cholesterol category. The mean age in each TC group was similar in men although analysis of covariance showed statistical significance. For women, there was a trend of increasing age with increasing cholesterol levels. There were significant differences in the mean values for albumin and BMI, with these being greatest in the higher cholesterol group in both genders. There were also significant differences in the prevalence of hypertension in both genders and in the prevalence of diabetes in women. In men, the highest TC group had the highest prevalence of diabetes and current drinker; however, they did not reach statistical significance by chi-square test because of small sample size of the highest TC group. The lowest TC group had the highest prevalence of smoking in men.

The total person-years studied were 159,293 with a mean follow-up period of 17.3 years. During the follow-up period there were 1841 deaths (992 males and 849 females). Of these, 36% ($N=666$) were due to cardiovascular disease that included 128 coronary heart disease deaths and 306 stroke deaths (intra-cerebral hemorrhage, $n=65$; cerebral infarction, $n=174$; others, $n=67$).

Among the total deaths, 30% ($n=558$) were due to cancer. The three major causes of cancer death were stomach cancer ($n=131$), lung cancer ($n=107$) and liver cancer ($n=50$), a total that represented 52% of deaths due to cancer. Of all the deaths, 34% ($n=617$) were due to non-cardiovascular or non-cancer diseases. There were 35 deaths due to non-cancer liver disease (liver cirrhosis, $n=26$), which represented approximately 5% of all-cause deaths when deaths due to liver cancer were included ($n=85$).

Table 2 shows the number of deaths and multivariable-adjusted HR for the major causes of death except for cardiovascular disease according to TC stratification. Mortality from cancer was not associated with TC levels in either gender although it was highest in the lowest TC group. The mortality from non-cancer or non-cardiovascular disease was also not associated with the TC level. We found there was a positive association between the lowest TC group and increased risk for all-cause mortality in men (HR = 1.21 (95% CI, 1.01–1.45), women (HR = 1.26; 95% CI, 0.99–1.60) and in the combined data from both genders (HR = 1.19; 95% CI, 1.03–1.37). The highest TC group also had an increased risk for all-cause mortality in men (HR = 1.44; 95% CI, 0.90–2.31), women (HR = 1.24; 95% CI, 0.90–1.71) and in the combined data from both genders (HR = 1.36; 95% CI, 1.05–1.77).

Table 3 shows the number of deaths and multivariable-adjusted HRs for cardiovascular and liver disease. Mortality from cardiovascular disease was the highest in the highest TC group in both genders, with significantly higher HR in

Table 2
The number of deaths and multivariable-adjusted HRs (95% CIs) for cancer, non-cardiovascular, non-cancer and all-cause mortality; according to serum total cholesterol level in a 17.3-year follow-up study, NIPPON DATA80

Baseline serum total cholesterol level (stratum mean), mmol/L	No. of persons	Person-years	Cancer		Non-cardiovascular, non-cancer		All-cause	
			No. of deaths	HR (95% CI)	No. of deaths	HR (95% CI)	No. of deaths	HR (95% CI)
Men								
<4.14 (3.74)	851	13768	92	1.22 (0.90, 1.64)	90	1.28 (0.94, 1.74)	259	1.21 (1.01, 1.45)
4.14–4.65 (4.39)	1000	17000	84	1.00	78	1.00	241	1.00
4.66–5.17 (4.91)	937	16057	74	1.08 (0.79, 1.47)	75	1.26 (0.92, 1.74)	223	1.18 (0.98, 1.42)
5.18–5.68 (5.41)	648	11113	44	1.01 (0.67, 1.46)	44	1.32 (0.90, 1.93)	140	1.25 (1.01, 1.55)
5.69–6.20 (5.90)	354	6192	22	0.88 (0.54, 1.41)	26	1.21 (0.77, 1.89)	76	1.08 (0.83, 1.41)
6.21–6.70 (6.41)	167	2872	13	1.12 (0.62, 2.03)	5	0.49 (0.20, 1.21)	34	1.02 (0.71, 1.47)
6.71– (7.30)	78	1365	6	1.20 (0.52, 2.76)	6	1.57 (0.67, 3.64)	19	1.44 (0.90, 2.31)
Women								
<4.14 (3.78)	952	16784	34	1.19 (0.76, 1.86)	42	1.34 (0.89, 2.00)	118	1.26 (0.99, 1.60)
4.14–4.65 (4.40)	1183	21011	47	1.00	56	1.00	165	1.00
4.66–5.17 (4.91)	1142	20011	53	0.96 (0.64, 1.43)	62	1.02 (0.71, 1.47)	185	0.98 (0.79, 1.21)
5.18–5.68 (5.40)	925	16155	46	0.88 (0.58, 1.33)	61	1.00 (0.69, 1.45)	171	0.92 (0.74, 1.21)
5.69–6.20 (5.91)	528	9252	23	0.68 (0.41, 1.14)	36	0.92 (0.60, 1.41)	106	0.92 (0.74, 1.14)
6.21–6.70 (6.40)	275	4751	10	0.58 (0.29, 1.16)	23	1.19 (0.73, 1.95)	54	0.88 (0.68, 1.12)
6.71– (7.20)	176	2960	10	0.88 (0.44, 1.77)	13	1.01 (0.55, 1.88)	50	1.24 (0.90, 1.71)
Men and women combined								
<4.14 (3.76)	1803	30552	126	1.21 (0.95, 1.55)	132	1.26 (0.99, 1.61)	377	1.19 (1.03, 1.37)
4.14–4.65 (4.39)	2183	38011	131	1.00	134	1.00	406	1.00
4.66–5.17 (4.91)	2079	36068	127	1.03 (0.80, 1.31)	137	1.16 (0.91, 1.48)	408	1.09 (0.95, 1.26)
5.18–5.68 (5.40)	1573	27268	90	0.96 (0.73, 1.26)	105	1.15 (0.89, 1.50)	311	1.07 (0.92, 1.25)
5.69–6.20 (5.91)	882	15444	45	0.78 (0.55, 1.10)	62	1.05 (0.77, 1.43)	182	0.98 (0.82, 1.17)
6.21–6.70 (6.40)	442	7623	23	0.89 (0.52, 1.27)	28	0.97 (0.64, 1.47)	88	0.96 (0.76, 1.22)
6.71– (7.23)	254	4325	16	1.01 (0.60, 1.72)	19	1.19 (0.73, 1.95)	69	1.36 (1.05, 1.77)

HR, hazard ratio; 95% CI, 95% confidence interval. HR was adjusted for age, serum albumin, body mass index, hypertension, diabetes, cigarette smoking category and alcohol intake category. Gender was also adjusted while a sex-combined analysis was performed.

Table 3
The number of deaths and multivariable-adjusted hazard ratios for cardiovascular and liver disease mortality according to serum total cholesterol level, NIPPON DATA80

Baseline serum total cholesterol level (stratum mean), mmol/L	No. of persons	Person-years	Cardiovascular						Liver disease	
			All		Coronary heart disease		Stroke		No. of deaths	HR (95% CI)
			No. of deaths	HR (95% CI)	No. of deaths	HR (95% CI)	No. of deaths	HR (95% CI)		
Men										
<4.14 (3.74)	851	13768	77	1.15 (0.84, 1.58)	10	1.07 (0.46, 2.51)	43	1.21 (0.78, 1.89)	32	2.74 (1.36, 5.52)
4.14–4.65 (4.39)	1000	17000	79	1.00	12	1.00	40	1.00	11	1.00
4.66–5.17 (4.91)	937	16057	74	1.24 (0.90, 1.71)	12	1.21 (0.54, 2.71)	32	1.08 (0.68, 1.73)	10	1.07 (0.45, 2.52)
5.18–5.68 (5.41)	648	11113	52	1.51 (1.05, 2.17)	12	2.11 (0.92, 4.84)	27	1.53 (0.92, 2.54)	3	0.59 (0.16, 2.15)
5.69–6.20 (5.90)	354	6192	28	1.19 (0.77, 1.85)	9	2.17 (0.89, 5.25)	11	0.97 (0.49, 1.90)	2	0.59 (0.13, 2.68)
6.21–6.70 (6.41)	167	2872	16	1.44 (0.83, 2.48)	7	3.74 (1.44, 9.76)	3	0.53 (0.16, 1.73)	0	–
6.71– (7.30)	78	1365	7	1.68 (0.77, 3.69)	3	3.77 (1.02, 13.9)	2	0.97 (0.23, 4.07)	0	–
Women										
<4.14 (3.78)	952	16784	42	1.21 (0.81, 1.79)	7	0.94 (0.36, 2.45)	17	1.05 (0.58, 1.92)	9	3.13 (1.04, 9.42)
4.14–4.65 (4.40)	1183	21011	62	1.00	12	1.00	30	1.00	5	1.00
4.66–5.17 (4.91)	1142	20011	70	1.00 (0.71, 1.42)	10	0.73 (0.31, 1.71)	28	0.77 (0.46, 1.30)	5	0.90 (0.26, 3.12)
5.18–5.68 (5.40)	925	16155	64	0.91 (0.64, 1.30)	12	0.92 (0.41, 2.08)	31	0.80 (0.48, 1.33)	2	0.35 (0.07, 1.83)
5.69–6.20 (5.91)	528	9252	47	1.03 (0.70, 1.52)	10	1.14 (0.48, 2.70)	22	0.85 (0.58, 1.49)	4	1.07 (0.28, 4.10)
6.21–6.70 (6.40)	275	4751	21	0.95 (0.58, 1.57)	3	0.74 (0.20, 2.68)	9	0.70 (0.33, 1.50)	2	1.25 (0.24, 6.61)
6.71– (7.20)	176	2960	27	1.84 (1.15, 2.93)	9	3.33 (1.35, 8.18)	11	1.31 (0.65, 2.67)	0	–
Men and women combined										
<4.14 (3.76)	1803	30552	119	1.11 (0.86, 1.42)	17	0.91 (0.49, 1.71)	60	1.14 (0.80, 1.62)	41	3.03 (1.70, 5.43)
4.14–4.65 (4.39)	2183	38011	141	1.00	24	1.00	70	1.00	16	1.00
4.66–5.17 (4.91)	2079	36068	144	1.12 (0.89, 1.42)	22	1.01 (0.56, 1.81)	60	0.92 (0.65, 1.30)	15	1.02 (0.50, 2.06)
5.18–5.68 (5.40)	1573	27268	116	1.13 (0.88, 1.46)	24	1.42 (0.79, 2.56)	58	1.06 (0.74, 1.52)	5	0.53 (0.19, 1.45)
5.69–6.20 (5.91)	882	15444	75	1.12 (0.84, 1.49)	19	1.67 (0.90, 3.11)	33	0.94 (0.61, 1.43)	6	0.97 (0.38, 2.51)
6.21–6.70 (6.40)	442	7623	37	1.14 (0.79, 1.65)	10	1.84 (0.86, 3.91)	12	0.69 (0.37, 1.29)	2	0.62 (0.14, 2.71)
6.71– (7.23)	254	4325	34	1.90 (1.29, 2.79)	12	3.81 (1.84, 7.91)	13	1.38 (0.75, 2.54)	0	–

HR, hazard ratio; 95% CI, 95% confidence interval. HR was adjusted for age, serum albumin, body mass index, hypertension, diabetes, cigarette smoking category and alcohol intake category. Gender was also adjusted while a sex-combined analysis was performed.

women and when the gender data were combined. The mortality for coronary heart disease suggested a positive graded relationship with TC when the gender data were combined. For men, the HR in the second highest TC group was 3.74 (95% CI, 1.44–9.76), while the HR in the highest TC group was 3.77 (95% CI, 1.02–13.9). For women, although a graded relationship was not observed, the highest TC group had a significantly increased risk of death from coronary heart disease. Mortality from stroke was not associated with TC levels in either gender. The mortality from cerebral hemorrhage was the highest in the lowest TC group in men (HR = 3.77; 95% CI, 1.35–10.5), while the mortality from cerebral infarction was not associated with TC levels in either gender (data not shown). The lowest TC group was positively associated with an increased risk for death from liver disease in men, women and the combined gender data.

The association between TC group and cause-specific mortality after excluding deaths within the first 5 years of follow-up was essentially similar to those shown in Table 2 and 3 (data not shown).

When all-cause mortality was calculated after exclusion of liver disease (Fig. 1), the increased HR in the lowest TC group disappeared (HR = 1.10, 95% CI, 0.95–1.28, for combined data of men and women). In contrast, in the combined data, the positive relationship between the highest TC group and all-cause mortality remained significant with an increase in HR (HR = 1.41, 95% CI, 1.12–1.38). After further excluding deaths within the first 5 years of follow-up, the magnitude of the HR in the lowest TC group decreased (HR = 1.05; 95% CI, 0.89–1.24), whereas the HR in the highest TC group increased even further (HR = 1.48; 95% CI, 1.12–1.96). Repeating these analyses on data grouped according to gender showed nearly identical results (data not shown).

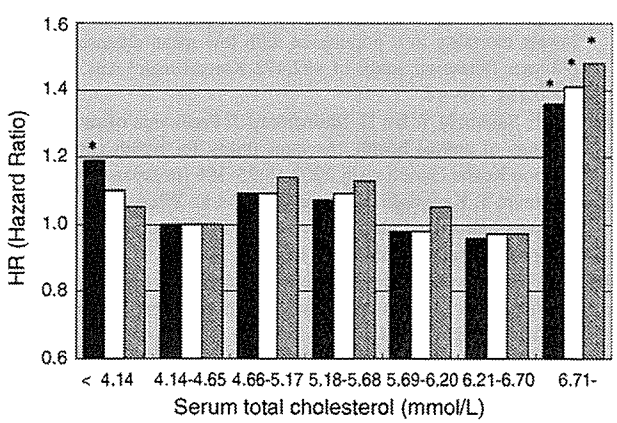


Fig. 1. Multivariable-adjusted hazard ratios (HR) for all-cause mortality grouped according to serum total cholesterol after adjustment for gender, age, serum albumin, body mass index, hypertension, diabetes, cigarette smoking and alcohol intake. Black bars show HR for all-cause mortality among all participants. White bars show HR for all-cause mortality after exclusion of deaths due to liver disease during the entire follow-up period. Hatched bars show HR for all-cause mortality after further exclusion of all-cause deaths within the first 5 years of follow-up. ($^{*}P < 0.05$).

In addition to stratifying participants based on clinical TC cut-off values, we also grouped all participants according to the quintile of serum TC. When we used the second quintile (4.16–4.59 mmol/L) as a reference group, we observed a significant increase in all-cause (HR = 1.21, 95% CI, 1.05–1.40) and liver disease mortality (HR = 2.91, 95% CI, 1.58–5.35) in the lowest TC quintile (<4.16 mmol/L, 161 mg/dl). This was similar to the results obtained in the lowest TC group (<4.14 mmol/L, 160 mg/dl) when clinical TC cut-off values were used to group the participants. However, the highest TC quintile (≥5.61 mmol/L, 217 mg/dl) was not associated with an increase in all-cause or any cause-specific mortality except for coronary heart disease (HR = 2.01; 95% CI, 1.16–3.51). When HR for all-cause mortality was calculated after exclusion of liver disease, the increased HR in the lowest TC quintile disappeared. Gender-specific analysis also showed similar results (data not shown).

4. Discussion

This 17.3-year cohort study of the Japanese population showed a positive association between the lowest (<4.14 mmol/L) or highest (≥6.71 mmol/L) TC levels and an increased risk of all-cause mortality. However, the relationship between low TC and all-cause mortality disappeared when deaths due to liver disease were excluded, with only the highest TC group showing a significant increase in all-cause mortality. The strengths of the present study were a high response rate in the baseline survey at which time several biological markers were measured and a long duration of follow-up of randomly selected subjects. The large number of person-years in the study also allowed us to use multivariable analysis to examine the relationship between high serum TC and all-cause mortality using cut-off points set higher than previous studies in Asian populations [5–8].

The prevalence of hepatitis C virus (HCV) infection in Japanese residents born before World War II has been estimated to be approximately 5–7%, [21,22] a level considerably higher than in Western countries [23–25]. Because the majority of our study participants belonged to the pre-World War II generation, the prevalence of HCV infection in our study cohort would be expected to be relatively high. It has recently been revealed that a low serum cholesterol level in individuals with chronic HCV infection is a predictor of both liver fibrosis [26] and liver cancer [9] Another study indicated that subjects with genotype 1b hepatitis C viral infection (the most common genotype of the HCV in Japan) had significantly lower serum cholesterol levels than those infected with hepatitis B virus or genotype 2a HCV, even in the precirrhosis period [27].

These results suggest that hypocholesterolemia in Japan is associated with the prevalence of persistent infection with HCV. Low serum TC may be a response to liver dysfunction caused by progressive fibrotic changes rather than a primary cause of liver fibrosis. We believe these findings may partly

explain the relationship we observed between low TC and all-cause death in Japan. An epidemic of HCV infection occurred mainly in the pre-war generation of the Japanese population as a result of commercial blood transfusions carried out during the two decades after World War II [28]. This interpretation is also supported by our previous finding that a history of earlier blood transfusion was associated with hypocholesterolemia in a rural Japanese community [29].

Most cohort studies in non-Western populations have failed to demonstrate a positive relationship between high serum TC and all-cause mortality [5–8]. We found participants with a TC level ≥6.71 mmol/L, a higher level than US criteria (≥6.21 mmol/L), had an increased risk of all-cause mortality, mainly as a consequence of coronary heart disease. It was reported in the 1960 and 1970s that a cohort of Japanese people born before World War II had markedly lower serum TC levels [30]. Although subjects may have had elevated TC at the baseline survey, it was not possible to determine the duration of elevated TC levels prior to the baseline measurement. A "lag time" between exposure to high serum TC levels and the occurrence of coronary heart disease may provide an explanation of the higher cut-off value for TC in the Japanese population. Accordingly, the effect of high serum TC on both coronary heart disease and all-cause mortality may be attenuated.

Similar to previous studies in Japan, we found no positive relationship between cholesterol levels and stroke [31,32]. In fact, we observed the highest mortality for cerebral hemorrhage in the lowest TC group in men. Some studies, [8,31] but not all, [33] reported that hypocholesterolemia was associated with a higher risk of cerebral hemorrhage. However, we were unable to determine if this was a causal relationship.

The present study had some limitations. First, a single cholesterol measurement at the baseline survey may have underestimated the relationship between TC and mortality due to regression dilution effects [34]. Second, we divided the population into seven TC groups with an unbalanced number of participants based on the combination of clinical criteria because the prevalence of hypercholesterolemia (6.71 mmol/L or greater) was very small in this population. Third, we did not measure antibodies against the hepatitis C virus, and non-fasting blood collection might have affected serum glucose levels. Fourth, the change in the ICD coding from version 9 to version 10 during follow-up period may have been a confounding factor in the diagnosis of the cause of death. However, ICD coding was done by specialists in the Ministry of Health and Welfare, not by researchers. Furthermore, mortality from stroke and cancer is known to be accurately reported on death certificates in Japan [35,36]. Although underestimation of coronary heart disease deaths during the use of ICD 9 is possible, [37] this should make it more difficult to show a positive association between high TC and death due to coronary heart disease. Thus, the positive association that we observed may be conservative.

In conclusion, as in the Western populations, we showed that high serum levels of TC in the Japanese general population were positively associated with all-cause mortality, although the cut-off point appeared to be higher in Japanese residents than Westerners. Furthermore, the relationship between hypocholesterolemia and liver diseases, such as liver cancer, liver cirrhosis and hepatitis, may increase all-cause mortality in hypocholesterolemic Japanese residents.

Acknowledgements

This study was supported by a research grant from the Ministry of Health, Labor and Welfare, 7A-2, H11-13-Chouju-046, H14-16-Chouju-003 and H17-18-Chouju-012. The authors thank all the staff of public health centers that cooperated with our study. The authors thank Masakazu Nakamura, Lipid Reference Laboratory, Osaka Medical Center for Health Science and Promotion, for his excellent control for lipid measurement.

References

[1] Kannel WB, Neaton JD, Wentworth D, et al. Overall and coronary heart disease mortality rates in relation to major risk factors in 325,348 men screened for the MRFIT. Multiple risk factor intervention trial. Am Heart J 1986;112:825–36.
[2] Stamler J, Daviglus ML, Garside DB, et al. Relationship between baseline serum cholesterol levels in three large cohorts of younger men to long-term coronary, cardiovascular, and all-cause mortality and longevity. JAMA 2000;284:311–8.
[3] Smith GD, Shipley MJ, Marmot MG, Rose G. Plasma cholesterol concentration and mortality. The Whitehall Study. JAMA 1992;267:70–6.
[4] Jacobs D, Blackburn H, Higgins M, et al. Report of the conference on low blood cholesterol: mortality associations. Circulation 1992;86:1046–60.
[5] Iso H, Naito Y, Kitamura A, et al. Serum cholesterol and mortality in a Japanese population. J Clin Epidemiol 1994;47:961–9.
[6] Song YM, Sung J, Kim JS. Which cholesterol level is related to the lowest mortality in a population with low mean cholesterol level: a 6.4-year follow-up study of 482,472 Korean men? Am J Epidemiol 2000;151:739–47.
[7] Irie F, Sairenchi T, Iso H, Shimamoto T. Prediction of mortality from findings of annual health checkups utility for health care programs. Nippon Koshu Eisei Zasshi 2001;48:95–108 [in Japanese].
[8] Okamura T, Kadowaki T, Hayakawa T, et al. What cause of mortality can we predict by cholesterol screening in the Japanese general population? J Intern Med 2003;253:169–80.
[9] Tanaka H, Tsukuma H, Yamano H, Oshima A, Shibata H. Prospective study on the risk of hepatocellular carcinoma among hepatitis C virus-positive blood donors focusing on demographic factors, alanine aminotransferase level at donation and interaction with hepatitis B virus. Int J Cancer 2004;112:1075–80.
[10] Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB. Cancer Incidence in Five Continents, vol. VIII. IARC Scientific Publications No. 155, Lyon: International Agency for Research on Cancer, 2002, p. 564–66.
[11] Okamura T, Hayakawa T, Kadowaki T, et al. Resting heart rate and cause-specific death in a 16.5-year cohort study of the Japanese general population. Am Heart J 2004;147:1024–32.
[12] Ueshima H, Choudhury SR, Okayama A, et al. Cigarette smoking as a risk factor for stroke death in Japan: NIPPON DATA80. Stroke 2004;35:1836–41.

[13] Okamura T, Hayakawa T, Kadowaki T, et al. A combination of serum low albumin and above-average cholesterol level was associated with excess mortality. J Clin Epidemiol 2004;57:1188–95.
[14] Nakamura Y, Okamura T, Tamaki S, et al. Egg consumption, serum cholesterol, and cause-specific and all-cause mortality: the National Integrated Project for Prospective Observation of Non-communicable Disease and Its Trends in the Aged, 1980 (NIPPON DATA80). Am J Clin Nutr 2004;80:58–63.
[15] Miyamatsu N, Kadowaki T, Okamura T, et al. Different effects of blood pressure on mortality from stroke subtypes depending on BMI levels: a 19-year cohort study in the Japanese general population, NIPPON DATA80. J Hum Hypertens 2005;19:285–91.
[16] Nakamura Y, Ueshima H, Okamura T, et al. Association between fish consumption and all-cause and cause-specific mortality in Japan: NIPPON DATA80, 1980–1999. Am J Med 2005;118:239–45.
[17] Nakamura M, Sato S, Shimamoto T. Current status of CDC lipid standardization and international needs for standardization in epidemiological studies and clinical trials in Japan. J Atheroscler Thromb 2004;11:35–7.
[18] Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). JAMA 2001;285:2486–97.
[19] Japan Atherosclerosis Society Guidelines for Diagnosis and Treatment of Atherosclerotic Cardiovascular Diseases. Japan Atherosclerosis Society, 2002 [in Japanese].
[20] The Ministry of Health and Welfare Manual for Health Examination under Health and Medical Service Law for the Elderly. Tokyo: Nihon Ijishinpou publisher; 1994 [in Japanese].
[21] Yoshizawa H. Trends of hepatitis virus carriers. Hepatol Res 2002;24:S28–39.
[22] Tanaka H, Hiyama T, Tsukuma H, et al. Prevalence of second generation antibody to hepatitis C virus among voluntary blood donors in Osaka, Japan. Cancer Causes Control 1994;5:409–13.
[23] Murphy EL, Bryzman S, Williams AE, et al. Demographic determinants of hepatitis C virus seroprevalence among blood donors. JAMA 1996;275:995–1000.
[24] Dubois F, Desenclos JC, Mariotte N, Goudeau A. Hepatitis C in a French population-based survey, 1994: seroprevalence, frequency of viremia, genotype distribution and risk factors. The Collaborative Study Group. Hepatology 1997;25:1490–6.
[25] Amin J, Gidding H, Gilbert G, et al. Hepatitis C prevalence—a nationwide serosurvey. Commun Dis Intell 2004;28:517–21.
[26] Forns X, Ampurdanes S, Llovet JM, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology 2002;36:986–92.
[27] Moriya K, Shintani Y, Fujie H, et al. Serum lipid profile of patients with genotype 1b hepatitis C viral infection in Japan. Hepatol Res 2003;25:371–6.
[28] Yoshizawa H. Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. Oncology 2002;62:8–17.
[29] Mao X, Okamura T, Choudhury SR, et al. What unfavorable factors are associated with low serum total cholesterol in a Japanese population? J Epidemiol 2002;12:271–9.
[30] Nichaman MZ, Hamilton HB, Kagan A, et al. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: distribution of biochemical risk factors. Am J Epidemiol 1975;102:491–501.
[31] Ueshima H, Iida M, Shimamoto T, et al. Multivariable analysis of risk factors for stroke. Eight-year follow-up study of farming villages in Akita, Japan. Prev Med 1980;9:722–40.
[32] Tanizaki Y, Kiyohara Y, Kato I, et al. Incidence and risk factors for subtypes of cerebral infarction in a general population: the Hisayama study. Stroke 2000;31:2616–22.
[33] Suh I, Jee SH, Kim HC, et al. Low serum cholesterol and hemorrhagic stroke in men: Korea Medical Insurance Corporation Study. Lancet 2001;357:922–5.
[34] MacMahon S, Peto R, Cutler J, et al. Blood pressure, stroke, and coronary heart disease, Part1: prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. Lancet 1990;335:765–74.
[35] Hasuo Y, Ueda K, Kiyohara Y, et al. Accuracy of diagnosis on death certificates for underlying causes of death in a long-term autopsy-based population study in Hisayama, Japan; with special reference to cardiovascular diseases. J Clin Epidemiol 1989;42:577–84.
[36] Ron E, Carter R, Jablon S, Mabuchi K. Agreement between death certificate and autopsy diagnoses among atomic bomb survivors. Epidemiology 1994;5:48–56.
[37] Saito I, Folsom AR, Aono H, et al. Comparison of fatal coronary heart disease occurrence based on population surveys in Japan and the USA. Int J Epidemiol 2000;29:837–44.

Original Article

hOGG1 Ser326Cys Polymorphism and Risk of Hepatocellular Carcinoma among Japanese

Tatsuhiko Sakamoto,[1] Yasuki Higaki,[1] Megumi Hara,[1] Masayoshi Ichiba,[2] Mikako Horita,[1,2] Toshihiko Mizuta,[3] Yuichiro Eguchi,[3] Tsutomu Yasutake,[3] Iwata Ozaki,[3] Kyosuke Yamamoto,[3] Shingo Onohara,[4] Seiji Kawazoe,[4] Hirohisa Shigematsu,[4] Shunzo Koizumi,[5] and Keitaro Tanaka.[1]

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.
J Epidemiol 2006; 16:233-239.

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

Received May 8, 2006, and accepted June 12, 2006.
This study was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology (Grant Nos. 11670344, 13220014, 14031216, and 15390188) and Grants-in-Aid for the Research on Hepatitis and for the Third Term Comprehensive Control Research for Cancer from the Ministry of Health, Labor and Welfare, Japan.
[1] Department of Preventive Medicine, Faculty of Medicine, Saga University.
[2] Department of Social and Environmental Medicine, Faculty of Medicine, Saga University.
[3] Department of Internal Medicine, Faculty of Medicine, Saga University.
[4] Department of Internal Medicine, Saga Prefectural Hospital Koseikan.
[5] Department of General Medicine, Faculty of Medicine, Saga University.
Address for correspondence: Tatsuhiko Sakamoto, MD, Department of Preventive Medicine, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga 849-8501, Japan. (e-mail: sakamott@med.saga-u.ac.jp)

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

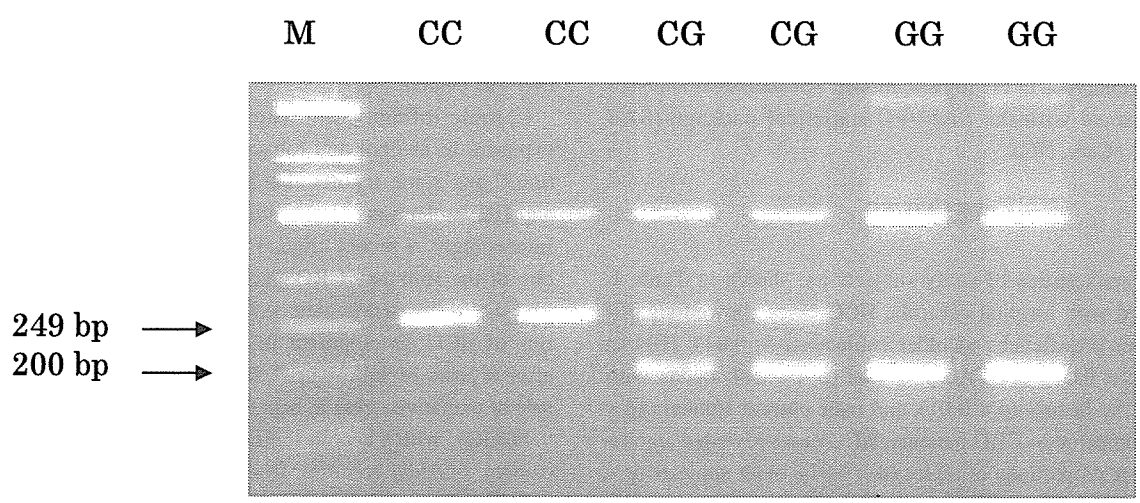
Research nurses interviewed study subjects on alcohol drinking and smoking habits using a uniform questionnaire. A history of heavy drinking was defined as having imbibed 69 g or more of ethanol per day for 10 or more years. We regarded "never smokers" as individuals who had never smoked or had smoked 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).

by a polymerase chain reaction (PCR) with confronting two-pair primers (PCR-CTPP) according to Ito et al[22] Genomic DNA (10-150 ng) was amplified in a volume of 25 μL with 0.18mM dNTPs, 12.5 pmol of each primer, 0.5 units of AmpliTaq Gold (Perkin-Elmer Corp., Foster City, CA), and 2.5 μL of 10×PCR buffer including 15 mM $MgCl_2$. The following 4 primers were used for each reaction: F1 (5'-CAGCCCAGACCCAGTGGACTC-3'), R1 (5'-TGGCTCCTGAGCATGGCGGG-3'), F2 (5'-CAGTGCCGACCTGCGCCAATG-3'), and R2 (5'-GGTAGTCACAGGGAGGCCCC-3'). PCR was conducted as follows: a 10 minute initial denature at 95℃, 30 cycles for 1 minute at 95℃, 1 minute at 64℃, and 1 minute at 72℃, and a 5 minute final extension at 72℃. PCR products were subjected to electrophoresis in 2% agarose gels and were visualized with ethidium bromide staining. The primer pair F1 and R1 produced the C allele (Ser326) band (252 bp), while F2 and R2 produced the G allele (326Cys) band (194 bp) (Figure 1). To validate the results, 10% were randomly selected for genotyping by using a PCR-restriction fragment length polymorphism analysis,[16] and the results were 100% concordant.

Statistical Analysis

Chi square tests were used for unadjusted comparisons based on frequency. The Wilcoxon's rank sum test was conducted to compare the distribution of age. Unconditional logistic regression models were used to estimate crude and adjusted odds ratios (ORs) of HCC and their 95 percent confidence intervals (CIs) for *hOGG1* genotypes by using dummy variables, with adjustment for potential confounders including sex, age category (40-49, 50-59, 60-69, and 70-79 years), heavy drinking history, smoking status (never, former, and current smokers), and HBsAg and HCVAb status. The gene-environment interaction on HCC risk was evaluated by including in the model the product terms of variables of interest (i.e., *hOGG1* genotype and heavy drinking history/smoking status), as well as main effects and covariates. Likelihood ratio tests were used to examine the overall statistical significance of a set of interaction terms. Tests of linear trend for *hOGG1* genotypes were performed by assigning an ordinal variable to the genotypes in the logistic model. All statistical analyses were performed with the SAS®/PC statistical package (SAS Institute Inc., Cary, NC).

RESULTS

Selected characteristics of study subjects are shown in Table 1. As compared with hospital controls, HCC cases showed significantly higher prevalences of older subjects ($p < 0.01$), HBsAg positives ($p < 0.01$), HCVAb positives ($p < 0.01$), males with heavy drinking history ($p < 0.01$), and male current smokers ($p = 0.03$). As compared to CLD patients, HCC cases revealed significantly greater proportions of males ($p < 0.01$), older subjects ($p < 0.01$), and males with heavy drinking history ($p < 0.01$).

The frequency of *hOGG1* genotype showed no significant difference between HCC cases and either control group ($p = 0.10$ or 0.23) (Table 1). The genotype distributions for hospital controls and CLD patients were in Hardy-Weinberg equilibrium ($p = 0.08$ and 0.14, respectively). After adjustment for sex, age, heavy drinking history, smoking, HBsAg, and HCVAb, the ORs (and 95% CIs) for the Ser/Cys, Cys/Cys, and Ser/Cys+Cys/Cys genotypes relative to the Ser/Ser genotype were estimated at 0.79 (0.35-1.79), 0.48 (0.18-1.27), and 0.68 (0.31-1.46) against hospital controls respectively,, and at 1.51 (0.96-3.37), 0.86 (0.50-1.47), and 1.25 (0.82-1.91),against CLD patients respectively.

Table 2 shows the adjusted ORs of HCC for *hOGG1* genotypes according to heavy drinking history and current smoking status. We could not detect any significant linear trend for the genotypes in any stratum. Among those without heavy drinking history, a significant risk excess for the Ser/Cys vs. Ser/Ser genotype (fully-adjusted OR = 1.82, 95% CI: 1.10-3.01) was observed between HCC cases and CLD patients, yet the risk for the Cys/Cys vs. Ser/Ser genotype was not elevated (OR = 0.90, 95% CI: 0.50-1.64). A similar tendency was observed among those without current smoking. In comparison of HCC cases with hospital controls, additional adjustment for HBsAg and HCVAb substantially altered the OR on some occasions (e.g., OR for Cys/Cys among current smokers), but with a very wide CI. No significant interaction was found between the *hOGG1* genotype and either heavy drinking history or current smoking. Although we conducted corresponding analyses based on daily amount of alcohol drinking and pack-years of smoking, the results were essentially identical (data not shown).

DISCUSSION

In the present study, we could not find any significant association between *hOGG1* Ser326Cys polymorphism and overall HCC risk. In subgroup analyses according to drinking and smoking habits, there was some risk increase for the Ser/Cys vs. Ser/Ser genotype, yet such a finding might be due to chance variation in the light of the absence of risk increase for the Cys/Cys vs. Ser/Ser genotype. In addition, no significant gene-alcohol or gene-smoking interaction was evident.

Chronic inflammation caused by hepatotropic viruses and exposure to alcohol and tobacco stimulate hepatic ROS generation,[7-9] and some reports also have demonstrated that both HCV and HBV infections could induce ROS without inflammation.[23,24] Interestingly, a recent clinical study reported that reducing iron, one of the sources of ROS generation, by phlebotomy and low iron diet decreased hepatic levels of 8-OHdG and eventually the risk of HCC development in patients with chronic hepatitis C after 6 years of follow-up.[25] These reports suggest an important role of oxidative stress in hepatocarcinogenesis.

hOGG1, which acts in the DNA base excision repair pathway, excises 8-OHdG resulting from oxidative stress. The Ser326Cys polymorphism in *hOGG1* may alter glycosylase function, and some studies showed that hOGG1 protein encoded by the 326Cys

Table 1. Selected characteristics of study subjects.

Factor	HCC cases n(%)	Hospital controls n(%)	CLD patients n(%)	P*	P†
Sex					
Male	141 (67.5)	180 (65.5)	205 (53.8)		
Female	68 (32.5)	95 (34.5)	176 (46.2)	0.64	<0.01
Age (year)					
40-49	6 (2.9)	42 (15.3)	73 (19.2)		
50-59	28 (13.4)	85 (30.9)	93 (24.4)		
60-69	76 (36.4)	86 (31.3)	136 (35.7)		
70-79	99 (47.4)	62 (22.6)	79 (9.2)	<0.01	<0.01
Median	69 yr	61 yr	61 yr	<0.01	<0.01
HBsAg					
Negative	190 (90.9)	269 (97.8)	346 (90.8)		
Positive	19 (9.1)	6 (2.2)	35 (9.2)	<0.01	0.97
HCVAb					
Negative	30 (14.4)	254 (92.4)	54 (14.2)		
Positive	179 (85.7)	21 (7.6)	327 (85.8)	<0.01	0.95
Heavy drinking history (male)					
No	95 (67.4)	158 (87.8)	170 (82.9)		
Yes	46 (32.6)	22 (12.2)	35 (17.1)	<0.01	<0.01
Heavy drinking history (female)					
No	65 (95.6)	94 (99.0)	172 (97.7)		
Yes	3 (4.4)	1 (1.1)	4 (2.3)	0.17	0.37
Smoking status (male)					
Never	24 (17.0)	50 (27.8)	54 (26.3)		
Former	51 (36.2)	67 (37.2)	76 (37.1)		
Current	66 (46.8)	63 (35.0)	75 (36.7)	0.03	0.07
Smoking status (female)					
Never	61 (89.7)	88 (92.6)	150 (85.2)		
Former	4 (5.9)	3 (3.2)	15 (8.5)		
Current	3 (4.4)	4 (4.2)	11 (6.3)	0.70	0.66
hOGG1 genotype					
Ser/Ser	56 (26.8)	73 (26.5)	105 (27.6)		
Ser/Cys	110 (52.6)	123 (44.7)	176 (46.2)		
Cys/Cys	43 (20.6)	79 (28.7)	100 (26.2)	0.10	0.23

* : Comparisons were made between HCC cases and hospital controls.

† : Comparisons were made between HCC cases and CLD patients.

HCC: hepatocellular carcinoma

CLD: chronic liver diseases

Table 2. Adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) of HCC for *hOGG1* genotypes according to heavy drinking history and current smoking status.

	HCC cases	Hospital controls	CLD patients	HCC cases vs. hospital controls		HCC cases vs. CLD patients	
	n(%)	n(%)	n(%)	OR* (95% CI)	OR† (95% CI)	OR* (95% CI)	OR† (95% CI)
Without heavy alcohol drinking history‡							
Ser/Ser	40 (25.0)	64 (25.4)	96 (28.1)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Ser/Cys	88 (55.0)	115 (45.6)	154 (45.0)	1.31 (0.77- 2.22)	0.73 (0.28- 1.93)	1.78 (1.07- 1.63)	1.82 (1.10- 3.01)
Cys/Cys	32 (20.0)	73 (29.0)	92 (26.9)	0.80 (0.43- 1.49)	0.42 (0.13- 1.28)	0.90 (0.50- 1.63)	0.90 (0.50- 1.64)
P for trend				0.51	0.13	0.84	0.86
With heavy alcohol drinking history‡							
Ser/Ser	16 (32.7)	9 (39.1)	9 (23.1)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Ser/Cys	22 (44.9)	8 (34.8)	22 (56.4)	1.56 (0.44- 5.59)	0.92 (0.17- 5.06)	0.50 (0.16- 1.50)	0.63 (0.18- 2.19)
Cys/Cys	11 (22.5)	6 (26.1)	8 (20.5)	1.05 (0.24- 4.61)	1.02 (0.10-10.06)	0.70 (0.18- 2.79)	0.84 (0.20- 3.84)
P for trend				0.87	0.99	0.54	0.81
P for interaction§				0.94	0.95	0.13	0.16
Without current smoking							
Ser/Ser	35 (25.0)	56 (26.9)	78 (26.4)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Ser/Cys	77 (55.0)	91 (43.8)	136 (46.1)	1.69 (0.95- 3.00)	1.00 (0.38- 2.59)	1.61 (0.95- 2.72)	1.65 (0.97- 2.81)
Cys/Cys	28 (20.0)	61 (29.3)	81 (27.5)	0.83 (0.42- 1.63)	0.60 (0.20- 1.84)	0.82 (0.44- 1.53)	0.86 (0.46-1.61)
P for trend				0.66	0.39	0.61	0.71
With current smoking							
Ser/Ser	21 (30.4)	17 (25.4)	27 (31.4)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Ser/Cys	33 (47.8)	32 (47.8)	40 (46.5)	0.77 (0.31- 1.92)	0.42 (0.07- 2.42)	1.04 (0.43- 2.52)	1.01 (0.41- 2.52)
Cys/Cys	15 (21.7)	18 (26.9)	19 (22.1)	0.81 (0.29- 2.32)	0.07 (0.003- 1.39)	1.00 (0.35- 2.84)	0.92 (0.32- 2.67)
P for trend				0.69	0.08	0.99	0.89
P for interaction§				0.15	0.70	0.52	0.69

*: Adjusted for sex, age category (40-49, 50-59, 60-69, and 70-79 years), and either smoking status (never, former, and current smokers) or heavy drinking history.

†: Adjusted for the above factors plus HBsAg and HCVAb.

‡: A "heavy alcohol drinking history" was defined as having imbibed ≥69 g ethanol per day for ≥10 years.

§: Calculated by the likelihood ratio test.

HCC : hepatocellular carcinoma

CLD : chronic liver diseases

had substantially lower DNA repair activity than that encoded by the 326Ser allele in an in vitro Escherichia coli complementation activity assay[14] and in human cells in *vivo*[15] whereas others did not find such a difference.[26,27] Thus, there is limited evidence of the genotype-phenotype relation, yet recent epidemiologic studies suggested that the putative low active allele (326Cys) was positively associated with lung, orolaryngeal, esophageal, stomach, and colon cancers,[16-20] but not with breast cancer.[28] To our knowledge, this is the first epidemiologic study on the association between the Ser326Cys polymorphism and HCC risk. Despite its high biological plausibility, however, we could not obtain any significant findings.

In this study, selection bias among controls could be responsible for the lack of association. However, we used two different control groups, and the results based on both control groups showed a similar tendency. Furthermore, the observed frequencies of the 326Ser allele (0.49 among hospital controls and 0.51 among CLD patients) were close to those in two earlier case-control studies among the Japanese (0.53 in both).[19,22] Given the sample size and the genotype frequency of hospital controls, we had an 83% chance of detecting a doubling of the risk for Ser/Cys+Cys/Cys (putative risk genotypes) vs. Ser/Ser (two-sided $p = 0.05$).

The difference of hOGG1 activity potentially caused by the Ser326Cys polymorphism may be compensated by higher induction of other cooperative enzymes (e.g., human MTH homolog 1[29] or human MutY homolog[30]) that prevent 8-OHdG-induced mutagenesis. In addition, regardless of the polymorphism, hOGG1 activity may be functionally inhibited by increased NO production resulting from chronic inflammation, which usually exists as the background of HCC, since NO mediated inhibition of hOGG1 activity has been shown in cholangiocarcinoma cell line.[31] On the other hand, Sugimura et al[32] reported that the risk for lung cancer associated with the *hOGG1* Cys/Cys genotype differed by histological subtypes, being elevated for squamous cell carcinoma but not for adenocarcinoma. HCC might represent a histological type unrelated to this genotype.

In conclusion, our results suggest that the *hOGG1* Ser326Cys polymorphism may not play a major role as an independent factor in hepatocarcinogenesis. Although this case-control study of moderate size is among the largest ones that have been reported on the association between HCC and genetic polymorphisms, we could not exclude the possibility of a weak association (e.g., OR < 2.0) with the *hOGG1* polymorphism and its interaction with environmental factors. Further large studies are needed to address these issues.

ACKNOWLEDGMENTS

We are grateful to S. Ohtsuka, Y. Fujigami, S. Yamashita, T. Koyanagi, and M. Tanaka for interviewing subjects and blood collection and to K. Takahashi for technical assistance. We also express our deep appreciation to Emeritus Prof. J. Tadano of the Department of Laboratory Medicine, Saga Medical School, and the staff of all the relevant departments for their kind cooperation.

REFERENCES

1. Tanaka K, Hirohata T, Koga S, Sugimachi K, Kanematsu T, Ohryohji F, et al. Hepatitis C and Hepatitis B in the etiology of hepatocellular carcinoma in the Japanese Population. Cancer Res 1991; 51: 2842-7.
2. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 1993; 328: 1797-801.
3. Shibata A, Fukuda K, Nishiyori A, Ogimoto I, Sakata R, Tanikawa K. A case-control study on male hepatocellular carcinoma based on hospital and community controls. J Epidemiol 1998; 8: 1-5.
4. Tanaka K, Hirohata T, Fukuda K, Shibata A, Tsukuma H, Hiyama T. Risk factors for hepatocellular carcinoma among Japanese women. Cancer Causes Control 1995; 6: 91-8.
5. Fearon ER. Human cancer syndromes: clues to the origin and nature of cancer. Science 1997; 278: 1043-50.
6. Marnett LJ. Oxyradicals and DNA damage. Carcinogenesis 2000; 21: 361-70.
7. Shimoda R, Nagashima M, Sakamoto M, Yamaguchi N, Hirohashi S, Yokota J, et al. Increased formation of oxidative DNA damage, 8-hydroxydeoxyguanosine, in human livers with chronic hepatitis. Cancer Res 1994; 54: 3171-2.
8. Albano E. Free radical mechanisms in immune reactions associated with alcoholic liver disease. Free Radic Biol Med 2002; 32: 110-4.
9. Asami S, Hirano T, Yamaguchi R, Tomioka Y, Itoh H, Kasai H. Increase of a type of oxidative DNA damage, 8-hydroxyguanine, and its repair activity in human leukocytes by cigarette smoking. Cancer Res 1996; 56: 2546-9.
10. Cheng KC, Cahill DS, Kasai H, Nishimura S, Loeb LA. 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G----T and A----C substitutions. J Biol Chem 1992; 267: 166-72.
11. Hazra TK, Hill JW, Izumi T, Mitra S. Multiple DNA glycosylases for repair of 8-oxoguanine and their potential in vivo functions. Prog Nucleic Acid Res Mol Biol 2001; 68: 193-205.
12. Boiteux S, Radicella JP. The human OGG1 gene: structure, functions, and its implication in the process of carcinogenesis. Arch Biochem Biophys 2000; 377: 1-8.
13. Sunaga N, Kohno T, Shinmura K, Saitoh T, Matsuda T, Saito R, et al. OGG1 protein suppresses G:C-->T:A mutation in a shuttle vector containing 8-hydroxyguanine in human cells. Carcinogenesis 2001; 22: 1355-62.
14. Kohno T, Shinmura K, Tosaka M, Tani M, Kim SR, Sugimura H, et al. Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involved in the repair of

8-hydroxyguanine in damaged DNA. Oncogene 1998; 16: 3219-25.
15. Yamane A, Kohno T, Ito K, Sunaga N, Aoki K, Yoshimura K, et al. Differencial ability of polymorphic OGG1 proteins to suppress mutagenesis induced by 8-hydroxyguanine in human cell in vivo. Carcinogenesis 2004; 25: 1689-94.
16. Park J, Chen L, Tockman MS, Elahi A, Lazarus P. The human 8-oxoguanine DNA N-glycosylase 1 (hOGG1) DNA repair enzyme and its association with lung cancer risk. Pharmacogenetics 2004; 14: 103-9.
17. Elahi A, Zheng Z, Park J, Eyring K, McCaffrey T, Lazarus P. The human OGG1 DNA repair enzyme and its association with orolaryngeal cancer risk. Carcinogenesis 2002; 23: 1229-34.
18. Xing DY, Tan W, Song N, Lin DX. Ser326Cys polymorphism in hOGG1 gene and risk of esophageal cancer in a Chinese population. Int J Cancer 2001; 95: 140-3.
19. Tsukino H, Hanaoka T, Otani T, Iwasaki M, Kobayashi M, Hara M, et al. hOGG1 Ser326Cys polymorphism, interaction with environmental exposures, and gastric cancer risk in Japanese populations. Cancer Sci 2004; 95: 977-83.
20. Kim JI, Park YJ, Kim KH, Kim JI, Song BJ, Lee MS, et al. hOGG1 Ser326Cys polymorphism modifies the significance of the environmental risk factor for colon cancer. World J Gastroenterol 2003; 9: 956-60.
21. Sakamoto T, Hara M, Higaki Y, Ichiba M, Horita M, Mizuta T, et al. Influence of alcohol consumption and gene polymorphisms of ADH2 and ALDH2 on hepatocellular carcinoma in a Japanese population. Int J Cancer 2006; 118: 1501-7.
22. Ito H, Hamajima N, Takezaki T, Matsuo K, Tajima K, Hatooka S, et al. A limited association of OGG1 Ser326Cys polymorphism for adenocarcinoma of the lung. J Epidemiol 2002; 12: 258-65.
23. Moriya K, Nakagawa K, Santa T, Shintani Y, Fujie H, Miyoshi H, et al. Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. Cancer Res 2001; 61: 4365-70.
24. Waris G, Siddiqui A. Regulatory mechanisms of viral hepatitis B and C. J Biosci 2003; 28: 311-21.
25. Kato J, Kobune M, Nakamura T, Kuroiwa G, Takada K, Takimoto R, et al. Normalization of elevated hepatic 8-hydroxy-2'-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. Cancer Res 2001; 61: 8697-702.
26. Dherin C, Radicella JP, Dizdaroglu M, Boiteux S. Excision of oxidatively damaged DNA bases by the human alpha-hOGG1 protein and the polymorphic alpha-hOGG1(Ser326Cys) protein which is frequently found in human populations. Nucleic Acids Res 1999; 27: 4001-7.
27. Janssen K, Schlink K, Gotte W, Hippler B, Kaina B, Oesch F. DNA repair activity of 8-oxoguanine DNA glycosylase 1 (OGG1) in human lymphocytes is not dependent on genetic polymorphism Ser326/Cys326. Mutat Res 2001; 486: 207-16.
28. Cai Q, Shu XO, Wen W, Courtney R, Dai Q, Gao YT, Zheng W. Functional Ser326Cys polymorphism in the hOGG1 gene is not associated with breast cancer risk. Cancer Epidemiol Biomarkers Prev. 2006; 15: 403-4.
29. Furuichi M, Yoshida MC, Oda H, Tajiri T, Nakabeppu Y, Tsuzuki T, et al. Genomic structure and chromosome location of the human mutT homologue gene MTH1 encoding 8-oxo-dGTPase for prevention of A:T to C:G transversion. Genomics 1994; 24: 485-90.
30. Slupska MM, Baikalov C, Luther WM, Chiang JH, Wei YF, Miller JH. Cloning and sequencing a human homolog (hMYH) of the Escherichia coli mutY gene whose function is required for the repair of oxidative DNA damage. J Bacteriol 1996; 178: 3885-92.
31. Jaiswal M, LaRusso NF, Nishioka N, Nakabeppu Y, Gores GJ. Human Ogg1, a protein involved in the repair of 8-oxoguanine, is inhibited by nitric oxide. Cancer Res 2001; 61: 6388-93.
32. Sugimura H, Kohno T, Wakai K, Nagura K, Genka K, Igarashi H, et al. hOGG1 Ser326Cys polymorphism and lung cancer susceptibility. Cancer Epidemiol Biomarkers Prev 1999; 8: 669-74.

Inverse association between coffee drinking and the risk of hepatocellular carcinoma: a case-control study in Japan

Keitaro Tanaka,[1,5] Megumi Hara,[1] Tatsuhiko Sakamoto,[1] Yasuki Higaki,[1] Toshihiko Mizuta,[2] Yuichiro Eguchi,[2] Tsutomu Yasutake,[2] Iwata Ozaki,[2] Kyosuke Yamamoto,[2] Shingo Onohara,[4] Seiji Kawazoe,[4] Hirohisa Shigematsu[4] and Shunzo Koizumi[3]

Departments of [1]Preventive Medicine, [2]Internal Medicine and [3]General Medicine, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga 849-8501; [4]Department of Internal Medicine, Saga Prefectural Hospital Koseikan, 1-12-9 Mizugae, Saga 840-8571, Japan

(Received September 15, 2006/Revised October 12, 2006/Accepted October 13, 2006/Online publication November 27, 2006)

Coffee use has consistently been associated with lower serum liver enzyme levels and a reduced risk of liver cirrhosis. A limited number of cohort and case-control studies also suggest a decreased risk of hepatocellular carcinoma (HCC) among coffee drinkers, but mostly without consideration of hepatitis virus infection. In the present case-control study, we recruited 209 incident HCC cases and three different controls (1308 community controls, 275 hospital controls, and 381 patients with chronic liver disease [CLD] without HCC), all of whom were aged 40–79 years and residents of Saga Prefecture, Japan. A questionnaire survey elicited information on coffee use during the last 1–2 years and 10 years before, and plasma hepatitis B surface antigen and antibodies to hepatitis C virus were tested for all but community controls. After adjustment for sex, age, heavy alcohol use, smoking status and hepatitis virus markers (except for community controls), coffee use during the last 1–2 years was associated with a decreased risk against any control group. For coffee use 10 years before, comparison between HCC cases and either community controls or CLD patients revealed a decreased risk; adjusted odds ratios for occasional use, 1–2 cups/day and ≥3 cups/day compared with no use were 0.33, 0.27 and 0.22 (*P* trend < 0.001), respectively, against community controls, and 0.86, 0.62 and 0.53 (*P* trend = 0.05), respectively, against CLD patients. These results suggest that coffee may protect against the development of HCC, yet further elaborate studies (hopefully, intervention studies) are warranted to corroborate these findings. (*Cancer Sci* 2007; 98: 214–218)

Chronic infections with hepatitis C and B viruses represent established risk factors for hepatocellular carcinoma (HCC), accounting for around 90% of occurrences of this malignancy in Japan.[1–3] However, little is known about the association between the risk of HCC and dietary habits, except for alcohol consumption.[4,5] Recently, coffee drinking has been linked to a decreased risk of HCC; three cohort studies[6–8] and three case-control studies[9–11] demonstrated that coffee drinkers were at a lower risk of HCC than non-coffee drinkers. However, only two case-control studies took into account hepatitis virus infection as a potential confounder.[10,11] For example, if hepatitis virus carriers who have a great risk of developing HCC tend to drink less coffee for any reason (e.g. impaired caffeine clearance due to their liver disease)[12] compared with healthy individuals, it would produce a seemingly protective association between coffee use and HCC.

To further explore the association between coffee drinking and HCC, we conducted a case-control study employing three different controls (community controls, hospital controls, and patients with chronic liver disease [CLD] without HCC). CLD patients were selected as controls because the majority of Japanese patients with HCC have preexisting CLD. Plasma hepatitis virus markers (except for community controls) and other major covariates such as alcohol consumption and cigarette smoking were measured and accommodated in our data analyses.

Materials and Methods

Subjects. The details of the study subjects excluding community controls have been described elsewhere.[13] Briefly, all study subjects were restricted to residents of Saga Prefecture, Japan, who were aged 40–79 years at the time of identification. Incident HCC patients ($n = 209$, participation rate [PR] = 92%) who were admitted or outpatients of two 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 ($n = 31$).

Community controls ($n = 1308$) were residents of Saga City, who were aged 40–79 years as of 31 December 2001. We randomly selected 1000 men and 1000 women from the resident register as follows. Based on computer-generated random numbers for page and line, we first identified corresponding reference lines in the register and then searched for eligible subjects on or after those lines. We asked these subjects to participate in a questionnaire survey by mail in March 2002. Sixty-three people later turned out to be ineligible (31 no delivery, nine erroneous sampling, 10 long absence, 12 inability to respond, one death), and 1338 people successfully responded with a net PR of 69% (1338/1937). Of the 1338 respondents, 30 were excluded due to missing information on current coffee use.

Hospital controls ($n = 275$, PR = 73%) 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: (1) men aged 50–79 years; (2) women aged 60–79 years; (3) men aged 40–49 years; and (4) 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. The 275 hospital controls had various, mostly minor, diseases ($n = 190$), undiagnosed symptoms ($n = 49$), or no definite abnormality ($n = 36$).

Patients with CLD ($n = 381$, 298 patients with chronic hepatitis and 83 patients with cirrhosis, PR = 96%) were out- or inpatients of the two hospitals, the same as the 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 parasitosis, congestive heart failure, or metabolic disorders) were excluded. These CLD patients had no evidence of HCC by radiological findings.

[5]To whom correspondence should be addressed. E-mail: tanakake@post.saga-med.ac.jp

doi: 10.1111/j.1349-7006.2006.00368.x

The study protocol was approved by the ethics committees of the above two hospitals. Written informed consent was obtained from all but community controls. For community controls, having returned the completed questionnaire was regarded as their consent.

Questionnaire survey. Except for community controls, coffee use, alcohol consumption and cigarette smoking were assessed based on an interview survey by research nurses using a uniform questionnaire. For community controls, we carried out a mail survey using a self-administered questionnaire including the same questions on the above lifestyle habits. With regard to coffee use, subjects were asked about their drinking frequency (never, occasional [less than one cup per day], or daily) during the last 1–2 years and 10 years before, with subsequent inquiry to daily users about the number of cups per day; 55 community controls had missing data on coffee use 10 years before. We did not obtain information on the type or brewing method of coffee. A history of heavy alcohol use was defined as having drunk ≥69 g of alcohol per day for ≥10 years. Current smokers were defined as those who currently smoked or stopped smoking less than 1 year before.

Serological tests. Venous blood was drawn from all but community controls, and plasma samples were tested for hepatitis B surface antigen (HBsAg) using a chemiluminescent immunoassay (CLIA; Dainabot, Tokyo, Japan), and for antibodies to hepatitis C virus (HCV) using a second-generation enzyme immunoassay (Abott HCV EIA II; Dainabot) at an external laboratory (SRL, Tokyo, Japan).

Statistical analysis. χ^2-tests and Student's t-tests were conducted for univariate analyses. Unconditional logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI) of HCC according to coffee drinking by using dummy variables, with adjustment for sex, age category (40–49, 50–59, 60–69 and 70–79 years), heavy alcohol use (ever vs never) and smoking status (current vs never/former smokers), as well as HBsAg and anti-HCV status (except for community controls). Linear trends for the association of increasing coffee use with other factors or HCC risk were assessed using the Mantel test (for proportions), linear regression analysis (for means), or logistic regression analysis (for OR) with an ordinal variable assigned for coffee consumption. Tests for interaction between coffee use and alcohol drinking/smoking habits on HCC risk were carried out by including an additional product term of two binary variables (i.e. coffee and either alcohol drinking or smoking) in the logistic model (the Wald test). All statistical analyses were carried out using the Stata statistical package (StataCorp, College Station, TX, USA).

Results

Table 1 shows selected characteristics of study subjects. Compared with at least one of the three control groups, HCC cases showed a significantly higher prevalence of men (against community controls or CLD patients), older subjects (against any control group), HBsAg positivity (against hospital controls), anti-HCV positivity (against hospital controls), men with a history of heavy alcohol use (against any control group), and male current smokers (against hospital controls). HCC cases included significantly less daily coffee users than any control group.

In Table 2, the characteristics of coffee drinkers among community controls and hospital controls are presented. No sex difference in coffee drinking was evident. Heavier coffee drinkers were younger and were more likely to be current smokers (except for female hospital controls). There was some indication of an inverse association between coffee use and either heavy alcohol use (among male community controls) or positivity for HBsAg or anti-HCV (among hospital controls), although it was not statistically significant.

We estimated the adjusted OR of HCC for coffee use during the last 1–2 years by comparing HCC cases with each control group (Table 3). Coffee use during the last 1–2 years was associated with a decreased risk against any control group, although the OR for ≥3 cups/day against hospital controls was unstable and not decreased after additional adjustment for HBsAg and anti-HCV.

Because HCC cases could have lately changed their coffee use due to their preexisting liver disease (e.g. impaired caffeine clearance)[12] we also evaluated the association with coffee use 10 years before (Table 4). In this analysis, the comparison between HCC cases and either community controls or CLD patients showed an inverse association between coffee drinking and HCC risk. Such a risk decrease was not observed against hospital controls, and the fully adjusted OR for ≥3 cups/day was rather elevated but very unstable, as illustrated by the wide 95% CI.

Finally, we examined whether the magnitude of the inverse association between coffee and HCC was modified by alcohol drinking and smoking status (Table 5). No significant difference in the adjusted OR for daily versus non-daily coffee users was observed according to daily alcohol use (<23 vs ≥23 g), heavy alcohol use (never vs ever), or smoking status (never/former vs current), as indicated by statistically insignificant P-values for interaction. The same was true for coffee use 10 years before (data not shown).

Table 1. Selected characteristics of study subjects

Characteristic	HCC cases	Community controls	Hospital controls	CLD patients
Number	209	1308	275	381
Male (%)	67.5	50.2**	65.5	53.8**
Mean age (years)	67.0	56.7**	60.6**	60.4**
HBsAg positive (%)	9.1	ND	2.2**	9.2
Anti-HCV positive (%)	85.6	ND	7.6**	85.8
Heavy alcohol use† (%)				
Male	32.6	12.0**	12.2**	17.1**
Female	4.4	2.9	1.1	2.3
Current smoker (%)				
Male	46.8	48.5	35.0*	36.6
Female	4.4	9.4	4.2	6.3
Daily coffee user (%)				
Male	9.9	51.4**	36.1**	34.6**
Female	10.3	48.2**	27.4**	23.3*

†Having drunk ≥69 g of alcohol per day for ≥10 years. *$P < 0.05$ compared with hepatocellular carcinoma (HCC) cases. **$P < 0.01$ compared with HCC cases. CLD, chronic liver disease; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; ND, not determined.

Discussion

The present study provides further evidence that coffee drinking is associated with a decreased risk of HCC. There are now several lines of accumulated evidence for possible beneficial effects of coffee on the liver: (1) a substantial number of cross-sectional and cohort studies have shown a very consistent inverse association between coffee consumption and serum liver enzyme levels;[14–26] (2) several cohort and case-controls studies have demonstrated a consistent protective association between coffee use and the risk of liver cirrhosis;[27–32] and (3) a few animal experiments have revealed that the incidence of liver tumor is lower in rodents given coffee than in those not given coffee.[33,34] Thus, the findings in this study, together with recent observations in several cohort and case-controls studies,[6–11] appear to carry sufficient biological plausibility.

Employing three different controls represents a unique characteristic of the present study. Theoretically, the results based on

Table 2. Characteristics of coffee users

Factor	Daily coffee use during last 1–2 years				*P* trend†
	None	Occasional	1–2 cups	≥3 cups	
Among 1308 community controls					
Number	230	427	387	264	
Male (%)	50.9	47.3	50.6	53.4	0.34
Mean age (years)	64.6	58.2	54.6	50.6	<0.001
Heavy alcohol use‡ (%)					
Male	14.5	12.9	12.8	7.8	0.11
Female	2.7	2.7	2.6	4.1	0.55
Current smoker (%)					
Male	32.5	43.1	49.5	68.1	<0.001
Female	3.5	7.1	9.4	18.7	<0.001
Among 275 hospital controls					
Number	96	88	60	31	
Male (%)	66.7	58.0	75.0	64.5	0.62
Mean age (years)	65.4	60.2	55.8	56.2	<0.001
Heavy alcohol use‡ (%)					
Male	12.5	13.7	15.6	0.0	0.43
Female	3.1	0.0	0.0	0.0	–
Current smoker (%)					
Male	21.9	37.3	48.9	40.0	0.01
Female	6.3	2.7	6.7	0.0	0.53
HBsAg/anti-HCV(+) (%)	12.5	10.2	8.3	3.2	0.13

†Based on the Mantel test or linear regression. ‡Having drunk ≥69 g of alcohol per day for ≥10 years. HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus.

Table 3. Adjusted odds ratios (OR) and 95% confidence intervals (CI) of hepatocellular carcinoma (HCC) for coffee use during the last 1–2 years by comparing 209 HCC cases with 1308 community controls, 275 hospital controls or 381 chronic liver disease (CLD) patients

Factor	Daily coffee use during last 1–2 years				Total	*P* trend
	None	Occasional	1–2 cups	≥3 cups		
Number (%)						
HCC cases	135 (64.6)	53 (25.4)	15 (7.2)	6 (2.9)	209 (100)	–
Community controls	230 (17.6)	427 (32.6)	387 (29.6)	264 (20.2)	1308 (100)	–
Hospital controls	96 (34.9)	88 (32.0)	60 (21.8)	31 (11.3)	275 (100)	–
CLD patients	175 (45.9)	94 (24.7)	66 (17.3)	46 (12.1)	381 (100)	–
Adjusted OR (95% CI) against						
Community controls†	1.00 (reference)	0.31 (0.21–0.46)	0.11 (0.06–0.21)	0.10 (0.04–0.24)	–	<0.001
Hospital controls†	1.00 (reference)	0.54 (0.34–0.87)	0.24 (0.12–0.48)	0.23 (0.08–0.61)	–	<0.001
Hospital controls‡	1.00 (reference)	0.42 (0.19–0.95)	0.23 (0.08–0.68)	1.08 (0.22–5.35)	–	0.03
CLD patients†	1.00 (reference)	0.86 (0.55–1.34)	0.42 (0.21–0.84)	0.28 (0.11–0.72)	–	0.001
CLD patients‡	1.00 (reference)	0.86 (0.55–1.35)	0.42 (0.21–0.84)	0.29 (0.11–0.75)	–	0.001

†Adjusted for sex, age, heavy alcohol use and smoking status. ‡Adjusted for sex, age, heavy alcohol use, smoking status, hepatitis B surface antigen and antibodies to hepatitis C virus.

community and hospital controls, who are recruited in usual settings of case-control studies, would correspond to the results from recent cohort studies in Japan.[6–8] We found a strong inverse association between coffee and HCC risk against community controls, although potential confounding by hepatitis virus infection could not be ruled out. The OR for coffee use against hospital controls were not clearly reduced but were even elevated for the highest intake category after additional adjustment for hepatitis virus markers. These OR were very unstable due to the low prevalence of hepatitis virus infection among hospital controls, and caution must be exercised in interpreting these findings. However, the results based on CLD patients showed a consistent tendency for coffee use during the last 1–2 years and 10 years before, regardless of adjustment for hepatitis virus markers. We speculate that the comparison between HCC cases and CLD patients may convey the most reliable information in terms of both confounding and precision issues. A recent case-control study demonstrated a similar protective association between coffee and HCC among patients with chronic hepatitis C.[11]

Several limitations of this study should be mentioned. First, HCC cases may have reduced coffee consumption because of their preexisting liver disease (e.g. impaired caffeine clearance[12] and gastrointestinal disorders accompanying liver disease). This can lead to a spurious protective association between coffee and HCC. In an attempt to address this issue, we examined the association with coffee intake 10 years before as well as the intake during the last 1–2 years, and found a somewhat weaker protective association against community controls and CLD patients. However, there still exists the possibility that coffee consumption 10 years before could have been affected by worsened liver

doi: 10.1111/j.1349-7006.2006.00368.x

Table 4. Adjusted odds ratios (OR) and 95% confidence intervals (CI) of hepatocellular carcinoma (HCC) for coffee use 10 years before by comparing 209 HCC cases with 1253 community controls, 275 hospital controls or 381 chronic liver disease (CLD) patients

Factor	Daily coffee use 10 years before				Total	*P* trend
	None	Occasional	1–2 cups	≥3 cups		
Number (%) of						
HCC cases	127 (60.8)	53 (25.4)	17 (8.1)	12 (5.7)	209 (100)	–
Community controls	268 (21.4)	496 (39.6)	268 (21.4)	221 (17.6)	1253 (100)	–
Hospital controls	129 (46.9)	73 (26.5)	48 (17.5)	25 (9.1)	275 (100)	–
CLD patients	166 (43.6)	102 (26.8)	59 (15.5)	54 (14.2)	381 (100)	–
Adjusted OR (95% CI) against						
Community controls†	1.00 (reference)	0.33 (0.22–0.48)	0.27 (0.15–0.48)	0.22 (0.11–0.43)	–	<0.001
Hospital controls†	1.00 (reference)	1.10 (0.68–1.78)	0.66 (0.33–1.32)	0.92 (0.40–2.14)	–	0.53
Hospital controls‡	1.00 (reference)	0.99 (0.42–2.32)	0.95 (0.31–2.89)	2.59 (0.58–11.56)	–	0.47
CLD patients†	1.00 (reference)	0.87 (0.56–1.36)	0.64 (0.33–1.23)	0.52 (0.25–1.10)	–	0.05
CLD patients‡	1.00 (reference)	0.86 (0.55–1.34)	0.62 (0.32–1.21)	0.53 (0.25–1.12)	–	0.05

†Adjusted for sex, age, heavy alcohol use and smoking status. ‡Adjusted for sex, age, heavy alcohol use, smoking status, hepatitis B surface antigen and antibodies to hepatitis C virus.

Table 5. Adjusted odds ratios (OR) and 95% confidence intervals (CI) of hepatocellular carcinoma (HCC) for daily versus non-daily coffee users according to alcohol drinking and smoking status

Alcohol drinking or smoking status	Community controls		Hospital controls		CLD patients	
	Ca/Co†	OR‡ (95% CI)	Ca/Co†	OR§ (95% CI)	Ca/Co†	OR§ (95% CI)
Alcohol <23 g/day	157/966	0.19 (0.11–0.35)	157/206	0.79 (0.25–2.46)	157/320	0.48 (0.25–0.95)
Alcohol ≥23 g/day	52/341	0.18 (0.07–0.44)	52/69	0.29 (0.07–1.31)	52/61	0.24 (0.08–0.70)
P interaction	–	0.71	–	0.77	–	0.44
Non-heavy alcohol user	160/1210	0.17 (0.09–0.30)	160/252	0.46 (0.15–1.39)	160/342	0.36 (0.18–0.70)
Heavy alcohol user	49/98	0.30 (0.12–0.75)	49/23	0.26 (0.03–2.30)	49/39	0.57 (0.18–1.87)
P interaction	–	0.27	–	0.43	–	0.29
Never/former smoker	140/929	0.23 (0.12–0.42)	140/208	0.56 (0.18–1.70)	140/295	0.58 (0.29–1.16)
Current smoker	69/379	0.14 (0.06–0.32)	69/67	0.17 (0.02–1.63)	69/86	0.19 (0.07–0.52)
P interaction	–	0.45	–	0.36	–	0.14

†Numbers of cases/controls. ‡Adjusted for sex, age and either smoking status (for OR by alcohol drinking) or heavy alcohol use (for OR by smoking status). §Adjusted for sex, age, hepatitis B surface antigen, antibodies to hepatitis C virus, and either smoking status (for OR by alcohol drinking) or heavy alcohol use (for OR by smoking status).

function at that time among HCC cases. Our comparison between HCC cases and CLD patients may get rid of this issue to some extent, yet intervention studies are needed to address it conclusively.

Second, selection bias could have been introduced in the present study. The catchment area for community controls (Saga City) differed from that for HCC cases (Saga Prefecture), but the results remained unchanged when we excluded the cases that resided outside Saga City in the data analysis (data not shown). We believe that the fairly high PR (69–96%) of study subjects reduced the possibility of self-selection bias. Third, information bias was unlikely because the potential beneficial effect of coffee was perhaps unknown in most study subjects as well as interviewers at the time of investigation. Fourth, misclassification of coffee use, particularly 10 years before, may have been present. Among community and hospital controls, the proportion of more frequent coffee drinkers (1–2 and ≥3 cups/day) during the last 1–2 years (Table 3) was higher than that 10 years before (Table 4), although heavier coffee drinkers were younger, as shown in Table 2. This may be due to underreporting of coffee consumption 10 years before or to generally increasing coffee consumption over the past decade. The possible underreporting, if any, was likely to be non-differential between cases and controls, and thus may have biased relevant OR towards unity. However, this issue would not seriously damage our interpretation.

Although potential confounding effects by known risk factors were taken into account in the data analyses, other possible confounders such as dietary factors (e.g. vegetable consumption)[35] could be relevant. We did not ascertain information on other food items and thus could not address this issue. To our knowledge, however, no specific food has been so strongly associated with HCC risk as to explain the inverse relationship between coffee and HCC by its confounding effect.

Because we previously observed a stronger inverse association between coffee consumption and serum liver enzyme levels (e.g. γ-glutamyltransferase) among male alcohol drinkers than among male non-alcohol drinkers,[21] we evaluated if the inverse association between coffee and HCC was modified by alcohol consumption as well as cigarette smoking. We found no evidence of such an effect modification, which accords with the results from a recent Italian case-control study.[10]

Potential candidates responsible for the inverse association between coffee and HCC may be caffeine,[36] coffee diterpenes (e.g. cafestol and kahweol),[37] antioxidants (e.g. chlorogenic acid)[33] or other unidentified ingredients. We could not evaluate the role of caffeine itself, but previous studies did not find an inverse association between HCC and green tea, another popular source of caffeine in Japan, indirectly suggesting no substantial role of caffeine.[6,7,11,38] Although we did not obtain information on the type or brewing method of coffee from study subjects, instant coffee is most popular in Japan, followed by filtered coffee,[39]

and the use of unfiltered coffee is negligible. As the levels of diterpenes are greatly reduced in instant and filtered coffee,[40] they are unlikely to be responsible for the observed inverse association. Tanaka *et al.* demonstrated that chlorogenic acid exerts an inhibitory effect on chemically induced hyperplastic liver cell foci in hamsters,[33] and some coffee antioxidants including chlorogenic acid may play a crucial protective role in human hepatocarcinogesis. Further studies incorporating genetic polymorphisms of enzymes involved in specific metabolic pathways of the above substances may afford a clue to the above issue.

As already discussed, the major limitation of the present study was a possible decrease of coffee use among HCC cases due to their advanced liver disease. Cohort studies incorporating hepatitis virus markers as well as the severity of liver disease and, hopefully, intervention studies targeting high-risk groups (e.g. patients with chronic hepatitis C) are warranted to establish the protective association between coffee drinking and HCC.

Acknowledgments

We express our deep appreciation to Emeritus Professor J. Tadano of the Department of Laboratory Medicine, Saga Medical School, and the staff of all the relevant departments for their kind cooperation. This study was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology (grant nos 11670344, 13220014, 14031216 and 15390188) and Grants-in-Aid for Research on Hepatitis and for Third Term Comprehensive Control Research for Cancer from the Ministry of Health, Labor and Welfare, Japan.

References

1 Nishioka K, Watanabe J, Furuta S *et al.* A high prevalence of antibody to the hepatitis C virus in patients with hepatocellular carcinoma in Japan. *Cancer* 1991; **67**: 429–33.
2 Tanaka K, Ikematsu H, Hirohata T, Kashiwagi S. Hepatitis C virus infection and risk of hepatocellular carcinoma among Japanese: possible role of type 1b (II) infection. *J Natl Cancer Inst* 1996; **88**: 742–6.
3 Ikai I, Arii S, Ichida T *et al.* Report of the 16th follow-up survey of primary liver cancer. *Hepatol Res* 2005; **32**: 163–72.
4 International Agency for Research on Cancer. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 44: Alcohol Drinking. Lyon: IARC, 1988.
5 Tanaka K, Hirohata T, Takeshita S *et al.* Hepatitis B virus, cigarette smoking and alcohol consumption in the development of hepatocellular carcinoma: a case-control study in Fukuoka, Japan. *Int J Cancer* 1992; **51**: 509–14.
6 Inoue M, Yoshimi I, Sobue T, Tsugane S. Influence of coffee drinking on subsequent risk of hepatocellular carcinoma: a prospective study in Japan. *J Natl Cancer Inst* 2005; **97**: 293–300.
7 Shimazu T, Tsubono Y, Kuriyama S *et al.* Coffee consumption and the risk of primary liver cancer: pooled analysis of two prospective studies in Japan. *Int J Cancer* 2005; **116**: 150–4.
8 Kurozawa Y, Ogimoto I, Shibata A *et al.* Coffee and risk of death from hepatocellular carcinoma in a large cohort study in Japan. *Br J Cancer* 2005; **93**: 607–10.
9 Gallus S, Bertuzzi M, Tavani A *et al.* Does coffee protect against hepatocellular carcinoma? *Br J Cancer* 2002; **87**: 956–9.
10 Gelatti U, Covolo L, Franceschini M *et al.* Coffee consumption reduces the risk of hepatocellular carcinoma independently of its aetiology: a case-control study. *J Hepatol* 2005; **42**: 528–34.
11 Ohfuji S, Fukushima W, Tanaka T *et al.* Coffee consumption and reduced risk of hepatocellular carcinoma among patients with chronic type C liver disease: a case-control study. *Hepatol Res* 2006; **36**: 201–8.
12 Cheng WS, Murphy TL, Smith MT *et al.* Dose-dependent pharmacokinetics of caffeine in humans: relevance as a test of quantitative liver function. *Clin Pharmacol Ther* 1990; **47**: 516–24.
13 Sakamoto T, Hara M, Higaki Y *et al.* Influence of alcohol consumption and gene polymorphisms of *ADH2* and *ALDH2* on hepatocellular carcinoma in a Japanese population. *Int J Cancer* 2006; **118**: 1501–7.
14 Arnesen E, Huseby NE, Brenn T, Try K. The Tromso Heart Study: distribution of, and determinants for, gamma-glutamyltransferase in a free-living population. *Scand J Clin Lab Invest* 1986; **46**: 63–70.
15 Nilssen O, Forde OH, Brenn T. The Tromso Study: distribution and population determinants of gamma-glutamyltransferase. *Am J Epidemiol* 1990; **132**: 318–26.
16 Casiglia E, Spolaore P, Ginocchio G, Ambrosio GB. Unexpected effects of coffee consumption on liver enzymes. *Eur J Epidemiol* 1993; **9**: 293–7.
17 Kono S, Shinchi K, Imanishi K, Todoroki I, Hatsuse K. Coffee and serum gamma-glutamyltransferase: a study of self-defense officials in Japan. *Am J Epidemiol* 1994; **139**: 723–7.
18 Nilssen O, Forde OH. Seven-year longitudinal population study of change in gamma-glutamyltransferase: the Tromso Study. *Am J Epidemiol* 1994; **139**: 787–92.
19 Pintus F, Mascia P. Distribution and population determinants of gamma-glutamyltransferase in a random sample of Sardinian inhabitants. *Eur J Epidemiol* 1996; **12**: 71–6.
20 Poikolainen K, Vartiainen E. Determinants of gamma-glutamyltransferase: positive interaction with alcohol and body mass index, negative association with coffee. *Am J Epidemiol* 1997; **146**: 1019–24.
21 Tanaka K, Tokunaga S, Kono S *et al.* Coffee consumption and decreased serum gamma-glutamyltransferase and aminotransferase activities among male alcohol drinkers. *Int J Epidemiol* 1998; **27**: 438–43.
22 Honjo S, Kono S, Coleman MP *et al.* Coffee drinking and serum gamma-glutamyltransferase: an extended study of self-defense officials of Japan. *Ann Epidemiol* 1999; **9**: 325–31.
23 Honjo S, Kono S, Coleman MP *et al.* Coffee consumption and serum aminotransferases in middle-aged Japanese men. *J Clin Epidemiol* 2001; **54**: 823–9.
24 Nakanishi N, Nakamura K, Nakajima K, Suzuki K, Tatara K. Coffee consumption and decreased serum gamma-glutamyltransferase: a study of middle-aged Japanese men. *Eur J Epidemiol* 2000; **16**: 419–23.
25 Nakanishi N, Nakamura K, Suzuki K, Tatara K. Effects of coffee consumption against the development of liver dysfunction: a 4-year follow-up study of middle-aged Japanese male office workers. *Ind Health* 2000; **38**: 99–102.
26 Ruhl CE, Everhart JE. Coffee and caffeine consumption reduce the risk of elevated serum alanine aminotransferase activity in the United States. *Gastroenterology* 2005; **128**: 24–32.
27 Klatsky AL, Armstrong MA. Alcohol, smoking, coffee, and cirrhosis. *Am J Epidemiol* 1992; **136**: 1248–57.
28 Corrao G, Lepore AR, Torchio P *et al.* The effect of drinking coffee and smoking cigarettes on the risk of cirrhosis associated with alcohol consumption: a case-control study. *Eur J Epidemiol* 1994; **10**: 657–64.
29 Corrao G, Zambon A, Bagnardi V, D'Amicis A, Klatsky A. Coffee, caffeine, and the risk of liver cirrhosis. *Ann Epidemiol* 2001; **11**: 458–65.
30 Gallus S, Tavani A, Negri E, La Vecchia C. Does coffee protect against liver cirrhosis? *Ann Epidemiol* 2002; **12**: 202–5.
31 Tverdal A, Skurtveit S. Coffee intake and mortality from liver cirrhosis. *Ann Epidemiol* 2003; **13**: 419–23.
32 Ruhl CE, Everhart JE. Coffee and tea consumption are associated with a lower incidence of chronic liver disease in the United States. *Gastroenterology* 2005; **129**: 1928–36.
33 Tanaka T, Nishikawa A, Shima H *et al.* Inhibitory effects of chlorogenic acid, reserpine, polyprenoic acid (E-5166), or coffee on hepatocarcinogenesis in rats and hamsters. *Basic Life Sci* 1990; **52**: 429–40.
34 Stalder R, Bexter A, Wurzner HP, Luginbuhl H. A carcinogenicity study of instant coffee in Swiss mice. *Food Chem Toxicol* 1990; **28**: 829–37.
35 Yu MW, Hsieh HH, Pan WH, Yang CS, Chen CJ. Vegetable consumption, serum retinol level, and risk of hepatocellular carcinoma. *Cancer Res* 1995; **55**: 1301–5.
36 Sharp DS, Benowitz NL. Re: 'Alcohol, smoking, coffee, and cirrhosis' and 'coffee and serum gamma-glutamyltransferase: a study of self-defense officials in Japan'. *Am J Epidemiol* 1995; **141**: 480–2.
37 Weusten-Van der Wouw MP, Katan MB, Viani R *et al.* Identity of the cholesterol-raising factor from boiled coffee and its effects on liver function enzymes. *J Lipid Res* 1994; **35**: 721–33.
38 Kurozawa Y, Ogimoto I, Shibata A *et al.* Dietary habits and risk of death due to hepatocellular carcinoma in a large scale cohort study in Japan. Univariate analysis of JACC study data. *Kurume Med J* 2004; **51**: 141–9.
39 All Japan Coffee Association. *A Basic Survey for Monitoring Trends in the Demand for Coffee (in Japanese)*. Tokyo: All Japan Coffee Association, 2005.
40 Urgert R, van der Weg G, Kosmeijer-Schuil TG, van de Bovenkamp P, Hovenier R, Katan MB. Levels of the cholesterol-elevating diterpenes cafestol and kahweol in various coffee brews. *J Agric Food Chem* 1995; **43**: 2167–72.

doi: 10.1111/j.1349-7006.2006.00368.x

Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Hepatology Research 36 (2006) 201–208
HEPATOLOGY RESEARCH
http://www.elsevier.com/locate/hepres

Coffee consumption and reduced risk of hepatocellular carcinoma among patients with chronic type C liver disease: A case–control study

Satoko Ohfuji [a,*], Wakaba Fukushima [a], Takashi Tanaka [a], Daiki Habu [b], Akihiro Tamori [b], Hiroki Sakaguchi [b], Tadashi Takeda [b], Norifumi Kawada [b], Shuichi Seki [b], Shuhei Nishiguchi [c], Susumu Shiomi [d], Yoshio Hirota [a]

[a] *Department of Public Health, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka, 545-8585, Japan*
[b] *Department of Hepatology, Osaka City University Graduate School of Medicine, Osaka, Japan*
[c] *Division of Hepatobiliary and Pancreatic Diseases, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan*
[d] *Department of Nuclear Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan*

Received 21 April 2006; received in revised form 5 July 2006; accepted 14 July 2006
Available online 17 August 2006

Abstract

Several studies have reported the role of coffee for hepatocellular carcinoma (HCC). However, no study investigated about the relation of coffee for HCC among individuals with a relevant risk factor, i.e., hepatitis C virus (HCV) infection. Thus, we conducted a hospital-based case–control study to assess an association between coffee and HCC, in which both 73 cases and 253 controls were patients with chronic type C liver disease. To consider potential changes in coffee intake due to progression of liver disease, the effect of coffee was estimated separately before and after first identification of liver disease. Odds ratios (OR) and 95% confidence intervals (CI) for HCC risk were calculated using the conditional logistic regression model. Coffee drinking on a daily basis (≥1 cup/day) revealed lowered ORs as compared with non-drinkers both before first identification of liver disease (OR 0.38; 95% CI: 0.13–1.12; $P=0.078$) as well as thereafter (OR 0.19; 95% CI: 0.05–0.71; $P=0.032$). Even after excluding subjects who reported a reduction in the frequency of coffee intake after first identification of liver disease, this negative correlation persisted (OR 0.35; 95% CI: 0.12–1.06; $P=0.063$). Taken together, coffee may be a protective factor for HCC among those infected with HCV.

Keywords: Coffee; Hepatocellular carcinoma; Hepatitis C virus; Risk factor; Case–control study

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide with morbidity increasing over the past two decades. The most important risk factors for HCC are chronic infection with hepatitis B or C viruses [1,2]. Particularly, infection with hepatitis C virus (HCV) is considered to play a big part in the future spread of HCC. It is because no effective vaccine for HCV is currently available, while HBV vaccine has been succeeding in control for the virus spread [3]. Accordingly, there are also reports that HCV infection may be responsible for the rising incidence of HCC in the US and in western Japan [4,5]. Thus, it is necessary to identify factors to control HCC among individuals infected with HCV.

As is generally known, the most important and effective way for HCC prevention is to receive an interferon treatment [6]. However, in practice, only half of patients can be expected to have a favorable response to the interferon treatment and some patients could not receive the treatment because of the progressed disease condition or side effect of the treatment [7,8]. Thus, other protective factors for HCC, in even those with hepatitis virus, must be demonstrated.

Recently, the concern with the protective effect of coffee for HCC development has been growing. To the best of

* Corresponding author. Tel.: +81 6 6645 3756; fax: +81 6 6645 3757.
E-mail address: satop@med.osaka-cu.ac.jp (S. Ohfuji).

1386-6346/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.hepres.2006.07.010

our knowledge, an inverse relationship was noted in three population-based cohort studies [9–11] and in two hospital-based case–control studies [12,13]; while no relationship was reported in two case–control studies [14,15]. The previous cohort studies, however, did not determine HCV infection status at baseline and failed to take into consideration the enormous effect of HCV infection. Furthermore, most case–control studies involved controls without liver disease, whose prevalence of HCV infection was quite low and the risk of developing HCC was also extremely low. Therefore, the association with the life-style characteristics detected in these studies might be controversial.

In addition, liver dysfunction is often accompanied by gastro-intestinal disorders or impaired caffeine clearance [16,17], and this may lead to a reduced consumption of coffee. Most previous studies, however, interpreted just from information of coffee drinking at one point without considering the potential changes in coffee consumption associated liver dysfunction. Through a general estimate about the duration between HCV infection and developing liver cirrhosis, i.e., more than 30 years [18–20], coffee drinking in recent past may be affected for liver dysfunction already manifested. This reduction of coffee consumption can bring about the apparent protective effect of coffee for HCC development.

Besides, no study has so far explored the association between coffee and HCC among individuals infected with HCV. Thus, we conducted a hospital-based case–control study, in which both cases and controls were patients with chronic type C liver disease. To consider the potential changes in coffee consumption due to liver disease progression, we collected information on coffee drinking habits separately before and after first identification of liver disease and estimated the risk of HCC based on the consumption during each period.

2. Methods

2.1. Selection of cases and controls

The method of present study was previously described [21]. We identified all consecutive patients with chronic type C liver disease who visited the Department of Hepatology at Osaka City University Hospital (OCUH) for clinical follow up between 1 November 2001 and 31 January 2002 (i.e., recruitment period). Exclusion criteria were as follows: patients with other types of liver disease (e.g., co-infection with HBV, primary biliary cirrhosis, auto-immune hepatitis, idiopathic portal hypertension, etc.); referred patients who had already been diagnosed with HCC at other hospitals; patients in poor health (e.g., liver failure, terminal stage of HCC, etc.). This resulted in 1159 patients who were regarded as a source population from which to identify HCC cases and controls.

There were 86 cases identified from hospital records that were firstly diagnosed with HCC between 1 November 1998 and 31 March 2002. The diagnosis of HCC was based either on histopathologic examination or on a positive result in at least one imaging study (CT, MRI, angiography) combined with an elevated serum alphafetoprotein level. For each case with HCC, we selected one to five control patients, matching for age (±2 years), gender, and the date of first OCUH visit (±2 years). Eventually, 86 cases and 333 controls were identified as candidates.

The study protocol was approved by the ethics committee at the Osaka City University Graduate School of Medicine.

2.2. Information collection

From 1 June 2002 to 31 December 2002 (i.e., study period), the physician-in-charge explained about this study to the candidate cases and controls each time they underwent regular medical examination. After obtaining informed consent verbally, patients were given a self-administered, mail-back questionnaire. We mailed a reminder to the non-respondents two times at monthly intervals. The questionnaire included items on demographic factors, past medical history (including surgery and blood transfusion as a surrogate for relevant infection), age at first identification of liver disease (e.g., abnormality of liver enzyme level or positive results for HCV infection, etc.), family history of liver diseases, smoking, alcohol drinking, consumption of caffeine-containing beverages, dietary habits, occupation, physical exercise, reproductive history, etc. Questions on caffeine-containing beverages (coffee, black tea and green tea) asked about consumption using 8 levels (never, 1–3 cup/month, 1 cup/week, 2–3 cup/week, 4–6 cup/week, 1 cup/day, 2 cup/day and ≥3 cup/day) during both time periods between relevant infection and first identification of liver disease, and between first identification of liver disease and beginning of the study period. These time periods are hereafter referred to as "before" and "after" first identification of liver disease.

Findings of abdominal ultrasonography and laboratory data at first OCUH visit were collected from medical records. At OCUH, findings of abdominal ultrasonography have been scored to show the disease severity on a semi-quantitative scale, designated the "US score". This score was the sum of the five leveled scores (0, 0.5, 1.0, 1.5 and 2.0) for the five variables (i.e., liver deformity, nature of the liver edge, nature of the liver surface, coarsening of intra-hepatic echo signals and size of the spleen). This was evaluated in patients with chronic type C liver disease and proved to be highly correlated with the degree of liver fibrosis according to the new European classification or Child-Turcotte criteria [22]. While we assessed "US score" >5.0 as indicating liver cirrhosis, the sensitivity and specificity of this approach to classifying the presence or absence of liver cirrhosis was estimated to be 83–97% and 91–96%, respectively [23,24]. Laboratory data included white blood cell, red blood cell, platelet count, total-bililbin, aspartate aminotransferase, alanine aminotransferase, total protein, albumin, alphafetoprotein, virus

titer of HCV-RNA, and fasting blood sugar, etc. The information on interferon therapy was also obtained from medical records.

2.3. Data analyses

We considered five variables to identify the timing of the disease course, as follows: age at relevant infection; age at first identification of liver disease; date of first OCUH visit; date of recruitment of study subjects; date at beginning of the study period. For the factor "age at relevant infection", we defined the following: if subjects had received a blood transfusion, the midterm age between the first and last transfusion was regarded as the time of infection. If subjects had not had any transfusions, but had undergone surgery, the midterm age between the first and last surgery was adopted [21].

In addition to the frequency of consumption of caffeine-containing beverages, cumulative consumption (cup/month of beverage × time periods) was also calculated separately before and after first identification of liver disease. The frequency of consumption and cumulative consumption were re-categorized into three levels according to the distribution of consumption for the controls, with the category boundaries drawn so as to make the size of groups as similar as possible.

The Chi-square test and Student's *t*-test were used to compare characteristics between cases and controls. The conditional logistic regression model was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for HCC risk. Trends were estimated as the slope when the categorical variables of interest were treated as quantitative variables. Variables which showed *P*-value less than 0.1 or seemed likely to correlate with coffee consumption were considered to be potential confounders for adjustment. In order to take into account the potential changes in beverage consumption due to liver disease progression, the effect for each beverages was estimated separately before and after first identification of liver disease. In addition, to minimize an apparent protective effect of coffee for HCC risk due to decreased consumption because of liver dysfunction, additional analyses were conducted excluding subjects who reported a lowered coffee intake after first identification of liver disease. This was achieved using an unconditional logistic regression model which included three matching variables along with potential confounders. All statistical analyses were performed using SAS version 8.2 (SAS Institute Inc.).

3. Results

Of 419 identified subjects, 10 (1 case and 9 controls) were subsequently found to be ineligible (e.g., co-infection with HBV, complete recovery from HCV infection, etc.). A further 41 (2 cases and 39 controls) did not visit OCUH during the study period. Information was not obtained from 23 subjects for the following reasons: 4 (3 cases and 1 control) died; 6 (3 cases and 3 controls) were in poor health; 13 (1 case and 12 controls) refused to participate. Of the remaining 345 subjects (76 cases and 269 controls) who answered the questionnaire (94%), a total of 326 (73 cases and 253 controls, 73 matched-set) maintained the initial matched combination, and comprised the subjects for the analysis.

Table 1 shows a comparison of selected characteristics of cases and matched controls. Information on the possible cause of HCV infection (i.e., relevant infection) was obtained from 65% of subjects. Both cases and controls took about 22 years from relevant infection until first identification of liver disease. A significant difference between cases and controls was observed in the mean duration from first identification of liver disease until the beginning of the study period (19.8 years versus 16.7 years). Cases had more family history of liver diseases, and received less interferon therapy (with marginal significance). Laboratory data and "US score" at first OCUH visit, which was 6–7 years before the beginning of the study period, indicated that cases were in a more severe condition than controls in the recent past.

Table 2 shows ORs for HCC according to the frequency of consumption of caffeine-containing beverages, adjusted for duration from first identification of liver disease until the study period, disease severity at first OCUH visit (US score, platelet count, aspartate aminotransferase, albumin, alphafetoprotein, fasting blood sugar) and experience of interferon therapy, etc. Higher coffee consumption before first identification of liver disease, was associated decreased ORs with a trend towards (compared with non-drinkers, OR at <1 cup/day, 0.61; 95% CI: 0.18–2.03 and OR at ≥1 cup/day, 0.38; 95% CI: 0.13–1.12). This negative correlation of coffee intake was more pronounced after identification of liver disease (OR at <1 cup/day, 0.57; 95% CI: 0.20–1.67 and OR at ≥1 cup/day, 0.19; 95% CI: 0.05–0.71) with a significant dose-response relationship ($P = 0.032$). Thus, higher consumption of coffee was associated with lowered ORs with a downward stepwise slope, irrespective of assessment before or after first identification of liver disease and obtained in both univariate and multivariate analysis. Both before and after first identification of liver disease, higher consumption of green tea was associated with elevated ORs, and the dose–response relation was statistically significant with a smaller *P*-value than that for coffee intake. However, the upward slope somewhat fluctuated. There was no association with black tea intake.

Table 3 shows ORs for HCC according to cumulative consumption of caffeine-containing beverages. Stable ORs could not be estimated before first identification of liver disease, since cumulative consumption was calculable for only 65% of subjects because of missing data on relevant infection. After first identification of liver disease, larger cumulative consumption of coffee was also associated with smaller ORs (OR at <5000 cups, 0.48; 95% CI: 0.18–1.29 and OR at ≥5000 cups 0.42; 95% CI: 0.15–1.22). Furthermore, the dose-response relation persisted with a marginal significance ($P = 0.098$). Cumulative intake of green tea suggested a positive association with HCC, but neither the OR at each level nor the result of the trend test achieved statistical signifi-