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Wakai K, Kurozawa Y, Shibata A, Fujita Y, Kotani K, Ogimoto I, Naito M, Nishio K, Suzuki H, Yoshimura T, Tamakoshi A for the JACC Study Group	Liver cancer risk, coffee and hepatitis C virus infection: a nested case-control study in Japan.	Br J Cancer	97	426-428	2007
Tanaka H, Uera F, Tsukuma H, Ioka A, Oshima A.	Distinctive change in male liver cancer incidence rate between the 1970s and 1990s in Japan: comparison with Japanese-Americans and US whites.	Jpn J Clin Oncol.	37	193-6	2007
Hara M, Tanaka K, Sakamoto T, Higaki Y, Mizuta T, Eguchi Y, Yasutake T, Ozaki I, Yamamoto K, Onohara S, Kawazoe S, Shigematsu H, Koizumi S	Case-control study on cigarette smoking and the risk of hepatocellular carcinoma among Japanese.	Cancer Sci	99	93-97	2008
Ohfuji S, Fukushima W, Tanaka T, Habu D, Takeda T, Tamori A, Sakaguchi H, Seki S, Kawada N, Nishiguchi S, Shiomi S, Hirota Y.	Does late evening meal reduce the risk of hepatocellular carcinoma among patients with chronic hepatitis C?	Hepatol Res			In press

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Serum aminotransferase level and the risk of hepatocellular carcinoma: A population-based cohort study in Japan

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ABSTRACT

Aminotransferase level is presumed to be a marker of hepatic inflammation, but uncertainty remains whether elevated aminotransferase levels are associated with an increased risk of hepatocellular carcinoma (HCC). We evaluated the risk of incidence of HCC by aminotransferase level in 19,812 middle-aged and older subjects with and without hepatitis virus infection from a large-scale population-based cohort study (JPHC Study Cohort II) in Japan. Hepatitis virus infection was identified at baseline in 1236 subjects, namely 737 (3.7%) with HCV, 479 (2.4%) with HBV, and 20 (0.1%) with both. By the end of follow-up, a total of 109 newly arising HCC cases were diagnosed (71 men, 38 women), of which 87 (79.8%) had evidence of viral etiology. Alanine aminotransferase (ALT) was concentration-dependently associated with an increased risk of HCC in both virus-positive and virus-negative subjects. Compared to virus-negative subjects with ALT levels of <30 IU/L, a significant increase in the risk of HCC was observed in virus-negative subjects with an ALT level >30 IU/L, and in virus-positive subjects with an ALT <30 IU/L, 30-69 IU/L and ≥70 IU/L (Hazard ratio (95% confidence interval): 9.4 (3.9-22.3), 15.2 (6.1-37.6), 180.5 (89.4-364.2), 454.2 (221.5-931.2), respectively; p for trend <0.001). In conclusion, our findings suggest that elevated ALT levels are strongly associated with the incidence of HCC regardless of hepatitis virus positivity. This finding indicates that ALT level is a good independent determinant of the need for intervention. Clinical application of these findings may help decrease HCC-associated mortality in hepatitis virus-endemic regions.

Keywords: hepatocellular carcinoma, hepatitis virus, prospective study, alanine aminotransferase, incidence

INTRODUCTION

In Japan, more than 30,000 people die of hepatocellular carcinoma (HCC) annually, making it the third-leading cause of death from malignant neoplasm in men and the fifth- in women (Kiyoyama *et al.*, 2004; Yoshizawa, 2002). Hepatitis C virus (HCV) infection and hepatitis B virus (HBV) infection are plausible as the two major causes of HCC. Both HCV and HBV bring about chronic necroinflammatory hepatic damage, the end result of which is cirrhosis and HCC. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are released into the blood from damaged hepatocytes after hepatocellular injury or death, and ALT and AST levels are presumed to be markers of hepatic inflammation.

Given that elevations in ALT or AST level provide important information on hepatocyte damage (Bacon et al., 2002) and that biopsy specimens reveal a close correlation between elevation and histologic

necroinflammation (Tarao et al., 2002), investigators have hypothesized an association between higher levels of ALT or AST and the development of HCC. Supporting this, two prospective studies in Japan showed that elevated ALT levels were strongly associated with the incidence of HCC in subjects positive for anti-HCV (Tanaka et al., 2004; Suruki et al., 2006). However, it remains uncertain whether elevated ALT or AST levels are associated with an increased risk of HCC in subjects without hepatitis virus infection, notwithstanding the known association of elevated ALT with conditions such as alcoholic liver disease, nonalcoholic fatty liver disease (NAFLD), hemochromatosis, and some types of metabolic disease.

Therefore, to gain further epidemiologic evidence on this issue, we evaluated the association between elevated levels of aminotransferase and the incidence of HCC, in a large-scale population-based cohort study in Japan, with particular attention to hepatitis virus positivity.

METHODS

Study Population

The Japan Public Health Center-based Prospective Study (JPHC Study) Cohort II was launched in 1993-1994 in registered Japanese inhabitants aged 40–69 years at the beginning of the respective baseline survey in six prefectural public health center (PHC) areas (n=68,980). Details of the study design have been described elsewhere (Tsugane *et al.*, 2001; Inoue *et al.*, 2005). The study protocol was approved by the Institutional Review Board of the National Cancer Center, Japan.

For the present analysis, a total of 19,812 subjects who responded to the questionnaire and provided a blood sample and health check-up data were enrolled.

Baseline survey

A baseline self-administered questionnaire survey on various lifestyle factors was conducted in 1993-1994 (response rate=82%). A total of 10 ml of blood was also provided voluntarily by 29% of subjects during health check-ups provided by the local government. The plasma and buffy layer were divided into four tubes holding 1.0 ml each (three tubes for plasma and one for the buffy layer) and stored at -80 °C.

Follow-up and identification of HCC

Subjects were followed from the baseline survey until December 31, 2005. Residence status, including survival, was confirmed through the residential registry. Resident and death registration are required by law in Japan and the registries are believed to be complete. Inspection of the resident registry is legally sanctioned by the resident registration law. The occurrence of HCC was determined by notification from hospitals in the study areas and data linkage with population-based cancer registries. Death certificates were used as a supplementary information source. In our cancer registry system, the proportion of cases for which information was available from death certificates only was 4.8%. This ratio was of satisfactory quality for the present study based on the international standard (Parkin *et al.*, 2002). The site of origin and histological type were coded using the International Classification of Diseases for Oncology, 3rd Edition (ICD-O-3; C22.0) (World Health Organization, 2000). We excluded subjects with no data on aminotransferase levels in blood samples taken during their health check-up. Through this procedure, a total of 109 newly diagnosed HCC cases were identified during follow-up as of December 31, 2005.

Laboratory analysis

Serum ALT, AST, and gamma-glutamyl transferase (GGT) levels were determined at the baseline health check-up. These items were measured in 23 laboratories in the cohort area, with accuracy control and standardization among the laboratories provided by the Japan Medical Association via its External Quality Control Survey. The upper limit of normal ALT was tentatively defined as 30 IU/L, as used in recent clinical or

prospective studies (Kunde *et al.*, 2005; Okanoue *et al.*, 2005).

Plasma samples were screened for anti-HCV using a third-generation immunoassay (Lumipulse II Ortho HCV, Ortho-Clinical Diagnostics K.K., Tokyo, Japan) (Abdel-Hamid *et al.*, 2002) and for hepatitis B virus antigen (HBsAg) by reversed passive hemagglutination with a commercial kit (Institute of Immunology Co., Ltd., Tokyo, Japan). The virus-positive group consisted of subjects positive for either or both anti-HCV and HBsAg.

Statistical analysis

P values for differences between groups were calculated using the Chi-square test or t-test. Person-years of follow-up were calculated from the date of baseline survey until the date of diagnosis of HCC, the date of a subject's death, the date of moving from a PHC area, or 31st December 2005, whichever occurred first. Multivariate-adjusted hazard ratios (HR) and corresponding 95% confidence intervals (CI) of HCC were estimated by the Cox proportional hazards model. The estimates were adjusted for the following potential confounding factors incorporated into the model: hepatitis virus positivity (HCV or HBV positive, negative), age at baseline (40-49, 50-59, 60-69 years), study area (six PHC areas), smoking status (never, past, current), weekly ethanol intake (none, 1-149g, 150g or more), body mass index (<23.0 kg/m^2, 23.0-24.9, 25.0-26.9, 27.0-29.9, ≥30.0) and coffee intake (almost never, 1-4 days/week, almost every day). Statistical analyses were performed using STATA version 9.2 (STATA Corporation, 2005).

RESULTS

Of 19,812 subjects, 737 were identified with HCV monoinfection (3.7%), 479 with HBV monoinfection (2.4%) and 20 with HCV and HBV coinfection (0.1%). The proportion of subjects with an ALT level ≥ 30 IU/L was 35.1% in the virus-positive group, and 11.0% in the virus-negative group. Current smokers and heavy alcohol drinkers tended to have elevated ALT levels regardless of virus-positive or –negative status. Further, obese subjects without hepatitis virus infection had higher ALT levels (Table I).

During the 234,016 person-years of follow-up (average follow-up period, 11.8 years) for the 19,812 subjects (6,920 men and 12,892 women), a total of 109 newly diagnosed cases of HCC (71 men, 38 women) were documented. Of these, 75 subjects had HCV monoinfection (68.8%), 10 had HBV monoinfection (9.2%), 2 had coinfection with HCV and HBV (1.8%), and 22 had no virus infection (20.9%).

After adjustment for potential confounding risk factors such as hepatitis virus positivity, sex, age, study area, weekly ethanol intake, body mass index and coffee intake, HCC was found to occur significantly more frequently in subjects with serum ALT levels ≥ 30 IU/L (HR = 13.5, 95% CI: 8.0-22.0), serum AST levels ≥ 30 IU/L (HR = 14.3, 95% CI: 8.0-25.8), and serum GGT levels ≥ 60 IU/L (HR = 5.5, 95% 95% CI: 3.5-8.8). ALT and AST levels increased in parallel (correlation coefficient=0.81). Most cases had both an abnormal ALT level and abnormal AST level, although a few had a normal ALT level and abnormal AST level or the converse. In contrast, the correlation of ALT and GGT was relatively low (correlation coefficient=0.43). We also observed that the association of incidence of HCC with elevated GGT level was relatively weak compared with that with ALT level after further adjustment for serum ALT (HR=2.1, 95% CI: 1.3-3.3) (Table II). On these bases, we restricted further analysis to ALT.

Compared to subjects in the normal range of ALT (< 30 IU/L), those with elevated levels (30-69 IU/L, 70-99 IU/L, 100 ≤ IU/L) had a significantly higher risk of developing HCC (HR = 10.5 (95% CI: 6.0-18.3), 25.2 (12.7-49.7), and 43.9 (22.7-84.8), respectively) after adjustment for virus positivity, age, sex, study area, smoking status, ethanol intake, body mass index, and coffee intake. We observed positive linear trends in HR according to level of ALT category ($P < 0.001$).

Among virus-positive subjects (virus-positive; anti-HCV and/or HBsAg-positive), elevated ALT (30-69 IU/L, 70-99 IU/L, 100- IU/L) was significantly associated with the incidence of HCC (HR = 12.0 (95%

CI: 5.8-24.9), 25.6 (11.3-58.1) and 37.1 (16.3-84.2), respectively) (Table III). Cumulative incidence of HCC at 10 years among virus-positive subjects was 1.1% for subjects with ALT < 30 IU/L, but 11.0% and 27.2% for those with ALT 30-69 IU/L and ALT ≥70 IU/L, respectively (Figure 1). This ALT-dependent increase in risk was identified in subset analyses of subjects with HCV (anti-HCV-positive), HBV (HBsAg-positive), and without hepatitis virus infection (virus-negative) (Table III). Furthermore, virus-positive subjects with normal ALT had a higher risk of HCC than virus-negative subjects with normal ALT (HR = 15.2 (95% CI: 6.1-37.6)). Virus-positive subjects with elevated ALT (30-69 IU/L, 70- IU/L) had an extremely elevated risk of HCC (HR = 180.5 (95% CI: 89.4-364.2), and 454.2 (221.5-931.2), respectively) (Table IV).

DISCUSSION

This population-based prospective study in Japan demonstrated that serum ALT level is concentration-dependently associated with an increased risk of HCC in both virus-positive and virus-negative subjects. Compared to virus-negative subjects with a normal ALT level (< 30 IU/L), virus-positive subjects with an ALT level ≥ 30 IU/L had a greater than 180-fold higher risk of HCC as well as higher cumulative incidence of HCC at 10 years. This finding suggests the need for anti-viral therapy in these patients to reduce the risk of HCC. Even virus-negative subjects with an ALT level ≥ 30 IU/L and virus-positive subjects with an ALT level < 30 IU/L showed a statistically significant risk of HCC, suggesting the necessity of regular follow-up.

An association between higher levels of ALT or AST and the development of HCC has been hypothesized, but few studies have investigated the link. In a prospective study in Japan, Tanaka *et al.* investigated the association between serum ALT level and incidence of HCC in 1927 voluntary blood donor subjects positive for anti-HCV and negative for HBsAg (Tanaka *et al.*, 2004). Results showed that elevated serum ALT level at blood donation was positively associated with the risk of HCC: compared to subjects at < 30 IU/L, those at 30-59 IU/L had a 6.2-fold increase in risk while those at > 60 IU/L had a 9.5-fold increase. This stepwise increase was statistically significant ($p<0.0001$). Although our cut-off and categorization of ALT were different, our present data also identified a stepwise increase in risk of HCC with increasing ALT in subjects with anti-HCV. Further Suruki *et al.*'s investigation of risk in a prospective community-based study in a single prefecture in Japan identified 667 anti-HCV-positive subjects and 52 cases of HCC on 10- years' follow-up (Suruki *et al.*, 2006). The risk of HCC was increased four-fold with abnormal (≥ 35 IU/L) compared to normal ALT levels (< 35 IU/L).

The long-term outcome of elevated ALT values in patients with virus infection remains uncertain. A second finding from Suruki *et al.*'s prospective study was that subjects with persistently abnormal ALT over multiple measurements during follow-up had a 19.8-fold risk of HCC compared to those with persistently normal values (Suruki *et al.*, 2006). Persico et al.'s prospective evaluation of disease progression in 37 HCV-infected patients found that chronic hepatitis with persistently normal ALT serum levels was mild and did not progress with time (Persico *et al.*, 2000). Another study which assessed hepatic fibrosis by liver biopsy in HCV patients with normal ALT levels showed weaker histological activity and a lower progression rate of fibrosis (Mathurin *et al.*, 1998). Regarding hepatocarcinogenesis, persistently high serum ALT levels were closely associated with a high incidence of HCC in patients with chronic HCV hepatitis (Tarao *et al.*, 2002), and with multicentric hepatocarcinogenesis in compensated cirrhosis patients with HCV infection in a follow-up study (Hayashi *et al.*, 2000).

Because subjects with hepatitis virus and a normal ALT range might be less likely to develop HCC, subjects with normal ALT have been routinely excluded from clinical trials with interferon therapy. However, several studies have reported marked liver lesions or fibrosis in HCV patients with persistently normal ALT levels (Martinot-Peinoux *et al.*, 2001; Paradot *et al.*, 2002), and such subjects in the present study had a 15-fold risk of HCC compared to non-infected subjects with normal ALT levels. Together, these findings advocate against the routine exclusion of patients with "normal" ALT levels from therapy. Zeuzem *et al.* reported that

combination therapy with peginterferon alfa-2a and ribavirin produced comparable sustained virological response rates and safety in patients with elevated ALT activity as in those with normal ALT (Zeuzem *et al.*, 2004). Treatment should be appropriate to the results of risk evaluation for items such as age, infectious period and virus genotype (Bacon *et al.*, 2002). From our results, the population-attributable fraction (Rockhill *et al.*, 1998) for HCC occurring during the study period attributable to virus-positive subjects with normal ALT can be estimated as 8.6%. Notwithstanding the low fraction of total HCC in this population, a decrease in incidence of 8.6% would be achieved if treatment were provided.

Further, subjects in our study who were neither anti-HCV- nor HBsAg-positive but had elevated ALT levels had a positive association with the incidence of HCC, albeit in a small number of cases. This association remained even after adjustment for alcohol consumption. To our knowledge this finding has not been previously reported.

The role of obesity in these findings also warrants mention. Elevated ALT can result from such etiologies as hepatitis virus infection and alcohol consumption to nonalcoholic fatty liver disease (NAFLD), autoimmune liver disease and metabolic disease (Yu and Keeffe, 2003). Of these, NAFLD has recently attracted attention because of its close association with obesity, metabolic syndrome and progression to cryptogenic cirrhosis (Caldwell *et al.*, 2004; Marchesini *et al.*, 2003; Clark and Diehl, 2003). NAFLD is often associated with obesity and is assumed to be a common underlying liver disease in non-viral infection patients with HCC in the United States (Marrero *et al.*, 2002). In our study, it is uncertain whether elevated ALT was caused by NAFLD because no histological evidence was obtained; nevertheless, the association between ALT level and incidence of HCC was identified after adjustment for body mass index, indicating that elevated levels cannot be explained by obesity or NAFLD only, and raising the possibility that unknown factors might have influenced the incidence of HCC. Our study identified a positive association between elevated ALT and the incidence of HCC in subjects with HBV infection, as previously reported (Bell *et al.*, 2005), but any interpretation of this finding requires caution owing to the small number of cases.

The strength of the present study is its population-based prospective design and low proportion of losses to follow-up (0.1%). Information was collected before the subsequent diagnosis of cancer, thereby avoiding the exposure recall bias inherent to case–control studies. Moreover, the proportion of losses to follow-up during the study period was negligible.

Nonetheless, several obvious limitations can be identified. First, we had no information on the clinical severity of hepatitis, or on the treatment of subjects with hepatitis virus infection before and during the study period. Interferon therapy, which decreases the risk of HCC in responding patients with HCV and HBV infection, has been available in Japan for the last decade, and it is possible that some of our infected subjects received this treatment. This in turn may have lead to the underestimation of HCC occurrence by responders, which would also bias the results toward the null. As most subjects were asymptomatic and lived in rural areas, however, most eligible subjects would not have received interferon therapy, and the influence of interferon, if present, is considered relatively small.

Second, evaluation by a single ALT measurement at baseline might have produced misclassification. Aminotransferase levels fluctuate on a day-to day basis, with exercise and with liver disease, particularly hepatitis virus infection, and the need for multiple measurement to ensure accuracy is well recognized (Green and Flamm, 2002). Further, without clinical information, subjects with severe liver injury in whom damaged hepatocytes are unable to produce ALT might have been classified into the normal group. If such misclassification were present, however, it would likely be nondifferential and would in any case lead to an underestimation of results.

Third, differences in the proportion of hepatitis virus positivity or in the dominant type of hepatitis virus may have resulted in geographical variation in blood biochemistry testing or incidence of HCC. We assumed that the association between ALT level and risk of HCC was not influenced by geographical area, but nevertheless adjusted for study area (PHC) on analysis. The small number of HCC cases prevents any conclusive

evaluation of this point, but we assume that the influence geographical area on the risk of HCC is not substantial.

Fourth, the failure to confirm HBV DNA means that occult HBV infection may have been present in noninfected HCC cases. Even following HBsAg clearance, some patients with chronic HBV infection develop HCC (Huo *et al.,* 1998). If present, however, such misclassification would be random, and in any case would bias the results toward the null.

Further, the present subjects were restricted to the 28.7% of the study subjects of the JPHC Study Cohort II group who provided blood samples. More women than men tend to participate in health checkup surveys provided by local governments. Participants often differ to nonparticipants in socioeconomic status and have a more favorable lifestyle profile, such as lower smoking rates, greater participation in physical exercise, and higher intake of green vegetables and fruits, particularly women (Iwasaki *et al.*, 2003), although the influence of these factors on the association between hepatitis virus related factors and HCC would not be substantial. In addition, the incidence of HCC in this study population was 46.6 cases per 100,000 people during the follow-up period, versus 64.2 cases per 100,000 people in the whole JPHC Study Cohort II, suggesting that subjects who were already under care for hepatitis infection may have been less willing to attend a health check-up. Together, these considerations mandate the need for caution in interpreting or generalizing these results.

In conclusion, this population-based prospective study in Japan showed that an increase in serum ALT level is positively associated with the incidence of HCC. Risk increased stepwise in an ALT concentration-dependent manner regardless of hepatitis virus infection status. Although any interpretation of these results requires close consideration of these methodological issues, this finding may indicate that ALT level is a good independent determinant of the need for intervention. Clinical application of this finding may contribute to a decrease in HCC-associated mortality.

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Appendix

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TABLE 1

Baseline characteristics

	Hepatitis virus-positive† n=1,236			Hepatitis virus-negative n=18,576		
	Serum ALT level		p**	Serum ALT level		p**
	<30 IU/L	≥30 IU/L		<30 IU/L	≥30 IU/L	
Number	802	434		16,528	2,048	
Age (years)*	58.3 (7.8)	58.2 (7.6)	0.41	57.3 (8.2)	55.4 (7.9)	0.001
Gender Men (%)	39.9	56.5	0.001	32.0	52.1	0.001
Current smoker (%)	24.0	29.8	0.001	15.7	23.5	0.001
Ethanol intake ≥150 g/week (%)	14.0	18.8	0.02	13.3	25.4	0.001
Body mass index ≥27 kg/m^2 (%)	10.6	13.4	0.30	11.0	26.4	0.001
Coffee intake, daily (%)	8.7	8.2	0.21	9.6	7.5	0.36

*, means (SD); **, p for difference; ALT, alanine amonotransferase; † anti-HCV-positive and/or HBsAg-positive.

TABLE 2

Hazard ratios (HR) and 95% confidence intervals (CI) of hepatocellular carcinoma by serum liver enzyme level

	Person-years	No. of cases	HR*		(95%CI)	
Serum ALT level						
<30 IU/L	205,509	20	1.0			
≥30 IU/L	28,507	89	13.5		(8.0-	22.0)
Serum AST level						
<30 IU/L	202,065	15	1.0			
≥30 IU/L	31,951	94	14.3		(8.0-	25.8)
Serum GGT level						
<60 IU/L	218,544	70	1.0			
≥60 IU/L	15,472	39	5.5	**	(3.5-	8.8)

* Adjusted for hepatitisvirus positivity (negative, positive), sex, years of age at baseline (40-49, 50-59, 60-69 years) and study area (six PHC areas), weekly ethanol intake (none, 1-149g, 150g and more), body mass index (<23.0 kg/m2, 23.0-24.9, 25.0-26.9, 27.0-29.9, ≥30.0) and coffee intake (almost never, 1-4 days/week, almost every day).

** HR = 2.1, 95%CI (1.3-3.3) after further adjustment for ALT level

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase

TABLE 3

Hazard ratios (HR) and 95% confidence intervals (CI) of HCC incidence by serum ALT level

	Person-years	No. of cases	HR	(95%CI)	P for trend
Serum ALT level					
Total* (n=19,812)					
-29 IU/L	205,509	20	1.0		
30-69 IU/L	25,361	48	10.5	(6.0- 18.3)	
70-99 IU/L	2,087	18	25.2	(12.7- 49.7)	
100- IU/L	1,059	23	43.9	(22.7- 84.8)	0.001
Virus-positive** (n=1,236)					
-29 IU/L	9,341	10	1.0		
30-69 IU/L	3,406	41	12.0	(5.8- 24.9)	
70-99 IU/L	571	17	25.6	(11.3- 58.1)	
100- IU/L	481	19	37.1	(16.3- 84.2)	0.001
HCV** (n=757)					
-29 IU/L	4,724	6	1.0		
30-69 IU/L	2,543	37	11.4	(4.7- 27.2)	
70-99 IU/L	485	16	25.1	(9.8- 64.8)	
100- IU/L	425	18	35.0	(13.4- 91.4)	0.001
HBV** (n=499)					
-29 IU/L	4,744	4	1.0		
30-69 IU/L	933	6	18.5	(3.7- 93.1)	
70- IU/L	165	2	35.0	(4.2- 293.1)	0.001
Virus-negative** (n=18,576)					
-29 IU/L	196,167	10	1.0		
30-69 IU/L	21,956	7	6.5	(2.2- 18.8)	
70- IU/L	2,095	5	60.5	(19.5- 187.9)	0.001

* Adjusted for hepatitis-virus positivity (negative, positive), sex, years of age at baseline (40-49, 50-59, 60-69 years) and study area (six PHC areas), weekly ethanol intake (none, 1-149g, 150g and more), body mass index (<23.0 kg/m2, 23.0-24.9, 25.0-26.9, 27.0-29.9, ≥30.0) and coffee intake (almost never, 1-4 days/week, almost every day).

** Adjusted for sex, years of age at baseline (40-49, 50-59, 60-69 years) and study area (six PHC areas), weekly ethanol intake (none, 1-149g, 150g and more), body mass index (<23.0 kg/m2, 23.0-24.9, 25.0-26.9, 27.0-29.9, ≥30.0) and coffee intake (almost never, 1-4 days/week, almost every day).

ALT, alanine aminotransferase; HCV, anti-HCV-positive; HBV, HBsAg-positive

TABLE 4

Hazard ratios (HR) and 95% confidence intervals (CI) of HCC incidence by serum ALT level and virus positivity

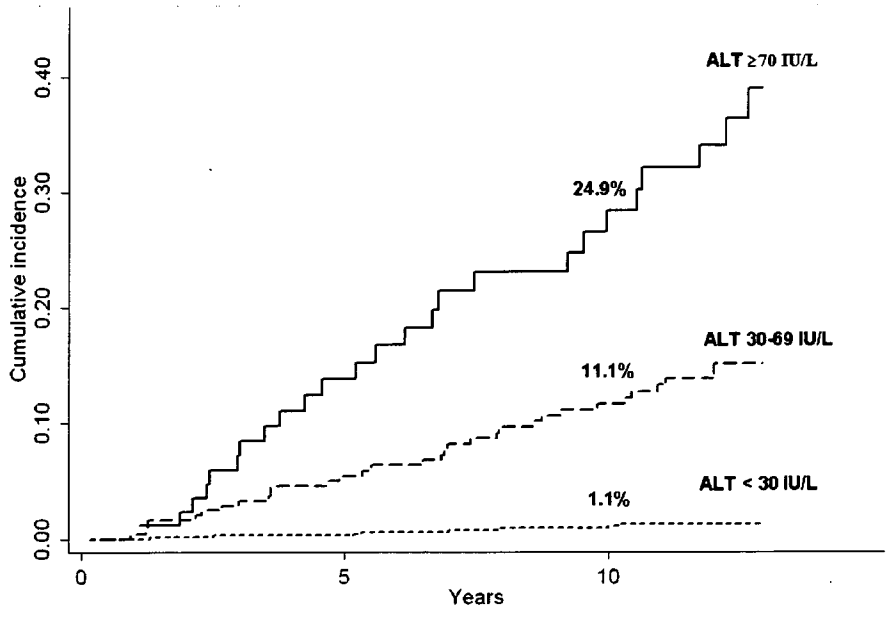
		Person-years	No. of cases	HR*	(95%CI)	P for trend
Serum ALT level						
Virus-negative**	-29 IU/L	196,167	10	1.0		
	30- IU/L	24,050	12	9.4	(3.9- 22.3)	
Virus-positive	-29 IU/L	9,341	10	15.2	(6.1- 37.6)	
	30-69 IU/L	3,406	41	180.5	(89.4- 364.2)	
	70- IU/L	1,052	36	454.2	(221.5- 931.2)	0.001

* Adjusted for gender, years of age at baseline (40-49, 50-59, 60-69 years) and study area (six PHC areas), weekly ethanol intake (none, 1-149g, 150g and more), body mass index (<23.0 kg/m2, 23.0-24.9, 25.0-26.9, 27.0-29.9, ≥30.0) and coffee intake (almost never, 1-4 days/week, almost every day).

ALT, alanine aminotransferase; **neither anti-HCV nor HBsAg.

FIGURE 1.

Cumulative incidence of hepatocellular carcinoma (HCC) among hepatitis virus-positive subjects by serum alanine aminotransferase (ALT) level.



Cumulative incidence of HCC at 10 years was 1.1% for subjects with ALT < 30 IU/L, but 11.0% and 27.2% for those with ALT 30-69 IU/L and ALT ≥70 IU/L, respectively. Elevated ALT levels showed a statistically significant association with the risk of HCC compared to normal ALT levels (ALT < 30 IU/L) after adjustment for age, sex, study area, smoking status, ethanol intake, body mass index, and coffee intake.

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Short Communication

Liver cancer risk, coffee, and hepatitis C virus infection: a nested case–control study in Japan

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We examined hepatocellular carcinoma mortality in relation to coffee consumption and anti-hepatitis C virus (HCV) antibody seropositivity in a nested case–control study involving 96 cases. The multivariate-adjusted odds ratios (95% confidence interval) for daily coffee drinkers *vs* non-drinkers were 0.49 (0.25–0.96), 0.31 (0.11–0.85), and 0.75 (0.29–1.92) in all cases, in HCV-positive and in HCV-negative individuals, respectively.

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Keywords: coffee; hepatocellular carcinoma; hepatitis C virus; cohort study; nested case–control study

The inverse associations between coffee consumption and the risk of hepatocellular carcinoma (HCC) have recently been reported not only from case–control studies (Gallus *et al*, 2002; Gelatti *et al*, 2005; Ohfuji *et al*, 2006; Montella *et al*, 2007; Tanaka *et al*, 2007) but also from Japanese cohort studies (Inoue *et al*, 2005; Kurozawa *et al*, 2005; Shimazu *et al*, 2005). Cohort studies are superior to case–control studies in avoiding recall and selection bias (Ohfuji *et al*, 2006). Previous prospective studies (Inoue *et al*, 2005; Kurozawa *et al*, 2005; Shimazu *et al*, 2005), however, did not consider the infection status of hepatitis C virus (HCV) at baseline. As HCV is the major cause of HCC in Japan and certain other countries (Heathcote, 2004), it would be important if protective factors against HCC could be found among the HCV-positive population. We therefore examined the relation of coffee use to risk of death from HCC by HCV infection status in a case–control study nested in a large cohort study in Japan.

MATERIALS AND METHODS

We carried out a nested case–control study as a part of the Japan Collaborative Cohort Study for Evaluation of Cancer Risk Sponsored by the Ministry of Education, Culture, Sports, Science and Technology of Japan (Mobusho), details of which are described elsewhere (Tamakoshi *et al*, 2005). It involved 110 792 individuals, aged 40–79 years at baseline, from 45 areas throughout Japan. A self-administered questionnaire on lifestyle and medical factors was distributed in 1988–1990 covering habitual coffee consumption, with possible responses including 'scarcely any', '1–2 cups per month', '1–2 cups per week', '3–4 cups per week', and 'almost every day'. Those who answered 'almost every day' were asked to report the number of cups consumed per day. The questionnaire was validated using four 3-day dietary records as a reference; the Spearman correlation coefficient was 0.79 (Iso *et al*, 2006).

In addition, those participants who underwent health-screening checks sponsored by municipalities were asked to donate blood samples at baseline and eventually, 39 242 subjects in 37 study areas did so (Tamakoshi *et al*, 2005), these being stored at −80°C until analysed. Informed consent was obtained individually from subjects, except in certain areas in which it was provided at the group level after details had been explained to community leaders. The Ethics Committee of Kurume University School of Medicine approved this study.

We used population registries in the municipalities to determine the vital and residential status of the subjects. Causes of death were confirmed by review of death certificates with permission from the Ministry of Internal Affairs and Communications. Cases eligible for the present study consisted of those who died of HCC, ICD-10 coded C22.0.

During follow-up through the end of 1999, 106 eligible cases were identified among the participants with serum samples, from which two were excluded with insufficient samples and eight without information on coffee consumption. Of the remaining 96 cases, 60 (62.5%) were positive for HCV Ab. As potential controls, sera of 11 513 subjects from the same geographical areas as the cases were also screened for HCV Ab. After excluding those with missing data on coffee drinking, we found 912 HCV-Ab-positive subjects (8.2%) and 10 175 HCV-Ab-negative ones. From these, we chose as many controls per case as possible, matching for age (same 5-year strata), sex, and HCV-Ab seropositivity, selecting 420

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HCV-Ab-positive controls (seven controls per case) and 3024 HCV-Ab-negative ones (84 controls per case).

Statistical analysis

Study participants were categorised into three groups by coffee consumption, that is, $\geqslant 1$ cup day^{-1}, <1 cup day^{-1} ('1–2 cups month^{-1}', '1–2 cups week^{-1}', or '3–4 cups week^{-1}'), and non-drinkers. Daily drinkers could not be further subdivided because of their small numbers. Odds ratios (OR) and the 95% confidence intervals (CI) by HCV-Ab positivity were estimated considering the matching using conditional logistic models (Breslow and Day, 1980). Multivariate-adjusted OR were also computed after adjustment for area, smoking and drinking habits, and history of diabetes mellitus and liver diseases. For alcohol drinking, subjects were categorised into never drinkers, former drinkers, or current drinkers who consumed <2 or $\geqslant 2$ Japanese drinks per day (one Japanese drink is equivalent to 23 g of ethanol) in this analysis. The linear trend in HCC risk was tested by treating the coffee consumption category as an ordinal variable. The heterogeneity in the association of coffee drinking by HCV status was statistically tested by incorporating a multiplicative interaction term between HCV status and the coffee consumption category in the model. Missing values for each covariate were treated as an additional category in the variable and were included in the model. All *P*-values were two-sided, and all the analyses were carried out using the Statistical Analysis System version 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Cases and controls were well matched on age and sex in both the HCV-positive and -negative groups. The mean ages $\pm$ s.d. were 62.9 ± 6.6, 62.4 ± 6.2, 63.6 ± 7.5, and 63.4 ± 7.3 years in the HCV-positive cases and controls and the HCV-negative cases and controls, respectively. Women accounted for 35.0% of both the HCV-positive cases and controls, and 36.1% of the HCV-negative cases and controls. Case subjects were more likely to currently smoke than controls in the HCV-positive group (56.6 *vs* 35.8%). Former drinkers and a history of diabetes mellitus and liver diseases were much more common in cases than in controls. In the HCV-positive cases, the proportions of former drinkers and those with diabetes and liver diseases were 28.6, 15.0, and 56.7%, respectively, against 6.6, 5.0, and 20.7% in the controls. The corresponding figures were 14.3, 13.9, and 27.8% in the HCV-negative cases and 4.4, 4.5, and 4.4% in the controls.

Drinking one or more cups of coffee per day was inversely associated with HCC mortality among all subjects (Table 1: multivariate-adjusted OR (OR2), 0.49; 95% CI, 0.25–0.96) and the anti-HCV-positive group (OR2, 0.31; 95% CI, 0.11–0.85). Although daily coffee drinkers in the HCV-negative group showed OR below unity, they did not reach statistical significance. The heterogeneity in the association of coffee drinking by HCV status was also not significant in the multivariate model ($P = 0.61$).

DISCUSSION

Coffee drinking was significantly associated with a decreased risk of death from HCC in all subjects and those infected with HCV. Our results from this prospective cohort study support the findings in some (Gelatti *et al*, 2005; Ohfuji *et al*, 2006), although not all (Montella *et al*, 2007), case–control studies that suggested a protective effect of coffee among HCV-positive individuals. Some patients with hepatitis or liver cirrhosis, however, may have decreased coffee consumption at their physician's advice or due to impaired caffeine metabolism in the liver (Hasegawa *et al*, 1989). Observational studies among subjects without active hepatitis or intervention

Table 1 OR and 95% CI for HCC mortality according to coffee consumption by anti-HCV-antibody seropositivity

Coffee consumption	All subjects					Anti-HCV-positive group					Anti-HCV-negative group				
	No. of cases	No. of controls	OR1[a]	95%CI	OR2[b]	95%CI	No. of cases	No. of controls	OR1[c]	95%CI	OR2[b]	95%CI	No. of cases	No. of controls	OR1[c]	95%CI	OR2[b]	95%CI
Non-drinkers	44	1163	1.00		1.00		28	147	1.00		1.00		16	1016	1.00		1.00	
<1 cup day^{-1}	34	1266	0.72	0.45–1.15	0.77	0.45–1.32	23	153	0.79	0.44–1.42	0.91	0.41–2.04	11	1113	0.62	0.29–1.35	0.65	0.29–1.46
$\geqslant 1$ cup day^{-1}	18	1015	0.49	0.28–0.86	0.49	0.25–0.96	9	120	0.39	0.18–0.87	0.31	0.11–0.85	9	895	0.63	0.28–1.45	0.75	0.29–1.92
P-value for trend				0.012		0.038				0.022		0.031				0.24		0.45

CI, confidence intervals; OR, odds ratios. [a]Considered for age, sex, and anti-HCV-antibody seropositivity using a conditional logistic model. [b]Further adjusted for area, smoking and drinking habits, and history of diabetes mellitus and liver diseases. [c]Considered for age and sex using a conditional logistic model.

Epidemiology

studies will further clarify the role of coffee in the possible prevention of HCV-related HCC. Further, because a nonsignificant inverse association was found between coffee consumption and HCC risk in HCV-negative individuals in the present study, investigations with more HCV-negative HCC cases are also warranted.

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Appendix A

Japan Collaborative Cohort Study Group The present investigators involved, with the co-authorship of this paper, in the JACC Study and their affiliations are as follows: Dr Akiko Tamakoshi (present chairman of the study group), Nagoya University Graduate School of Medicine; Dr Mitsuru Mori, Sapporo Medical University School of Medicine; Dr Yutaka Motohashi, Akita University School of Medicine; Dr Ichiro Tsuji, Tohoku University Graduate School of Medicine; Dr Yosikazu Nakamura, Jichi Medical School; Dr Hiroyasu Iso, Graduate School of Medicine, Osaka University; Dr Haruo Mikami, Chiba Cancer Center; Dr Yutaka Inaba, Juntendo University School of Medicine; Dr Yoshiharu Hoshiyama, University of Human Arts and Sciences; Dr Hiroshi Suzuki, Niigata University School of Medicine; Dr Hiroyuki Shimizu, Gifu University School of Medicine; Dr Hideaki Toyoshima and Dr Kenji Wakai, Nagoya University Graduate School of Medicine; Dr Shinkan Tokudome, Nagoya City University Graduate School of Medical Sciences; Dr Yoshinori Ito, Fujita Health University School of Health Sciences; Dr Shuji Hashimoto, Fujita Health University School of Medicine; Dr Shogo Kikuchi, Aichi Medical University School of Medicine; Dr Akio Koizumi, Graduate School of Medicine and Faculty of Medicine, Kyoto University; Dr Takashi Kawamura, Kyoto University Center for Student Health; Dr Yoshiyuki Watanabe, Kyoto Prefectural University of Medicine, Graduate School of Medical Science; Dr Tsuneharu Miki, Graduate School of Medical Science, Kyoto Prefectural University of Medicine; Dr Chigusa Date, Faculty of Human Life and Environment, Nara Women's University; Dr Kiyomi Sakata, Wakayama Medical University; Dr Youichi Kurozawa, Tottori University Faculty of Medicine; Dr Norihiko Hayakawa, Research Institute for Radiation Biology and Medicine, Hiroshima University; Dr Takesumi Yoshimura, Fukuoka Institute of Health and Environmental Sciences; Dr Akira Shibata, Kurume University School of Medicine; Dr Naoyuki Okamoto, Kanagawa Cancer Center; Dr Hideo Shio, Moriyama Municipal Hospital; Dr Yoshiyuki Ohno, Asahi Rosai Hospital; Dr Tomoyuki Kitagawa, Cancer Institute of the Japanese Foundation for Cancer Research; Dr Toshio Kuroki, Gifu University; and Dr Kazuo Tajima, Aichi Cancer Center Research Institute.

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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

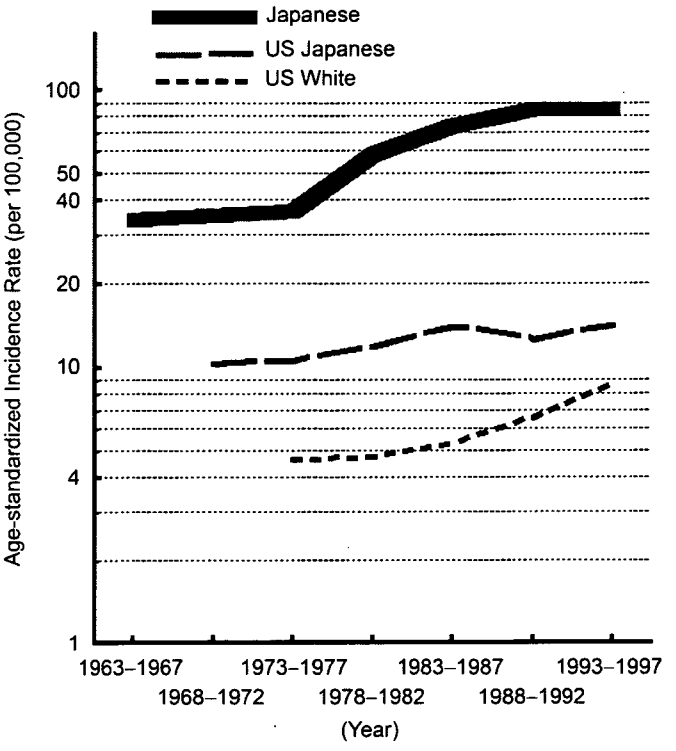

Figure 1. Trends in age-standardized incidence rates of liver cancer in Japanese males, Japanese-American (US Japanese) males and US white males.

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).

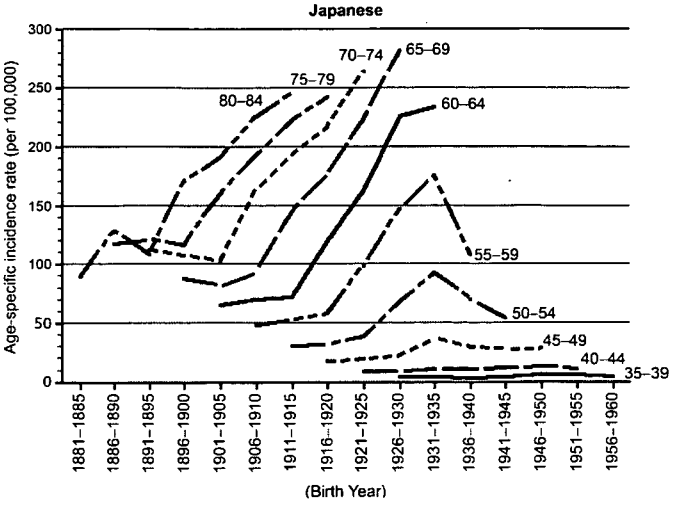

Figure 2. Age-specific incidence rates of liver cancer according to year of birth from 1881 to 1960 in Japanese males in Japan.

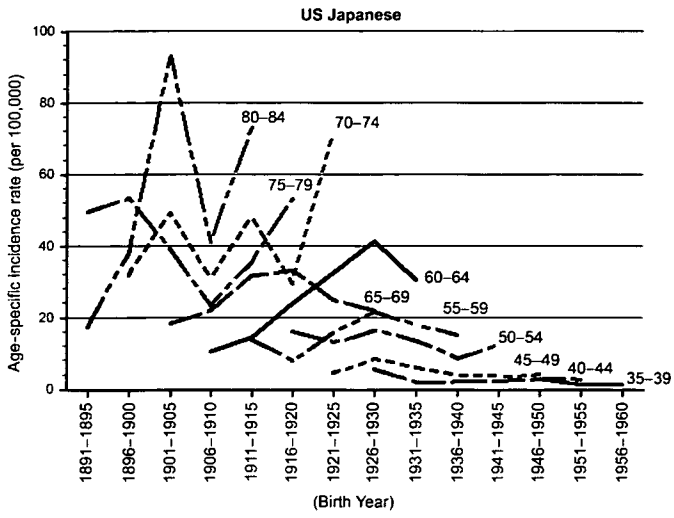

Figure 3. Age-specific incidence rates of liver cancer according to year of birth from 1891 to 1960 in Japanese-American males (US Japanese).

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.

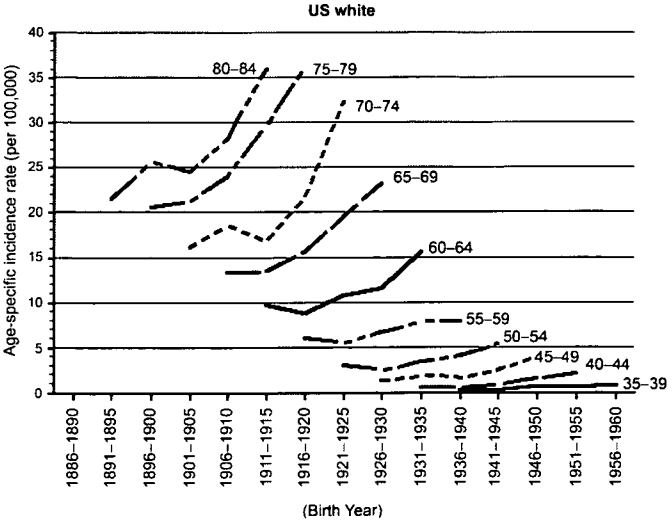

Figure 4. Age-specific incidence rates of liver cancer according to year of birth from 1891 to 1960 in US white males.

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Conflict of interest statement

None declared.

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Case–control study on cigarette smoking and the risk of hepatocellular carcinoma among Japanese

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Emerging epidemiologic data suggest that cigarette smoking may increase the risk of hepatocellular carcinoma (HCC), yet considerable controversies (e.g. inconsistent dose–response relationships) still exist with this association. We examined whether smoking was associated with HCC risk in a case–control study including 209 incident HCC cases and two different control groups (256 hospital controls and 381 patients with chronic liver disease [CLD] without HCC). Comparison of HCC cases with CLD patients, but not with hospital controls, demonstrated a significantly increased risk of HCC for current smokers. After adjustment for sex, age, heavy drinking history and hepatitis virus markers, odds ratios (and 95% confidence intervals) for former and current smokers relative to never smokers were 1.0 (0.6–1.7) and 2.5 (1.4–4.6), respectively, against CLD patients, as compared with 0.8 (0.3–2.3) and 1.8 (0.6–5.1), respectively, against hospital controls. In terms of pack-years during lifetime, dose–response relationship was not evident against either control group (*P* trend = 0.43), but it became clearer for more recent cigarette use among CLD patients. For example, regarding cumulative cigarette consumption during the last 5 years, adjusted odds ratios (and 95% confidence intervals) for 1–4 and 5+ pack-years relative to no use were 1.9 (1.1–3.6) and 2.8 (1.5–5.2) (*P* trend = 0.003), respectively. These results suggest that cigarette smoking may play a crucial role in the late stage of HCC development and that CLD patients may benefit from their earliest smoking cessation. (*Cancer Sci* 2008; 99: 93–97)

Chronic infections with hepatitis C and B viruses are two major causative factors for hepatocellular carcinoma (HCC) in Japan, where more than 90% of HCC occurrences are attributable to at least either infection.[1,2] However, there is considerable experimental and epidemiologic evidence indicating that HCC development is a multistage process and is influenced by other environmental and genetic factors, and tobacco use has been suspected as one such candidate.[3]

Recently, the International Agency for Research on Cancer classified liver cancer as a tobacco-related malignancy.[4] However, the following issues remain to be resolved. First, the dose–response relationship between smoking and HCC risk has been unclear in most epidemiologic studies (particularly, case–control studies).[5] Second, possible confounding by hepatitis virus infection has not been considered in most studies (especially, cohort studies). Third, potential virus and smoking interactions have not fully been explored. For example, initial case–control studies suggested an increased risk of HCC among smokers that are seronegative for hepatitis B surface antigen (HBsAg),[6,7] whereas a recent nested case–control study revealed an elevated risk among smokers seropositive for antibody to hepatitis C virus (anti-HCV).[8] In addition, study populations have substantially been heterogeneous (e.g. almost healthy individuals, hepatitis virus carriers, or patients with chronic liver disease [CLD]), making the target population of possible smoking intervention indefinite.

In an attempt to address the above issues, we conducted a case–control study of HCC including two different control groups (hospital controls and patients with CLD without HCC); the former represents a conventional control group when the natural history of disease is unknown, and the latter was selected based on the clinically established finding that the majority of HCC patients, at least in Japan, have pre-existing CLD.[1]

Materials and Methods

Subjects. The details of the study subjects and methods have been described elsewhere.[9] In brief, all study subjects were restricted to being residents of Saga prefecture, Japan, who were 40–79 years old at the time of identification. During a 3-year period between April 2001 and March 2004, we identified 226 incident cases with HCC from among in- or outpatients of two main hospitals in Saga City (Saga Medical School Hospital and Saga Prefectural Hospital), of whom 209 (92%) agreed to participate. Confirmation of their HCC diagnosis was based on biopsy (n = 59), angiography (n = 123), or other imaging methods (n = 27). Of the 209 cases, 198 (95%) had pre-existing CLD (cirrhosis 167, chronic hepatitis 31).

Hospital controls were first-time visitors at the general outpatient clinic of Saga Medical School Hospital between May 2001 and April 2003, who had no evidence of HCC. From among consecutive visitors, research nurses identified eligible controls based on the following order of priority: (i) men aged 50–79 years; (ii) women aged 60–79 years; (iii) men aged 40–49 years; and (iv) women aged 40–59 years. This order was determined by the sex and age distribution of deaths from liver cancer in Saga Prefecture in 1998. Of 379 eligible outpatients contacted, 275 (73%) agreed to participate. These controls had various, mostly minor, diseases (n = 190), undiagnosed symptoms (n = 49), and no definite abnormality (n = 36). We excluded 19 controls whose final diagnoses for their current visits were smoking-related diseases,[10] (cancer seven, coronary heart disease two, chronic obstructive pulmonary disease five, chronic pharyngitis/laryngitis two, gastric ulcer three), leaving 256 controls for data analysis.

Patients with CLD without HCC were out- or inpatients of the two hospitals between September 2001 and March 2004. Patients with special types of CLD (primary and secondary biliary cirrhosis, autoimmune hepatitis, and liver disease due to parasitosis, congestive heart failure, or metabolic disorders) were excluded. Of 397 eligible patients contacted, 381 (96%) agreed to participate. These CLD patients had chronic hepatitis (n = 298; 266 with hepatitis C alone, 20 with hepatitis B alone,

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