB-HCC decreased 2 years after surgery and later (Fig. 3, top). On the other hand, in more advanced stages, such as TNM stages III and IV, most recurrences happened in the early follow-up years in both HC-HCC and HB- HCC patients (Fig. 3,

TABLE 4. Multivariate Analysis and Adjusted Hazard Ratios of Prognostic Factors for Disease-Free Survival on HCC Patients Who Underwent Hepatic Resection

Variable	No. of Patients	Hazard Ratio	95% CI	*P*
Viral hepatitis				
B type:C type	66:351†	1.93†	1.27–2.93†	0.002†
ALT (u/l)				
<80:≥80	227:190	1.14	0.87–1.49	0.36
ICG R15 (%)*				
<20:≥20	249:161	1.17	0.69–1.49	0.25
TNM stage by LCSGJ				
I + II:III + IV	339:88†	2.15†	1.55–2.97†	<0.0001†
Blood loss (ml)				
≤1000:≥1000	210:207	1.22	0.89–1.66	0.20
Surgical margin (mm)				
≥10:<10	176:241†	1.34†	1.00–1.80†	0.049†
Edmondson's grade				
I:II + III	79:262†	1.48†	1.01–2.15†	0.04†
Extracapsular invasion				
No:yes	161:186	1.06	0.78–1.45	0.70
Venous invasion				
No:yes	210:130	1.27	0.95–1.71	0.10

HCC, hepatocellular carcinoma; ICG-R 15, indocyanine green 15-min retention test (normal range ≤10%); LCSGJ, Liver Cancer Study Group of of Japan.

*Three patients were excluded because of ICG excretion abnormalities, and four were not measured. There are several deficits in microscopic factors because of necrosis by preoperative TAE.

†Factor with significant differences.

bottom). These findings may suggest that it is necessary to change the treatment strategy for HCC patients not only by the stage of progression or liver function but also the difference of viral hepatitis type. Namely, intrahepatic metastasis is considered to be the main form of recurrence in advanced stages of HCC. Therefore, for the more advanced stages of HCC, such as stages III or IV, regardless of the difference of hepatitis virus, we should pay attention to the appearance of intrahepatic metastasis in the early follow-up years after surgery, and an anticancer therapy such as chemotherapy may be recommended to reduce this type of recurrence. On the other hand, mainly for HC-HCC in less-advanced HCC, such as stages I or II, we should make a close follow-up to check intrahepatic recurrence by multicentricity even 2 years after surgery or later, and antiviral treatment such as interferon with or without ribavirin might be useful for preventing a second primary occurrence.[32]

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Distinctive Change in Male Liver Cancer Incidence Rate between the 1970s and 1990s in Japan: Comparison with Japanese-Americans and US Whites

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Objective: To characterize the time trend of the male liver cancer incidence rate in Japan.
Methods: We obtained data on male liver cancer incidence rates from the 'Cancer Incidence in Five Continents (CI5) Series'. Data from the population-based cancer registries of Miyagi, Osaka, Nagasaki, Hiroshima, Saga and Yamagata between 1962 and 1997 were combined and used as the data for the Japanese. To characterize the time trend in rate, we chose and combined the data on Japanese-Americans from the cancer registries of Hawaii and Los Angeles County, California between 1968 and 1997. Data on US whites who participated in the Surveillance, Epidemiology, and End Results program in 1973–1997 were obtained from the Data Series. The age-standardized incidence rate (ASR) and birth-cohort-specific rate were calculated in the three groups using a computer program in 'CI5 Vols I–VIII'.
Results: Among Japanese males in Japan, the ASR increased sharply starting in the mid 1970s and leveled off in the mid 1990s. In contrast, among both the Japanese-Americans and US whites, the ASR continued to increase throughout the observation period. Among the US whites, an increasing trend was more apparent during 1983–97 than during 1973–87. The trend by birth cohort among Japanese males in Japan clearly showed that there was a peak incidence among men aged 45–59 years. They had been born between 1931 and 1935.
Conclusions: The present calculations clarified the distinctive time trend of liver cancer between the 1970s and 1990s in Japanese males. A possible explanation for the observed trend is discussed.

Key words: liver cancer – Japanese – incidence – population-based cancer registry – emigrant

INTRODUCTION

Over the last 30 years, liver cancer has been the third leading cause of cancer death among Japanese males (23 421 deaths in 2004) (1). Ninety-five per cent of liver cancer cases consist of hepatocellular carcinoma (2), which is mainly caused by chronic hepatitis C virus (HCV) infection rather than chronic hepatitis B virus (HBV) infection in Japan (3). Liver cancer is more common in males than in females (4) although the prevalence of HCV infection between males and females is similar in Japan (5). The geographic difference in liver cancer incidence is positively correlated with the geographic pattern of the prevalence of HCV infection among the general population of Japan (6). By molecular clock analysis of the sequences of HCV isolates, it has been hypothesized that a major spread of HCV infection in Japan occurred in the 1940s and 1960s, while in the USA it occurred in the late 1960s and 1970s (7). This might yield different trends of liver cancer incidence rates between the two countries.

Comparison of liver cancer incidence trends among Japanese in Japan, Japanese-Americans, and another population in the USA may provide interesting results from an epidemiological and public health perspective. Thus, we studied the trends in male liver cancer incidence rates in the three groups using data from population-based cancer registries.

METHODS

We obtained data on the incidence rate of male liver cancer from the CD-ROM of the 'Cancer Incidence in Five

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Continents (CI5) Vols I–VIII' (8), which is supported by the International Agency of Research on Cancer (Lyon, France). This is a computer program that provides access to data in the CI5 Series. The data in CI5 including the incidence data of cancer together with the corresponding population data, had been submitted from population-based cancer registries worldwide, which had standard data quality (9). We used the data of the cancer registries of Miyagi, Osaka, Nagasaki, Hiroshima, Saga and Yamagata between 1962 and 1997 when these data were available as the data of Japanese in Japan. The data on Japanese-Americans were obtained from the cancer registries of Hawaii and Los Angeles County, California between 1968 and 1997, because these were the only two registries with Japanese immigrants in which consecutive data were available in the CI5 Series. The third group was white Americans who participated in the Surveillance, Epidemiology, and End Results (SEER) program between 1973 and 1997.

The data were selected from the CD-ROM and the subgroups within the three groups were combined to calculate the incidence of liver cancer in each group. Trends in age-standardized incidence rates (ASRs) of male liver cancer for five calendar years (world population as the standard population), and trends in 5-year birth-cohort-specific rates were calculated in the three groups. Classification of liver cancer titled malignant neoplasm of liver and intrahepatic bile ducts in the CI5 in 1963–67 (Vol. II), 1968–77 (Vols III–IV), 1978–92 (Vols V–VII) and 1993–97 (Vol. VIII) was coded to the International Classification of Diseases (ICD) 7th (155.0), 8th (155), 9th (155) and 10th (C22) Revision, respectively. All of the calculations were performed by a computer program in the 'CI5 Vols I–VIII' (8).

RESULTS

Figure 1 shows the time trends of the age-standardized incidence rate of liver cancer among the Japanese males, Japanese-American males and US white males. Among Japanese males in Japan, the ASR increased slowly from 34.2 to 36.7 per 10^5 between 1963 and 1977. From the mid 1970s, the incidence rate rose sharply until the early 1990s to 85.9 per 10^5 and then it leveled off in the mid 1990s. Among Japanese-Americans, the ASR increased slowly throughout the observation period from 10.3 to 14.2 per 10^5. Among US whites, there was an increasing trend in the ASR during the observation period. The trend was more apparent during 1983–97 (5.3–8.6 per 10^5) than during 1973–87 (4.6–5.3).

The time trends of the age-specific incidence rate of liver cancer by birth cohort are shown in Figs 2–4. The horizontal axis shows years of birth. Among the Japanese in Japan, the incidence rate seemed to be constant between the ages of 35 and 44 years in the birth cohort between 1921 and 1960 (Fig. 2). The incidence rates at ages 45–59 were highest in the birth cohort between 1931 and 1935. Among

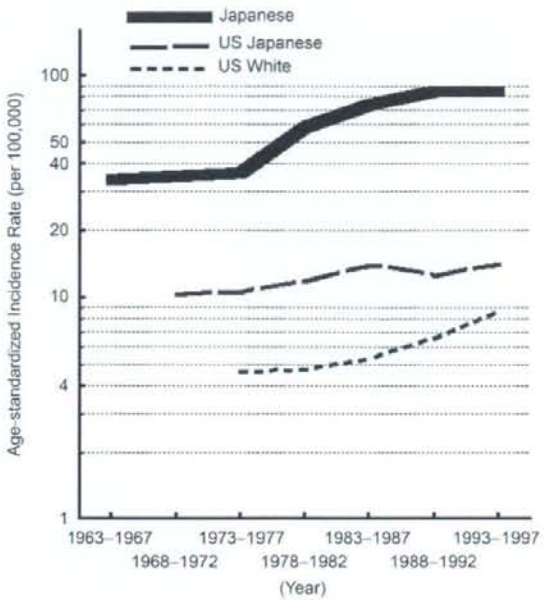

Figure 1. Trends in age-standardized incidence rates of liver cancer in Japanese males, Japanese-American (US Japanese) males and US white males.

Japanese-Americans, the incidence rates seemed to be highest in birth cohorts around 1901–1905 and 1926–1930, although they were fluctuating because of the small number of the incidence in this population (Fig. 3). Among US whites, all of the age-specific incidence rates (35–84 years) among those born from 1891 to 1960 increased as the birth cohort descended (Fig. 4).

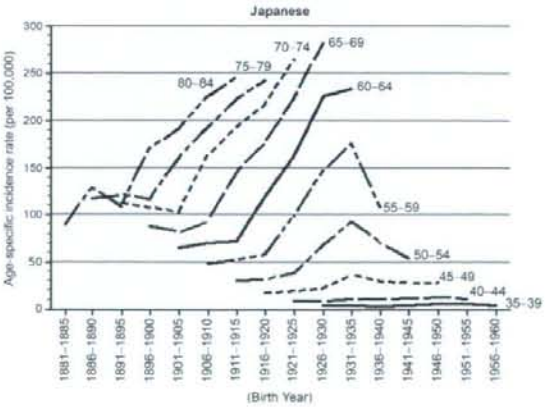

Figure 2. Age-specific incidence rates of liver cancer according to year of birth from 1881 to 1960 in Japanese males in Japan.

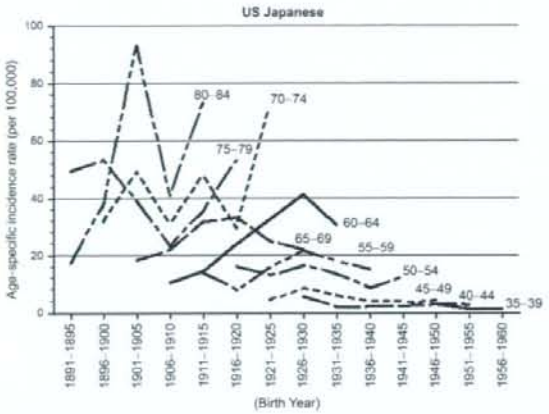

Figure 3. Age-specific incidence rates of liver cancer according to year of birth from 1891 to 1960 in Japanese-American males (US Japanese).

DISCUSSIONS

Our study demonstrated that the ASR of liver cancer among Japanese males in Japan has changed remarkably between the 1970s and 1990s. It increased sharply starting in the mid 1970s and it had more than doubled by the early 1990s, but then it leveled off in the mid 1990s. The time trends by birth cohort clearly showed that the rate was the highest among people born between 1931 and 1935 and with ages of 45 years and over. A similar birth cohort effect on liver cancer mortality in Japanese male has been reported (10). How did this effect appear? The prevalence of HCV infection among Japanese males in Japan was thought to be highest among the generation born around 1931–1935 based on data on first-time blood donor candidates (11), although data on older Japanese individuals are not available (5, 12). This assumption is supported by the recent study on molecular tracing of the HCV epidemic in Japan that reported that exponential spread of HCV-1b infection started in the 1940s (7), which coincided with an outbreak of parenteral amphetamine use in the devastated society after the Second World War (11, 13). The spread was considered to be amplified through blood transfusions and parenteral medical procedures in the 1950s and 1960s (11, 13), but it subsequently ended by the early 1990s at the latest, as evidenced by the very low incidence of HCV infection among repeat blood donors (14, 15). It is realistic to consider that Japanese males born between 1931 and 1935, who were adolescents in the early 1950s, were the most susceptible to HCV transmission from these circumstances.

As for Japanese-Americans, the first group of Japanese emigrated to the USA before 1924, when immigration of Japanese into the USA was prohibited by the 'Quota Immigration Amendment Act'. Therefore, the next generations of Japanese-Americans were free from HCV epidemics within Japan that yielded different trends of rates between Japanese in Japan and Japanese-Americans. The ASR of liver cancer among US whites has increased since the mid 1980s, although the rates are the lowest among the three groups. This finding may also be attributed to the previous finding that the spread of HCV-1a, a dominant genotype in the USA (16), began in 1965 based on molecular tracing of the HCV epidemic in the USA (7). This is approximately 25 years after the HCV outbreak started in Japan (7).

In conclusion, the present calculation of liver cancer incidence rates shows a distinctive time trend between the 1970s and 1990s in Japanese males. The trend was affected by the birth cohort effect which was possibly attributed to HCV outbreaks in Japan. If this trend is maintained, the male Japanese liver cancer incidence rate is likely to further decline in the current decade.

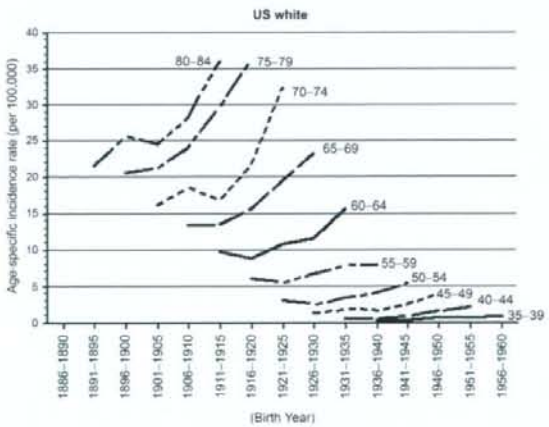

Figure 4. Age-specific incidence rates of liver cancer according to year of birth from 1891 to 1960 in US white males.

Acknowledgement

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Conflict of interest statement
None declared.

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ARTICLE | Annals of Internal Medicine

Declining Incidence of Hepatocellular Carcinoma in Osaka, Japan, from 1990 to 2003

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Background: Japan has the highest incidence rate of primary liver cancer attributed to chronic hepatitis C virus (HCV) infection among developed countries. Molecular clock analysis of HCV sequences revealed that the spread of HCV took place earlier in Japan than in other countries. This might influence recent temporal trends in hepatocellular carcinoma (HCC) incidence.

Objective: To characterize the contribution of HCV-related hepatocellular carcinoma (HCC) to recent changes in HCC incidence in Osaka, Japan.

Design: Population-based survey.

Setting: Osaka Cancer Registry and 10 hospitals in Osaka.

Participants: 63 862 patients with HCC that was diagnosed between 1981 and 2003 in Osaka Prefecture, including 5253 HCV-seropositive patients with HCC that was diagnosed between 1990 and 2003 at 10 hospitals.

Measurements: Incidence of HCC and estimated incidence rate of HCV-related HCC, measured by multiplying the prevalence of anti-HCV by the corresponding HCC incidence rate.

Results: Between 1981 and 2003, peak incidence of HCC among men age 50 to 59 years, 60 to 69 years, and 70 to 79 years occurred in 1986, 1995, and 2000, respectively, with marked downward trends thereafter (average annual change, −7.9, −22.3, and −12.4 per 100 000 persons, respectively). Similar trends were observed in women. Estimated sex- and age-specific incidence of HCV-related HCC (per 100 000 persons) decreased from 255 to 92 cases at the maximum in men age 60 to 69 years and from 61 to 34 cases in women age 60 to 69 years, whereas estimated incidence of non-HCV–related HCC did not change between 1990 and 2003.

Limitation: Infection was determined only by HCV seropositivity.

Conclusion: The incidence of HCC in Osaka started to decrease by 2000, mainly because of decreased HCV-related HCC.

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Primary liver cancer was the fifth most common cancer worldwide by 2000, with approximately 551 000 new cases recorded (1). In most countries, hepatocellular carcinoma (HCC) comprises 85% to 90% of primary liver cancer cases. With some exceptions, developed countries, including the United States, have been experiencing an increase in the incidence of primary liver cancer, considered to be due at least in part to increased prevalence of chronic hepatitis C virus (HCV) infection (2).

Japan has had one of the highest incidence rates of primary liver cancer among developed countries (age-standardized incidence rate in 1995, 25.5 per 100 000 men and 7.7 per 100 000 women) (3). Approximately 90% of liver cancer cases are HCC, which, in Japan, is mainly caused by chronic HCV infection rather than chronic hepatitis B virus infection (4). A recent report on the age-standardized incidence of primary liver cancer among Japanese men, which was calculated from 6 population-based cancer registries, showed a sharp increase that started in the mid-1970s but leveled off in the mid-1990s (5). These distinctive trends were thought to be due to the spread of HCV infection, which began in the 1920s and increased after World War II (6–8). Thus, HCV penetrated Japan earlier than Spain, Egypt, the United States, the former Soviet Union, South Africa, and Hong Kong, as evidenced by molecular clock analysis of the sequences of HCV isolates (8). However, recent temporal trends regarding incidence rates of HCC and the contribution of HCV infection have not been clearly documented in the Japanese population.

We analyzed temporal trends for HCC incidence rates between 1981 and 2003 in Osaka Prefecture (population in 2005, 8.8 million) and interpreted these in the context of HCV infection rates.

See also:

Print
Editors' Notes 821

Web-Only
Conversion of graphics into slides

Methods

Data Collection on Incident HCC Cases

We obtained data on incident HCC cases from the Osaka Cancer Registry, which was established by the Osaka Prefectural Government in 1962. The registry collects reports on patients with newly diagnosed cancer, including demographic and cancer-related information, from all medical institutions in Osaka Prefecture (9). These have been routinely supplemented by death certificates gathered

by the Osaka Prefectural Government (9). For patients with cancer who were enrolled in the registry on the basis of their death certificate, we contacted the issuing hospital to obtain information on diagnosis and treatment and to establish the date of HCC incidence, which we determined to be the time of diagnosis at that hospital. We site-coded the data according to the International Classification of Diseases for Oncology, Third Edition (10). We included patients with HCC (codes 8170 through 8180). The protocol was approved by the ethics committee of the Osaka Medical Center for Cancer and Cardiovascular Diseases.

From 1981 to 2003, 48 166 men and 15 696 women with HCC were documented in the Osaka Cancer Registry. We calculated the annual age-standardized incidence rates of HCC (world population as a standard population) by sex between 1981 and 2003. To characterize temporal trends for HCC, we assessed 10-year, age-specific incidence rates of HCC between 1981 and 2003 in individuals age 50 to 79 years. We studied these particular age-specific rates because most HCV-related HCC cases in the Japanese population occur between the ages of 50 and 79 years (4). We used the annual population estimates from 1981 to 2003, which were based on the average population in each sex and age category for the Osaka Prefecture during the particular period, as denominators for calculating incidence rates. The annual population estimates were based on data from the 1980, 1985, 1990, 1995, 2000, and 2005 Japanese population censuses, with linear interpolation for the years in between.

Statistical Analysis

To identify years when a statistically significant change in the slope of the temporal trend in the incidence occurred, we applied the joinpoint regression model by using the Joinpoint Regression Program, version 3.0 (U.S. National Cancer Institute, Bethesda, Maryland). We assumed constant variance and uncorrelated errors (11) because we could not detect heteroskedasticity by the White test or autocorrelation by the Durbin-Watson test in men or women in any age group.

We computed the estimated slopes describing the average annual change of incidence rate per 100 000 persons and the corresponding 95% CIs for each trend by fitting a piecewise regression line to the rates, using calendar year as a regression variable. We used the permutation test method to identify years when a statistically significant change had occurred ($P < 0.05$) and set the number of randomly permuted data sets at 4499. We set the number of joinpoints to a minimum of 0 and a maximum of 3 in the Joinpoint Regression Program.

Data Collection on Prevalence of HCV Infection among Patients with HCC

The Osaka Cancer Registry does not collect serologic data on HCV infection in the registered patients. Therefore, we used data on HCV seropositivity from patients with HCC that was diagnosed at 10 hospitals in Osaka Prefecture (1 university hospital, 2 cancer centers, and 7 general hospitals) to estimate the prevalence of HCV infection in patients with HCC. We considered the HCC diagnosis confirmed when the patient had positive histologic or positive radiologic results by enhanced computed tomography or hepatic angiography. We collected data on the patient's sex, date of birth, date of diagnosis between 1990 and 2003, first Chinese letter of the family name, and presence of hepatitis B surface antigen and antibody to hepatitis C (anti-HCV) as assessed by any commercially available kit. We did not collect the full first and family name for reasons of confidentiality. Because anti-HCV testing first became available in Japan in 1990, we collected data on patients whose HCC diagnosis was between 1990 and 2003. One investigator checked for duplication of the data set, because some patients might have been registered multiple times among the participating hospitals as a result of referrals and recurrence of HCC. We defined HCV-related HCC as occurring in patients who were HCV-seropositive at the time of diagnosis.

We calculated the sex-specific, age-specific (50 to 59, 60 to 69, or 70 to 79 years), and period-specific (1990 to 1992, 1993 to 1995, 1996 to 1998, 1999 to 2001, or 2002 to 2003) prevalences of HCV seropositivity for patients with HCC. We then multiplied prevalence rates by the corresponding strata of the HCC incidence rate obtained from the Osaka Cancer Registry data. Thus, we derived the denominators from the general population in Osaka through the denominators of the HCC incidence rate and obtained the numerators by multiplying the prevalence rates by the HCC incidence rate. We calculated the incidence rate of non–HCV-related HCC by subtracting HCV-related HCC from total HCC. Thus, we describe trends for the estimated incidence rates of HCV-related

Context

Hepatitis C virus (HCV) infection in Japan began to spread during the 1920s, increased after World War II with an explosion in parenteral amphetamine use and paid blood donation, and decreased in the 1950s to 1960s with voluntary blood donation and penalties against amphetamine use. Evidence linking the trends in HCV infection to hepatocellular carcinoma rates in Japan is limited.

Contribution

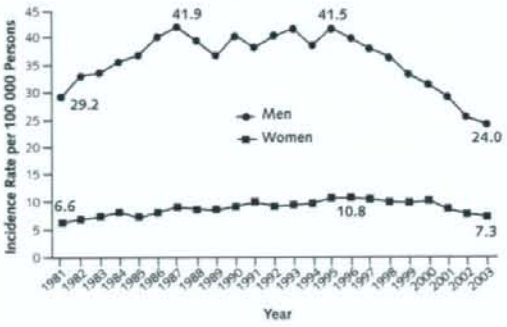
Data from the Osaka Cancer Registry and 10 Osaka hospitals suggest that hepatocellular carcinoma rates began to decrease in 2000, mainly because of a decrease in HCV-associated cancer.

Implication

Control of HCV transmission within a population seems to be followed by a decrease in hepatocellular carcinoma.

—The Editors

Figure 1. **Trends in age-standardized (world population) incidence of hepatocellular carcinoma in Osaka, Japan, 1981–2003.**



and non–HCV-related HCC between 1990 and 2003 in Osaka Prefecture. We calculated the CI of the estimated rates by multiplying the lower and upper limits of the CI of the prevalence based on SE by the corresponding HCC incidence rate.

Role of the Funding Source

This study was supported by the Osaka Prefectural Government between 1990 and 2000 and Grants-in-Aid for Hepatitis Research of the Japanese Ministry of Health, Labor, and Welfare. There is no conflict of interest in the study. The funding sources had no role in the collection, management, or analysis of data.

RESULTS

The age-standardized incidence rate of HCC in men increased between 1981 and 1987 from 29.2 to 41.9 cases per 100 000 persons, then fluctuated until 1995. After that, it steadily decreased to 24.0 cases per 100 000 persons in 2003 (**Figure 1**). Among women, the age-standardized incidence rate of HCC increased between 1981 and 1996 from 6.6 to 10.8 cases per 100 000 persons, then gradually decreased to 7.3 cases per 100 000 persons in 2003 (**Figure 1**).

Figure 2 shows the trends in the incidence of HCC among men and women age 50 to 59 years, 60 to 69 years, and 70 to 79 years in Osaka between 1981 and 2003. The HCC incidence rate increased from 1981 to 1986 among men age 50 to 59 years, from 1981 to 1995 among men age 60 to 69 years, and from 1981 to 2000 among men age 70 to 79 years (average annual change of the incidence rate [per 100 000 persons], 10.0, 10.7, and 6.2, respectively) (**Table 1**). A striking downward trend occurred after the year of peak incidence in the 3 age groups (−7.9 until 1996, −22.3 until 2003, and −12.4 until 2003, respectively). Among men age 50 to 59 years, there was a second joinpoint (a change from rapid to moderate decrease) in 1996, resulting in a slope of −3.1 until 2003. Among women age 50 to 59 years, 60 to 69 years, and 70 to 79 years, the incidence rates of HCC peaked in 1991, 1997, and 2000, respectively (**Table 1**). The rates in women seemed to increase slightly from 1981 until the year of the joinpoint, with slopes of 0.43, 2.07, and 3.10, respectively. Thereafter, HCC incidence rates in women decreased through 2003 at a statistically significant average annual rate of −0.9, −5.7, and −7.9, respectively (**Table 1**).

Figure 2. **Joinpoint analysis of the incidence rate of hepatocellular carcinoma among individuals age 50 to 79 years in Osaka, Japan, 1981–2003.**

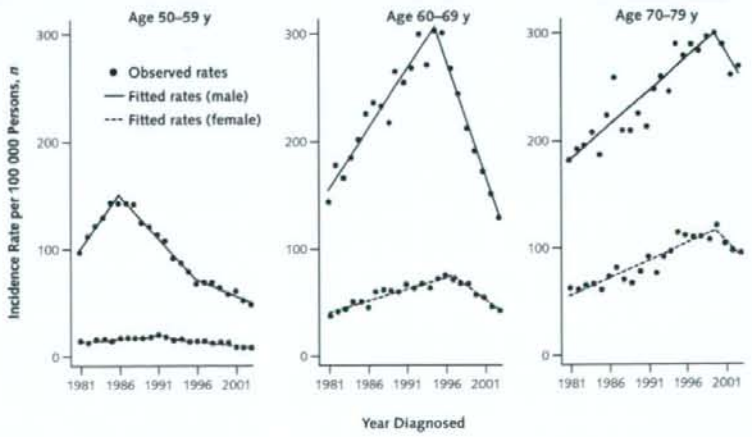


Table 1. **Joinpoint Analysis of the Hepatocellular Carcinoma Incidence Rate per 100 000 Persons in Osaka, Japan, 1981–2003**

Age Range	Peak Year	Incidence Rate per 100 000 Persons	Trend 1		Trend 2		Trend 3	
			Years	Slope (95% CI)	Years	Slope (95% CI)	Years	Slope (95% CI)
Men								
50–59 y	1986	142.0	1981–1986	10.0 (8.2 to 11.8)*	1986–1996	−7.9 (−8.6 to −7.1)*	1996–2003	−3.1 (−4.2 to −2.1)*
60–69 y	1995	299.6	1981–1995	10.7 (9.1 to 12.3)*	1995–2003	−22.3 (−26.0 to −18.6)*	–	–
70–79 y	2000	296.4	1981–2000	6.2 (4.8 to 7.5)*	2000–2003	−12.4 (−35.7 to 10.9)	–	–
Women								
50–59 y	1991	19.7	1981–1991	0.4 (0.2 to 0.7)*	1991–2003	−0.9 (−1.1 to −0.7)*	–	–
60–69 y	1997	68.5	1981–1997	2.1 (1.7 to 2.4)*	1997–2003	−5.7 (−7.3 to −4.1)*	–	–
70–79 y	2000	118.1	1981–2000	3.1 (2.5 to 3.7)*	2000–2003	−7.9 (−18.1 to 2.4)	–	–

* $P < 0.001$.

Table 2 shows the prevalence of anti-HCV antibodies among 5253 patients age 50 to 79 years with HCC that was diagnosed at 10 hospitals in Osaka between 1990 and 2003. The prevalence was highest in men with HCC that was diagnosed in 1993 to 1995 (82.4%). The proportion of HCV-seronegative patients ranged from 18% to 29% through the observation period. The prevalence of anti-HCV was almost constant (81% to 83%) among women with HCC that was diagnosed between 1993 and 2003 (Table 2).

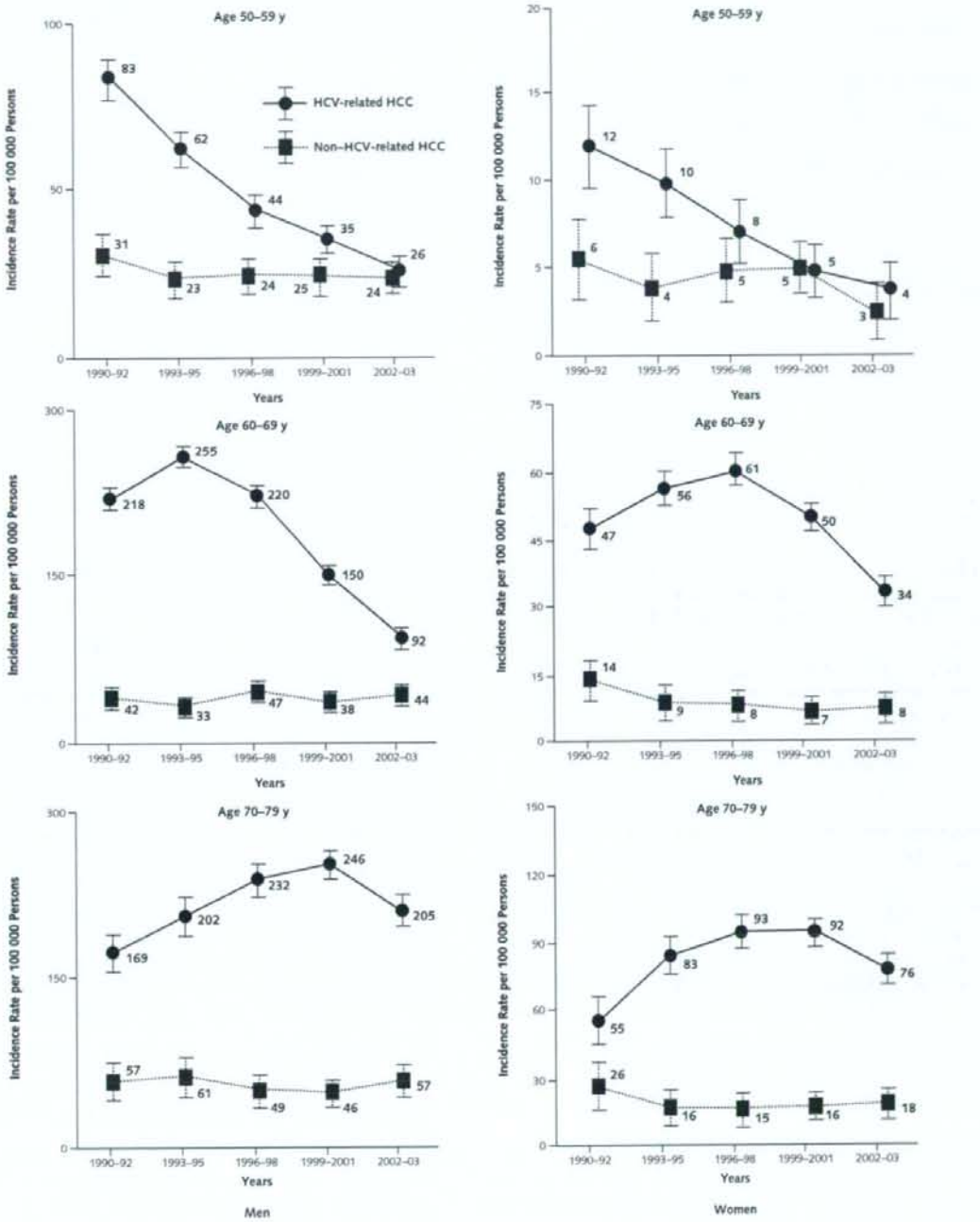
Figure 3 shows changes in the estimated incidence rate of HCV-related and non–HCV-related HCC from 1990 to 2003. Among men, the estimated incidence rate of HCV-related HCC steadily decreased among Osaka residents age 50 to 59 years from 83 (95% CI, 77 to 89) cases per 100 000 persons in 1990 to 1992 to 26 (CI, 21 to 30) cases per 100 000 persons in 2002 to 2003. Among men age 60 to 69 years, incidence seemed to peak (255 [CI, 247 to 264] cases per 100 000 persons) from 1993 to 1995. Among men age 70 to 79 years, the incidence rate increased from 1990 to 1992 (169 [CI, 153 to 186] cases per 100 000 persons) to 1999 to 2001 (246 [CI, 234 to 258] cases per 100 000 persons) and leveled off afterward. The estimated incidence rate of HCV-related HCC among women age 50 to 59 years decreased from 12.4 (CI, 10.1 to 14.7) cases per 100 000 persons during 1990 to 1992 to 4.2 (CI, 2.5 to 5.8) cases per 100 000 persons during 2002 to 2003, whereas among women age 60 to 69 years, the incidence peaked (61 [CI, 57 to 64] cases per 100 000 persons) during 1996 to 1998. The trend in women age 70 to 79 years seemed to be similar to that in men of the same age: increasing during the 1990s and leveling off in the early 2000s (Figure 3). The estimated incidence rate of non–HCV-related HCC was lower than that of HCV-

Table 2. **Prevalence of Anti-HCV among 5253 Patients Age 50 to 79 Years with Hepatocellular Carcinoma at 10 Hospitals in Osaka, Japan, 1990–2003***

Variable	1990–1992		1993–1995		1996–1998		1999–2001		2002–2003	
	Patients, *n*	Prevalence (±SE), %	Patients, *n*	Prevalence (±SE), %	Patients, *n*	Prevalence (±SE), %	Patients, *n*	Prevalence (±SE), %	Patients, *n*	Prevalence (±SE), %
Men										
Anti-HCV(+)	602	78.3 ± 1.5	677	82.4 ± 1.3	651	78.7 ± 1.4	709	76.6 ± 1.4	385	70.9 ± 1.9
Anti-HCV(+) and HBsAg(+)	18	2.3 ± 0.5	17	2.1 ± 0.5	11	1.3 ± 0.4	16	1.7 ± 0.4	8	1.5 ± 0.5
Anti-HCV(+) and HBsAg(−)	584	75.9 ± 1.5	660	80.3 ± 1.4	640	77.4 ± 1.5	693	74.8 ± 1.4	377	69.4 ± 2.0
Anti-HCV(−)	167	21.7 ± 1.5	145	17.6 ± 1.3	176	21.3 ± 1.4	217	23.4 ± 1.4	158	29.1 ± 1.9
Anti-HCV(−) and HBsAg(+)	60	7.8 ± 1.0	57	6.9 ± 0.9	71	8.6 ± 1.0	106	11.4 ± 1.0	68	12.5 ± 1.4
Anti-HCV(−) and HBsAg(−)	107	13.9 ± 1.2	88	10.7 ± 1.1	105	12.7 ± 1.2	111	12.0 ± 1.1	90	16.6 ± 1.6
Total	769	100.0	822	100.0	827	100.0	926	100.0	543	100.0
Women										
Anti-HCV(+)	165	73.0 ± 3.0	211	82.7 ± 2.4	248	82.9 ± 2.2	274	80.8 ± 2.1	200	81.0 ± 2.5
Anti-HCV(+) and HBsAg(+)	8	3.5 ± 1.2	2	0.8 ± 0.6	5	1.7 ± 0.7	2	0.6 ± 0.4	2	0.8 ± 0.6
Anti-HCV(+) and HBsAg(−)	157	69.5 ± 3.1	209	82.0 ± 2.4	243	81.3 ± 2.3	272	80.2 ± 2.2	198	80.2 ± 2.5
Anti-HCV(−)	61	27.0 ± 3.0	44	17.3 ± 2.4	51	17.1 ± 2.2	65	19.2 ± 2.1	47	19.0 ± 2.5
Anti-HCV(−) and HBsAg(+)	21	9.3 ± 1.9	17	6.7 ± 1.6	29	9.7 ± 1.7	29	8.6 ± 1.5	18	7.3 ± 1.7
Anti-HCV(−) and HBsAg(−)	40	17.7 ± 2.5	27	10.6 ± 1.9	22	7.4 ± 1.5	36	10.6 ± 1.7	29	11.7 ± 2.0
Total	226	100.0	255	100.0	299	100.0	339	100.0	247	100.0

* HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus.

Figure 3. **Trends in estimated incidence rates of hepatitis C virus (*HCV*)–related and non–HCV-related hepatocellular carcinoma (*HCC*) in Osaka, Japan, 1990–2003.**



Information on anti-HCV status only became available after 1989. Error bars indicate 95% CIs.

related HCC in most strata. We observed no distinctive changes in the temporal trends for non–HCV-related HCC during the study period.

Discussion

Our analysis of HCC incidence in the Japanese population between 1981 and 2003 identified calendar years in which significant changes in temporal trends occurred. The HCC incidence rates in men and women age 50 to 59 years peaked during 1986 and 1991, respectively; in men and women age 60 to 69 years during 1995 and 1997, respectively; and in men and women age 70 to 79 years in 2000. We also found that temporal trends for HCC incidence between 1990 and 2003 by age group were mainly determined by trends in the incidence rates of HCV-related HCC.

The most likely explanation for these observations is the particular mode of HCV transmission in Japanese society. According to a study on molecular tracing of endemic HCV (8), the exponential spread of HCV-1b infection, a dominant genotype of HCV in Japan, started in the 1920s. This was associated with treatment of *Schistosoma japonicum* beginning in 1921 (12). Later, HCV infection coincided with an increase in parenteral amphetamine use in the devastated country during and after World War II (6, 7). Subsequently, viral spread was considered to be amplified through blood transfusions and parenteral medical procedures in the 1950s and 1960s (6, 7). Data on first-time blood donor candidates in Osaka indicate that the prevalence of anti-HCV antibodies among those born in 1925 to 1935 was much higher (7% to 10%) than that in the younger generation born in 1936 to 1955 (13). It is plausible that Japanese people born between 1925 and 1935, who were adolescents in the early 1950s, were most susceptible to HCV transmission under these circumstances. Age groups with peak incidence of HCC in men and women in the current study (1986 and 1991, respectively, for 50 to 59 years; 1995 and 1997, respectively, for 60 to 69 years; and 2000 for 70 to 79 years) included the generation for which prevalence of anti-HCV was high in Osaka (born in 1925 and 1935) (13). Stiffening of legal penalties against amphetamine use starting in 1954 and conversion from paid to voluntary blood donation in the late 1960s may have reduced HCV transmission, thereby resulting in the lower prevalence of HCV infection in generations born after 1935. Indeed, the spread of HCV in Japan essentially ended by the early 1990s at the latest, as evidenced by the current very low incidence of HCV infection among repeat blood donors (14, 15). Better detection methods introduced in the early 1980s for HCC in patients with cirrhosis through ultrasonography and measurement of α-fetoprotein may have contributed to the apparent increase in the incidence of HCC found in this study. However, the distinctive changes we observed in the age-specific incidence of HCC during the 1990s through the early 2000s cannot be explained by the increased ability to detect HCC, because the different joinpoints in age-specific incidence rates would not be derived from a single period effect of detection of HCC.

Increases in the incidence of and deaths from liver cancer in the 1970s to 1990s have been reported in Japan (5, 16), Australia (2), the United Kingdom (17), France (2, 18), Italy (2, 18), and the United States (2, 19). The increases in Japan and the United States are attributable to increased seroprevalence of HCV (6, 13, 20, 21), whereas this relationship has not been clearly established in the other countries.

Certain limitations of this study should be considered. First, because cancer reporting in Osaka is not mandated by law, HCC could have been underreported. However, because it is fatal, most of the unreported cases should have been detected by examination of the death certificate. In addition, because the proportion of persons with HCC included only on the basis of their death certificate was almost constant (22% to 25%) during the observation period (22–24), such underreporting would not be expected to affect the temporal trends for HCC incidence rates shown in our study. Second, the proportion of HCV-seropositive patients among the 5253 cases diagnosed at 10 hospitals might differ somewhat from the entire cohort of patients with HCC in Osaka. However, all Japanese patients, including those with HCC, have easy access to hospitals because of the national medical insurance system, and the 10 participating hospitals did not select patients with HCC on the basis of their etiologic background. Therefore, it is realistic to suppose that selection bias on prevalence of anti-HCV among these 5253 patients would have been limited. Finally, the temporal trends seen in the present study might differ from those among the entire Japanese population. We previously reported age-specific incidence rates of liver cancer by birth year in Japanese men between 1962 and 1997 (5) by using 6 population-based cancer registries from Cancer Incidence in Five Continents (9) (registries for Miyagi, Yamagata, Osaka, Hiroshima, Saga, and Nagasaki). Our previous study found the peak incidence of HCC among those born between 1931 and 1935 (5). In addition, the age-dependent prevalence of anti-HCV among first-time blood donors in Osaka (13) was similar to those in other areas of Japan (25). These findings may indicate that the timing of the outbreak of HCV infection and its reduction were similar in the different geographic areas of the country.

In conclusion, our calculation of HCC incidence rates demonstrated that they are already decreasing in both sexes in Osaka, Japan. That the outbreak of HCV infection in Japan after World War II and its termination occurred earlier in Japan than in the rest of the world is the most likely explanation for these observations. These findings confirm that HCV-related HCC is a preventable disease that can be decreased by controlling parenteral HCV transmission. In the early 1990s, interferon therapy for patients

with chronic HCV infection was started in Japan to reduce the risk for HCC (26, 27). A nationwide, community-based anti-HCV screening system targeting individuals age 40 to 70 years was introduced by municipal governments in Japan in 2002. Further observation of the temporal trends of HCC incidence is needed to assess the efficacy of these interventions in Japan.

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Original Article

hOGG1 Ser326Cys Polymorphism and Risk of Hepatocellular Carcinoma among Japanese

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BACKGROUND: The Ser326Cys polymorphism in human oxoguanine glycosylase 1 (hOGG1), which is involved in the repair of 8-hydroxy-2-deoxyguanine in oxidatively damaged DNA, has been associated with susceptibility to certain cancers, but has not been examined in causation of hepatocellular carcinoma (HCC).
METHODS: We conducted a case-control study to investigate whether this polymorphism was related to HCC risk with any interaction with alcohol consumption and cigarette smoking. Genotyping was performed by a polymerase chain reaction with confronting two-pair primers among 209 newly diagnosed HCC cases, 275 hospital controls, and 381 patients with chronic liver disease (CLD) without HCC.
RESULTS: Overall, the *hOGG1* genotype was not significantly associated with HCC; adjusted odds ratios (and 95% confidence intervals) for the Ser/Cys and Cys/Cys genotypes compared with the Ser/Ser genotype were 0.79 (0.35-1.79) and 0.48 (0.18-1.27) against hospital controls, and 1.51 (0.96-3.37) and 0.86 (0.50-1.47) against CLD patients. We could not detect any significant gene-alcohol interaction ($p = 0.95$ or 0.16) or gene-smoking interaction ($p = 0.70$ or 0.69).
CONCLUTIONS: These results suggest that the *hOGG1* Ser326Cys polymorphism may not play a major role as an independent factor in hepatocarcinogenesis.
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Key words: Carcinoma, Hepatocellular; human 8-oxoguanine glycosylase 1; Polymorphism (Genetics).

The major causative factor of hepatocellular carcinoma (HCC) is chronic infection with hepatitis C virus (HCV) and hepatitis B virus (HBV) in Japan.[1,2] Alcohol intake and cigarette smoking have also been implicated in the etiology of HCC.[3,4] Although the biological mechanisms underlying these factors are not fully understood, one of the proposed mechanisms represents the involvement of oxidative DNA damage which can induce mutations leading to cancer.[5,6] Chronic hepatic inflammation caused by hepatitis viruses and exposure to alcohol and tobacco stimulate the generation of hepatic reactive oxygen species (ROS) causing oxidative DNA damage.[7-9]

Among many types of oxidative DNA damage, 8-hydroxy-2-deoxyguanine (8-OHdG) is highly mutagenic because of its propensities to mispair with adenine during DNA replication and to cause ultimately GC to TA transversion.[10,11] The human 8-oxoguanine glycosylase 1 (hOGG1) encoded by the *hOGG1* gene

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located on chromosome 3p25/26 has an activity to remove directly 8-OHdG from DNA as a part of the base excision repair pathway.[12,13] The Ser326Cys polymorphism in exon 7 of *hOGG1* has been related to glycosylase function and an individual's ability to repair damaged DNA.[14,15]

Although recent studies[16-20] suggested that the low active *hOGG1* allele (326Cys) was positively associated with the risk of several cancers while showing interactions with environmental factors, the association between this polymorphism and HCC has not been examined so far. Therefore, we conducted this case-control study including 209 HCC cases and two different controls (275 hospital controls and 381 patients with chronic liver disease [CLD] without HCC); CLD patients were selected as control subjects because most HCC patients in Japan have preexisting CLD.

METHODS

Subjects

The details of this study have been described elsewhere.[21] Briefly, all study subjects were restricted to residents of Saga Prefecture, Japan, who were aged 40 to 79 years. Incident HCC cases (n = 209, participation rate = 92%), who were admitted or outpatients of 2 main hospitals in Saga City (Saga Medical School Hospital and Saga Prefectural Hospital) between April 2001 and March 2004, were recruited as case subjects; 198 cases (95%) had preexisting cirrhosis (n = 167) or chronic hepatitis (n = 31). Hospital controls (n = 275, participation rate = 73%) were first-time visitors at the general outpatient clinic of Saga Medical School Hospital between May 2001 and April 2003; these controls were selected so that the sex and age distribution of them would be similar to that of deaths from liver cancer in Saga Prefecture in 1998.21 They had various diseases (n = 190), undiagnosed symptoms (n = 49), or no definite abnormality (n = 36). Patients with CLD (298 patients with chronic hepatitis and 83 patients with cirrhosis, participation rate = 96%) were out- or inpatients of the hospitals same as HCC cases between September 2001 and March 2004; patients with special types of CLD (primary and secondary biliary cirrhosis, autoimmune hepatitis, and liver disease due to parasitotis, congestive heart failure, or metabolic disorders) were excluded. All control subjects had no evidence of HCC.

The study protocol was approved by the ethics committees of the above two hospitals, and written informed consent to the use of their blood and clinical information for this study was obtained from all subjects.

Interviews

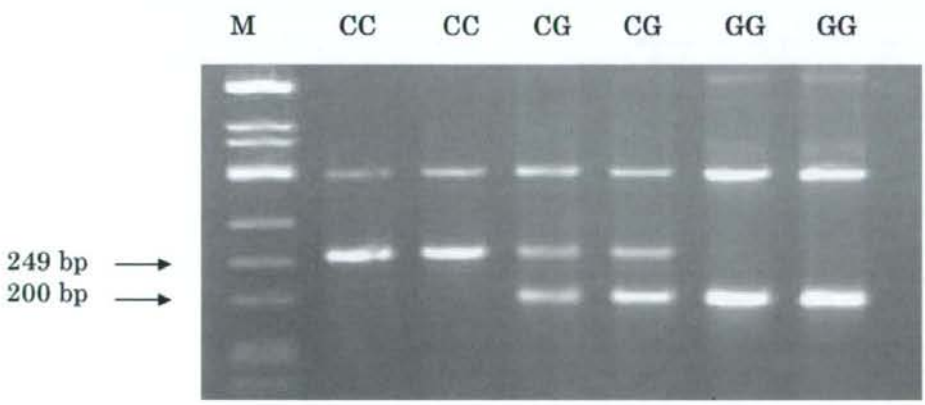
Research nurses interviewed study subjects on alcohol drinking and smoking habits using a uniform questionnaire. A history of heavy drinking was defined as having imbibed 69 g or more of ethanol per day for 10 or more years. We regarded "never smokers" as individuals who had never smoked or had smoked for less than 1 year, "former smokers" as those who stopped smoking 1 or more years before the interview, and "current smokers" as those who currently smoked or stopped smoking less than 1 year prior to the interview. The cumulative amount of smoking was calculated as pack-years.

Serologic Tests and Genotyping

Venous blood was drawn, and plasma samples were tested for hepatitis B surface antigen (HBsAg) by a chemiluminescent immunoassay (CLIA; Dainabot, Tokyo, Japan) and for antibodies to HCV (HCVAb) by a 2nd-generation enzyme immunoassay (Abott HCV EIA II; Dainabot, Tokyo).

DNA was extracted from buffy coat preparations by using a commercial kit (QIAmp DNA Blood Mini kit; QIAGEN Inc, Tokyo). The *hOGG1* Ser326Cys polymorphism was genotyped

Figure 1. PCR-CTPP analysis for the hOGG1 polymorphism at codon 326 in exon 7.



The amplified PCR products are 252 bp for the C allele (326Ser) and 194 bp for the G allele (326Cys).