2.3. 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 antibody 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). Genotyping of *IL-1B* −31T/C and *TNF-A* −1031C/T polymorphisms was carried out by polymerase chain reaction with confronting two-pair primers (PCR-CTPP) according to Hamajima et al. [9,24]. *TNF-A* −308G/A polymorphism was analyzed by PCR-restriction fragment length polymorphism (PCR-RFLP) according to Ho *et al* [17]. We confirmed the above results for several specimens with different genotyping patterns by direct sequencing. In addition, about 10% random samples were rechecked by the same method (PCR-CTPP or PCR-RFLP).

2.4. Statistical analyses

χ^2 tests were used for unadjusted comparisons based on frequency. Unconditional logistic regression analyses were conducted to estimate odds ratios (ORs) and their 95 percent confidence intervals (CIs) of HCC for *IL-1B* and *TNF-A* genotypes and drinking and smoking habits with adjustment for sex, age category (40–49, 50–59, 60–69, and 70–79 years), heavy drinking history (never and ever), smoking status (never, former, and current smokers), and HBsAg and HCVAb status. Tests of linear trend for *IL-1B* and *TNF-A* genotypes were performed by assigning an ordinal variable to the genotype in the logistic model. Interactions between each genotype and alcohol/tobacco on HCC risk were evaluated by the likelihood ratio test. All statistical analyses were performed with

Table 1
Selected characteristics of study subjects

Factor	HCC cases no.(%)	Hospital controls no. (%)	CLD patients no.(%)	P[a]	P[b]
Sex				0.64	<0.01
Male	141 (67.5)	180 (65.5)	205 (53.8)		
Female	68 (32.5)	95 (34.5)	176 (46.2)		
Age (year)				<0.01	<0.01
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)		
HBsAg				<0.01	0.97
Negative	190 (90.9)	269 (97.8)	346 (90.8)		
Positive	19 (9.1)	6 (2.2)	35 (9.2)		
HCVAb				<0.01	0.95
Negative	30 (14.4)	254 (92.4)	54 (14.2)		
Positive	179 (85.7)	21 (7.6)	327 (85.8)		
Heavy drinking history, male				<0.01	<0.01
No	95 (67.4)	158 (87.8)	170 (82.9)		
Yes	46 (32.6)	22 (12.2)	22 (17.1)		
Heavy drinking history, female				0.17	0.37
No	65 (95.6)	94 (99.0)	172 (97.7)		
Yes	3 (4.4)	1 (1.1)	4 (2.3)		
Smoking status, male				0.03	0.07
Never smoker	24 (17.0)	50 (27.8)	54 (26.3)		
Former smoker	51 (36.2)	67 (37.2)	76 (37.1)		
Current smoker	66 (46.8)	63 (35.0)	75 (36.7)		
Smoking status, female				0.70	0.66
Never smoker	61 (89.7)	88 (92.6)	150 (85.2)		
Former smoker	4 (5.9)	3 (3.2)	15 (8.5)		
Current smoker	3 (4.4)	4 (4.2)	11 (6.3)		

[a] Comparisons were made between HCC cases and hospital controls.
[b] Comparisons were made between HCC cases and CLD patients.

Table 2
Adjusted ORs of HCC for IL-1B −31 and TNF-A −1031 and −308 genotypes

	HCC cases no. (%)	Hospital controls no. (%)	CLD patient no. (%)	OR[a],[b] (95% CI)	OR[a],[c](95% CI)
IL-1B −31					
C/C	41 (19.6)	66 (24.0)	95 (25.2)	1.00 (reference)	1.00 (reference)
C/T	101 (48.3)	146 (53.1)	176 (45.7)	0.84 (0.35–2.03)	1.46 (0.90–2.37)
T/T	67 (32.1)	63 (22.9)	110 (29.1)	1.32 (0.48–3.65)	1.46 (0.86–2.47)
P for trend				0.53	0.14
TNF-A −1031					
T/T	151 (72.3)	196 (71.3)	264 (69.3)	1.00 (references	1.00 (reference)
T/C	49 (23.4)	69 (25.1)	109 (28.6)	0.35 (0.16–0.78)	0.75 (0.48–1.15)
C/C	9 (4.3)	10 (3.6)	8 (2.1)	2.35 (0.40–13.75)	1.51 (0.49–4.66)
P for trend				0.15	0.53
TNF-A −308					
G/G	205 (98.1)	270 (98.2)	371 (97.4)	1.00 (reference)	1.00 (reference)
G/A	4 (1.9)	5 (1.8)	10 (2.6)	9.13 (0.91–91.63)	0.86 (0.24–3.10)

[a] Adjusted for sex, age category, HBsAg, HCVAb, heavy drinking history, and smoking status.
[b] Comparisons were made between HCC cases and hospital controls.
[c] Comparisons were made between HCC cases and CLD patient.

the SAS/PC statistical package (SAS Institute Inc., Cary, NC).

3. Results

Basic characteristics of study subjects are shown in Table 1. As compared with at least either control group, HCC cases presented significantly higher proportions of males (against CLD patients), older subjects (against both control groups), HBsAg positives (against hospital controls), HCVAb positives (against hospital controls), males with a heavy drinking history (against both control groups), and male current smokers (against hospital controls).

Table 2 shows the distribution of *IL-1B* and *TNF-A* genotypes among study subjects and associated ORs of HCC against either control group. When HCC cases were

Table 3
Adjusted ORs of HCC for *IL-1B* −31 genotype according to drinking and smoking status

	HCC cases no. (%)	Hospital controls no. (%)	CLD patients no. (%)	OR[a,b] (95% CI)	OR[a,c] (95% CI)
Never drinker					
C/C	12 (15.4)	38 (24.4)	50 (25.5)	1.00 (reference)	1.00 (reference)
C/T	38 (48.7)	80 (51.3)	86 (43.9)	1.50 (0.30–7.45)	1.70 (0.76–3.77)
T/T	28 (35.9)	38 (24.4)	60 (30.6)	1.80 (0.31–10.58)	2.46 (1.05–5.76)
P for trend				0.51	0.03
Current/former drinker					
C/C	29 (22.1)	28 (23.5)	45 (24.3)	1.00 (reference)	1.00 (reference)
C/T	63 (48.1)	66 (55.5)	90 (48.7)	0.63 (0.23–1.77)	1.28 (0.69–2.38)
T/T	39 (29.8)	25 (21.0)	50 (27.0)	1.10 (0.33–3.74)	1.08 (0.54–2.17)
P for trend				0.89	0.79
Never/former smoker					
C/C	26 (18.6)	47 (22.6)	68 (23.1)	1.00 (reference)	1.00 (reference)
C/T	68 (48.6)	111 (53.4)	130 (48.6)	0.76 (0.26–2.20)	1.50 (0.85–2.66)
T/T	46 (32.9)	50 (24.0)	97 (32.9)	1.02 (0.31–3.39)	1.33 (0.72–2.45)
P for trend				0.91	0.44
Current smoker					
C/C	15 (21.7)	19 (28.4)	27 (31.4)	1.00 (reference)	1.00 (reference)
C/T	33 (47.8)	35 (52.2)	46 (53.5)	1.27 (0.16–10.31)	1.53 (0.60–3.90)
T/T	21 (30.4)	13 (19.4)	13 (15.1)	1.76 (0.15–20.50)	2.54 (0.81–7.95)
P for trend				0.65	0.11

[a] Adjusted for sex, age category, HBsAg, HCVAb, and either smoking status or heavy drinking history.
[b] Comparisons were made between HCC cases and hospital controls.
[c] Comparisons were made between HCC cases and CLD patients.

compared with hospital controls, ORs were very unstable after adjustment for HBsAg and HCVAb in addition to other covariates, as evidenced by wide CIs. This was because only 2% and 8% of hospital controls tested positive for HBsAg and HCVAb, respectively. Multiple logistic regression analyses did not reveal any significant trends in HCC risk associated with the *IL-1B* −31 or *TNF-A* −1031 genotype. For the *TNF-A* −308 genotype, the minor A allele was too few to make meaningful analyses.

Table 3 presents the HCC risk for the *IL-1B* −31 genotype according to drinking and smoking status. Based on comparison of HCC cases with CLD patients, we detected a significant trend in HCC risk associated with the genotype among never drinkers ($P = 0.03$); the adjusted ORs (and 95% CIs) for the C/T and T/T genotypes compared with the C/C genotype were 1.70 (0.76–3.77) and 2.46 (1.05–5.76), respectively. Such an association was not observed in former and current drinkers combined. A similar upward tendency was observed among current smokers (P for trend = 0.11); the corresponding ORs (and 95% CIs) were 1.53 (0.60–3.90) and 2.54 (0.81–7.95), respectively. Similar but statistically insignificant results were obtained by the comparison between HCC cases and hospital controls. Regarding the *TNF-A* −1031 and −308 genotypes, we did not find any appreciable differences by drinking or smoking status (data not shown).

Finally, we evaluated if the HCC risk due to heavy alcohol intake and current smoking, both of which were risk factors for HCC in this study [23,25], differed by the *IL-1B* −31 genotype based on comparison of HCC cases with CLD patients (Fig. 1). The HCC risk associated with heavy alcohol intake decreased with increasing T allele; the adjusted ORs (and 95% CIs) for heavy drinking history among C/C, C/T, and T/T carriers were 5.70 (1.72–18.92), 3.13 (1.35–7.24), and 0.80 (0.28–2.33), respectively (P for interaction = 0.10). Conversely, the risk associated with current smoking increased with increasing T allele; the corresponding ORs (and 95% CIs) were 1.62 (0.53–4.95), 2.21 (1.07–4.58), and 5.66 (1.91–16.73), respectively (P for interaction = 0.55).

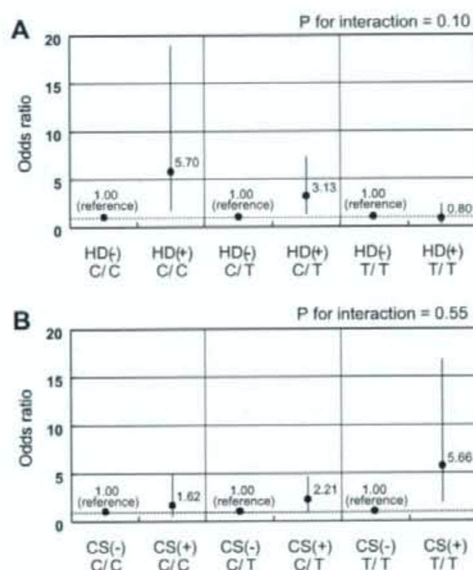

Fig. 1. Adjusted ORs (and 95% CIs) of HCC for either heavy drinking history (HD, Fig. 1A) or current smoking (CS, Fig. 1B) according to *IL-1B* −31 genotype. Comparisons were made between HCC cases and CLD patients, and ORs were adjusted for sex, age category, HBsAg, HCVAb, and either smoking status or heavy drinking history. P values for interaction were calculated by the likelihood ratio test in unconditional logistic regression analysis.

4. Discussion

The present study assessed the association between *IL-1B* and *TNF-A* gene polymorphisms and HCC. IL-1β and TNF-α are proinflammatory cytokines with multiple biological effects and play major roles in inflammation-linked tumor development. Both can induce inflammation, which leads to tissue damage resulting in increased cellular turnover. Nitric oxide and reactive oxygen species from inflammatory cells may induce DNA damage, which increases the possibility of the emergence of cells possessing a high risk of malignant transformation. In addition, these proinflammatory cytokines can activate NF-κB and AP-1 transcription factors, which not only induce inflammatory mediators, cytokines, and growth factors but also regulate cell proliferation, antiapoptosis, and immune response in target tumors and pretumorous cells [8]. Therefore, we hypothesized that gene polymorphisms of *IL-1B* and *TNF-A* might be associated with the HCC risk. Several epidemiological studies have examined this association although the results have been controversial [10–13,17]. In addition, no studies but one [12] evaluated potential interactions between the genes and environmental factors such as alcohol and tobacco.

In this study, from the comparison between HCC cases and CLD patients, we noticed that the HCC risk associated with the *IL-1B* −31T/C polymorphism increased with increasing T allele among never drinkers and current smokers although this polymorphism did not significantly affect the overall risk. Similarly, the risk associated with heavy alco-

hol intake and current smoking was different according to the polymorphism. The *IL-1B* −31T/C polymorphism is included in a TATA-box and has been shown to affect DNA–protein interactions in vitro; the −31T allele, which preserves the TATA-box, was reported to have a five-fold elevated binding activity with the transcription initiation factor [26]. Accordingly, this allele may enhance IL-1β production in the liver and may cause higher susceptibility to HCC.

Alcohol intake and cigarette smoking are also known to influence the production of inflammatory cytokines. There is evidence showing that moderate alcohol intake in humans reduces the production of IL-1β and TNF-α through inhibition of NF-κB [20] and that in vitro exposure of cultured cells to ethanol in high concentrations reduced the IL-1β and TNF-α induced cytokine generation by inhibiting translocation of NF-κB subunits to the nucleus [27]. Smoking has been shown to increase the production of IL-1β and TNF-α by macrophages [21,28]. These properties might contribute to the potential risk modification for the *IL-1B* −31T/C genotype by alcohol and tobacco although the precise mechanisms remain to be elucidated.

The association of the *IL-1B* −31 genotype with HCC was not statistically significant based on comparison between HCC cases and hospital controls although the point estimates of ORs against hospital controls demonstrated similar tendencies with those against CLD patients. One possible reason may be that the ORs against hospital controls became very unstable after adjustment for hepatitis virus markers that were positive in less than 10% of hospital controls.

The *TNF-A* −1031T/C and −308G/A polymorphisms were not associated with HCC in this study. *TNF-A* is known to have five biallelic single-nucleotide polymorphisms in the promoter region at −1031T/C, −863C/A, −857C/T, −308G/A, and −238G/A [16]. In the Japanese, the −308A and −238A alleles are rare [16], and −1031T/C is tightly linked with −863C/A [29]. The −308A allele has been associated with an eight-fold higher transcriptional activation in vitro [30] and higher TNF-α production in human whole blood cell culture stimulated by lipopolysaccharide [31]. A recent study reported a positive association between this polymorphism and HCC [17], but we could not detect such an association. As another candidate, we examined the −1031T/C polymorphism for which a possible difference of *TNF-A* expression [29,32] and potential relationships with several diseases among the Japanese [18,19] were reported, yet no significant relation was observed.

In conclusion, we found that the impact of *IL-1B* −31T/C polymorphism on HCC was different by drinking and smoking status among CLD patients, and this polymorphism appeared to modify the HCC risk in relation to alcohol and tobacco. Further well-designed large studies are required to confirm these results.

Acknowledgment

We 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. This study was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology (Grant No. 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.

References

[1] K. Tanaka, T. Hirohata, S. Koga, K. Sugimachi, T. Kanematsu, F. Ohryohji, H. Nawata, H. Ishibashi, Y. Maeda, H. Kiyokawa, K. Tokunaga, Y. Irita, S. Takeshita, Y. Arase, N. Nishino, Hepatitis C and hepatitis B in the etiology of hepatocellular carcinoma in the Japanese population, Cancer Res. 51 (1991) 2842–2847.

[2] H. Tsukuma, T. Hiyama, S. Tanaka, M. Nakao, T. Yabuuchi, T. Kitamura, K. Nakanishi, I. Fujimoto, A. Inoue, H. Yamazaki, T. Kawashima, Risk factors for hepatocellular carcinoma among patients with chronic liver disease, N. Engl. J. Med. 328 (1993) 1797–1801.

[3] F. Donato, A. Tagger, U. Gelatti, G. Parrinello, P. Boffetta, A. Albertini, A. Decarli, P. Trevisi, M.L. Ribero, C. Martelli, S. Porru, G. Nardi, Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women, Am. J. Epidemiol. 155 (2002) 323–331.

[4] International Agency for Research on Cancer, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 83, Tobacco Smoke and Involuntary Smoking, IARC, Lyon, France, 2004.

[5] S. Takano, O. Yokosuka, F. Imazeki, M. Tagawa, M. Omata, Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients, Hepatology 21 (1995) 650–655.

[6] K. Tarao, Y. Rino, S. Ohkawa, A. Shimizu, S. Tamai, K. Miyakawa, H. Aoki, T. Imada, K. Shindo, N. Okamoto, S. Totsuka, Association between high serum alanine aminotransferase levels and more rapid development and higher

rate of incidence of hepatocellular carcinoma in patients with hepatitis C virus-associated cirrhosis, Cancer 86 (1999) 589–595.

[7] L.M. Coussens, Z. Werb, Inflammation and cancer, Nature 420 (2002) 860–867.

[8] A. Yoshimura, Signal transduction of inflammatory cytokines and tumor development, Cancer Sci. 97 (2006) 439–447.

[9] N. Hamajima, K. Matsuo, T. Saito, K. Tajima, K. Okuma, K. Yamao, S. Tominaga, Interleukin 1 polymorphisms, lifestyle factors, and *Helicobacter pylori infection*, Jpn. J. Cancer Res. 92 (2001) 383–389.

[10] Y. Tanaka, T. Furuta, S. Suzuki, E. Orito, A.E. Yeo, N. Hirashima, F. Sugauchi, R. Ueda, M. Mizokami, Impact of interleukin-1beta genetic polymorphisms on the development of hepatitis C virus-related hepatocellular carcinoma in Japan, J. Infect. Dis. 187 (2003) 1822–1825.

[11] Y. Wang, N. Kato, Y. Hoshida, H. Yoshida, H. Taniguchi, T. Goto, M. Moriyama, M. Otsuka, S. Shiina, Y. Shiratori, Y. Ito, M. Omata, Interleukin-1beta gene polymorphisms associated with hepatocellular carcinoma in hepatitis C virus infection, Hepatology 37 (2003) 65–71.

[12] C.C. Chen, S.Y. Yang, C.J. Liu, C.L. Lin, Y.F. Liaw, S.M. Lin, S.D. Lee, P.J. Chen, C.J. Chen, M.W. Yu, Association of cytokine and DNA repair gene polymorphisms with hepatitis B-related hepatocellular carcinoma, Int. J. Epidemiol. 34 (2005) 1310–1318.

[13] N. Hirankarn, I. Kimkong, P. Kummee, P. Tangkijvanich, Y. Poovorawan, Interleukin-1beta gene polymorphism associated with hepatocellular carcinoma in hepatitis B virus infection, World J. Gastroenterol. 12 (2006) 776–779.

[14] A. Vatay, L. Bene, A. Kovacs, Z. Prohaszka, C. Szalai, L. Romics, B. Fekete, I. Karadi, G. Fust, Relationship between the tumor necrosis factor alpha polymorphism and the serum C-reactive protein levels in inflammatory bowel disease, Immunogenetics 55 (2003) 247–252.

[15] T. Aoki, T. Hirota, M. Tamari, K. Ichikawa, K. Takeda, T. Arinami, M. Shibasaki, E. Noguchi, An association between asthma and TNF−308G/A polymorphism: meta-analysis, J. Hum. Genet. 51 (2006) 677–685.

[16] S. Kamizono, Y. Hiromatsu, N. Seki, T. Bednarczuk, H. Matsumoto, A. Kimura, K. Itoh, A polymorphism of the 5′ flanking region of tumour necrosis factor alpha gene is associated with thyroid-associated ophthalmopathy in Japanese, Clin. Endocrinol. 52 (2000) 759–764.

[17] S.Y. Ho, Y.J. Wang, H.L. Chen, C.H. Chen, C.J. Chang, P.J. Wang, H.H. Chen, H.R. Guo, Increased risk of developing hepatocellular carcinoma associated with carriage of the TNF2 allele of the −308 tumor necrosis factor-alpha promoter gene, Cancer Causes Control 15 (2004) 657–663.

[18] M. Nishimura, S. Kuno, R. Kaji, H. Kawakami, Influence of a tumor necrosis factor gene polymorphism in Japanese patients with multiple system atrophy, Neurosci. Lett. 374 (2005) 218–221.

[19] Y. Soga, F. Nishimura, H. Ohyama, H. Maeda, S. Takashiba, Y. Murayama, Tumor necrosis factor-alpha gene (TNF-alpha) −1031/−863, −857 single-nucleotide polymorphisms (SNPs) are associated with severe adult periodontitis in Japanese, J. Clin. Periodontol. 30 (2003) 524–531.

[20] P. Mandrekar, D. Catalano, B. White, G. Szabo, Moderate alcohol intake in humans attenuates monocyte inflammatory responses: inhibition of nuclear regulatory factor kappa B and induction of interleukin 10, Alcohol Clin. Exp. Res. 30 (2006) 135–139.

[21] P.S. Tappia, K.L. Troughton, S.C. Langley-Evans, R.F. Grimble, Cigarette smoking influences cytokine production and antioxidant defences, Clin. Sci. 88 (1995) 485–489.

[22] I. Ikai, S. Arii, T. Ichida, K. Okita, M. Omata, M. Kojiro, K. Takayasu, Y. Nakanuma, M. Makuuchi, Y. Matsuyama, Y. Yamaoka, Report of the 16th follow-up survey of primary liver cancer, Hepatol. Res. 32 (2005) 163–172.

[23] T. Sakamoto, M. Hara, Y. Higaki, M. Ichiba, M. Horita, T. Mizuta, Y. Eguchi, T. Yasutake, I. Ozaki, K. Yamamoto, S. Onohara, S. Kawazoe, H. Shigematsu, S. Koizumi, K. Tanaka, Influence of alcohol consumption and gene polymorphisms of ADH2 and ALDH2 on hepatocellular carcinoma in a Japanese population, Int. J. Cancer 118 (2006) 1501–1507.

[24] N. Hamajima, A. Shibata, N. Katsuda, K. Matsuo, H. Ito, T. Saito, K. Tajima, S. Tominaga, Subjects with TNF-A-857TT and −1031TT genotypes showed the highest *Helicobacter pylori* seropositive rate compared with those with other genotypes, Gastric Cancer 6 (2003) 230–236.

[25] M. Hara, K. Tanaka, T. Sakamoto, Y. Higaki, T. Mizuta, Y. Eguchi, T. Yasutake, I. Ozaki, K. Yamamoto, S. Onohara, S. Kawazoe, H. Shigematsu, S. Koizumi, Case-control study on cigarette smoking and the risk of hepatocellular carcinoma among Japanese, Cancer Sci. 99 (2008) 93–97.

[26] E.M. El-Omar, M. Carrington, W.H. Chow, K.E. McColl, J.H. Bream, H.A. Young, J. Herrera, J. Lissowska, C.C. Yuan, N. Rothman, G. Lanyon, M. Martin, J.F. Fraumeni Jr., C.S. Rabkin, Interleukin-1 polymorphisms associated with increased risk of gastric cancer, Nature 404 (2000) 398–402.

[27] A.S. Johansson, J. Liden, S. Okret, J.E. Palmblad, Effects of ethanol on cytokine generation and NFkappaB activity in human lung epithelial cell, Biochem. Pharmacol. 70 (2005) 545–551.

[28] S. Nagai, M. Takeuchi, K. Watanabe, H. Aung, T. Izumi, Smoking and interleukin-1 activity released from human alveolar macrophages in healthy subjects, Chest 94 (1988) 694–700.

[29] T. Higuchi, N. Seki, S. Kamizono, A. Yamada, A. Kimura, H. Kato, K. Itoh, Polymorphism of the 5′-flanking region of the human tumor necrosis factor (TNF)-alpha gene in Japanese, Tissue Antigens 51 (1998) 605–612.

[30] A.G. Wilson, J.A. Symons, T.L. McDowell, H.O. McDevitt, G.W. Duff, Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation, Proc. Natl. Acad. Sci. USA 94 (1997) 3195–3199.

[31] E. Louis, D. Franchimont, A. Piron, Y. Gevaert, N. Schaaf-Lafontaine, S. Roland, P. Mahieu, M. Malaise, D. De Groote, R. Louis, J. Belaiche, Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans, Clin. Exp. Immunol. 113 (1998) 401–406.

[32] C.C. Lu, B.S. Sheu, T.W. Chen, H.B. Yang, K.H. Hung, A.W. Kao, C.H. Chuang, J.J. Wu, Host TNF-alpha −1031 and −863 promoter single nucleotide polymorphisms determine the risk of benign ulceration after H. pylori infection, Am. J. Gastroenterol. 100 (2005) 1274–1282.

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

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 ($\geq$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.
© 2006 Elsevier Ireland Ltd. All rights reserved.

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-

Table 1
Comparison of selected characteristics between cases and controls

Characteristics	Level	n (%)		P-value
		Case (N = 73)	Control (N = 253)	
Mean age (years)		68.9	68.3	0.384
Gender	Male	35 (47)	131 (52)	0.434
Possible cause of infection[a]	Transfusion	24 (33)	97 (38)	0.980
	Operation	22 (30)	69 (27)	
Mean duration until first identification of liver disease (years)				
From relevant infection[a]		21.8	21.6	0.926
Mean duration until beginning of the study period (years)				
From relevant infection[a]		38.2	35.5	0.136
From first identification of liver disease		19.8	16.7	0.023
From first OCUH visit		7.1	6.4	0.135
Family history of liver diseases	Present	28 (38)	69 (27)	0.069
Interferon therapy	Present	18 (25)	91 (36)	0.072
Smoking	Never	36 (49)	125 (49)	0.960
	Former	19 (26)	64 (25)	
	Current	18 (25)	64 (25)	
Alcohol drinking	Never	32 (44)	96 (38)	0.137
	Former	23 (32)	67 (27)	
	Current	18 (25)	90 (36)	
Mean volume of cumulative ethanol consumption (g/dL)		232	334	0.396
Body mass index	≥22.5	31 (42)	131 (52)	0.216
Platelet count ($\times 10^4\ \mu L^{-1}$)	<10	29 (40)	28 (11)	0.000
Aspartate aminotransferase (IU/L)	≥80	40 (55)	96 (38)	0.010
Alanine aminotransferase (IU/L)	≥80	48 (66)	143 (57)	0.159
Albumin (g/dL)	<3.5	10 (14)	9 (4)	0.001
Alphafetoprotein (ng/mL)	≥20	25 (34)	37 (15)	0.000
Fasting blood sugar (mg/dL)	≥126	13 (18)	18 (7)	0.005
US score	Severe	51 (70)	90 (36)	0.000

[a] Data from 46 cases and 166 controls because of missing information.

cance. Cumulative consumption of black tea did not indicate an association, either. Thus, these results suggest that coffee drinking (both frequency of intake and cumulative intake) is associated with a decreased risk of HCC.

Additionally, we examined the changes in frequency of consumption of coffee after first identification of liver disease. The proportion of subjects who reported a decreased frequency of coffee drinking was nearly the same in both cases and controls (27% versus 24%). After excluding these subjects from the analysis, ORs inversely associated with frequency of intake of coffee were also observed (OR at <1 cup/day, 0.99; 95% CI: 0.35–2.79; $P = 0.987$ and OR at ≥1 cup/day, 0.35; 95% CI: 0.12–1.06; $P = 0.063$).

4. Discussion

The present study reveals that coffee intake may decrease the risk of HCC among patients with chronic type C liver disease. There are several studies which also report similar results, but they have two major limitations. First, these studies did not adequately control for status of HCV infection although this is an overwhelming risk factor for HCC. Therefore, the association detected between any variables including coffee and HCC might have been largely affected. This problem could occur in cohort studies with no baseline data on HCV infection or in case–control studies with a substantial difference in prevalence of HCV infection between the compared groups. The present study, however, could overcome this limitation, since both cases and controls were patients with HCV infection.

Second, it has been pointed out that the decreased consumption of coffee due to already-developed liver dysfunction may bring about the apparent protective effect of coffee for HCC. However, the present study made it possible to analyze the data on coffee consumption by separating them into two periods, i.e., before and after first identification of liver disease. The decreased OR with increasing coffee intake is shown by the downward stepwise slope during both periods (Table 2). Furthermore, this inverse relationship was also observed even after excluding the subjects who reported a reduced coffee consumption after liver disease identification. Thus, it can be considered that prior coffee consumption influenced subsequent development of HCC.

However, this study may be underpowered to detect relevant association before first identification of liver disease. If there are some potential changes in coffee drinking habit due to progression of liver disease, that habit before first identification of liver disease would represent original true coffee drinking pattern. The negative association between the fre-

Table 2
Odds ratio for hepatocellular carcinoma according to frequency of consumption of the caffeine-containing beverages, calculated before and after first identification of liver disease: Japan

Variable/level	n (%)		Univariate		Multivariate[a]	
	Case (N = 73)	Control (N = 253)	OR (95% CI)	P-value	OR (95% CI)	P-value
Before first identification of liver disease						
Coffee						
Non-drinker	25 (34)	63 (25)	1		1	
<1 cup/day	19 (26)	74 (29)	0.71 (0.35–1.44)	0.343	0.61 (0.18–2.03)	0.420
≥1 cup/day	29 (40)	116 (46)	0.67 (0.36–1.27)	0.219	0.38 (0.13–1.12)	0.078
			Trend: P = 0.235		Trend: P = 0.171	
Black tea						
Non-drinker	31 (43)	124 (49)	1		1	
<2 cup/day	22 (30)	63 (25)	1.30 (0.70–2.40))	0.405	1.26 (0.43–3.71)	0.671
≥2 cup/day	20 (27)	66 (26)	1.12 (0.57–2.19)	0.750	0.68 (0.22–2.13)	0.509
			Trend: P = 0.671		Trend: P = 0.739	
Green tea						
≤1 cup/day	12 (16)	67 (27)	1		1	
2 cup/day	16 (22)	56 (22)	1.67 (0.70–3.98)	0.248	5.90 (1.32–26.3)	0.020
≥3 cup/day	45 (62)	130 (51)	1.93 (0.92–4.04)	0.081	4.08 (1.20–13.9)	0.024
			Trend: P = 0.089		Trend: P = 0.053	
After first identification of liver disease						
Coffee						
Non-drinker	27 (37)	59 (23)	1		1	
<1 cup/day	25 (34)	83 (33)	0.66 (0.34–1.30)	0.234	0.57 (0.20–1.67)	0.307
≥1 cup/day	21 (29)	111 (44)	0.42 (0.21–0.85)	0.016	0.19 (0.05–0.71)	0.014
			Trend: P = 0.016		Trend: P = 0.032	
Black tea						
Non-drinker	28 (38)	111 (44)	1		1	
<2 cup/day	26 (36)	66 (26)	1.31 (0.72–2.39)	0.375	1.67 (0.59–4.69)	0.334
≥2 cup/day	19 (26)	76 (30)	0.88 (0.44–1.77)	0.728	0.64 (0.18–2.33)	0.500
			Trend: P = 0.845		Trend: P = 0.907	
Green tea						
≤1 cup/day	12 (16)	66 (26)	1		1	
2 cup/day	14 (19)	51 (20)	1.63 (0.68–3.93)	0.274	6.95 (1.38–35.2)	0.019
≥2 cup/day	47 (64)	136 (54)	1.98 (0.96–4.10)	0.065	4.93 (1.37–17.8)	0.015
			Trend: P = 0.067		Trend: P = 0.029	

[a] Model includes: duration from first identification of liver disease, body mass index at first identification of liver disease, disease severity at first OCUH visit (US score, platelet count, aspartate aminotransferase, albumin, alphafetoprotein, fasting blood sugar), family history of liver disease, interferon therapy, smoking, alcohol drinking, and other caffeine-containing beverage.

quency of this original drinking pattern and HCC persisted with a marginal significance. Thus, it seems to tell that the natural coffee drinking habit before liver disease may also affect an individual's risk for HCC.

When interpreting the present results, the following three major limitations should be discussed. The first limitation is that a selection bias might be introduced, since the source population consisted of patients who had survived to the recruitment period. The patients who developed HCC but died before the recruitment period were not included in case series, although cases were defined as those patients who had been firstly diagnosed with HCC in the recent past, i.e., within 3 years. However, there are previous studies which report significantly lower mortality rates among daily coffee drinkers than among non-drinkers [25–28]. Supposed a hypothetical situation that cases excluded because of death were included in this study, the prevalence of non-coffee-drinkers would increase in the hypothetical case series and OR would be decrease. Thus, this selection bias may operate to bias the association toward the null, but not lead to exaggerated results.

The second limitation is that the time point of relevant infection could be estimated for only 65% of subjects, although the time since HCV infection is a major risk factor for HCV-associated HCC. Therefore, we performed an analysis controlling for the duration from first identification of liver disease instead of duration since infection. This is because the mean duration from infection to first identification was similar in cases and controls, at 21.8 and 21.6 years, respectively, although there were some missing data (Table 1). Besides, the severity of liver disease at the first OCUH visit was also controlled for in the analysis, under the assumption that the longer the time that has passed since HCV infection, the more severe the liver disease becomes.

Table 3
Odds ratio for hepatocellular carcinoma according to cumulative consumption of the caffeine-containing beverages, calculated before and after first identification of liver disease: Japan

Variable/level (cups)	n (%)		Univariate		Multivariate[a]	
	Case	Control	OR (95% CI)	P-value	OR (95% CI)	P-value
Before first identification of liver disease[b]						
Coffee						
Non-drinker	15 (33)	49 (30)	1		1	
<5000	16 (35)	52 (31)	0.86 (0.35–2.11)	0.739	0.38 (0.05–2.69)	0.331
≥5000	15 (33)	65 (39)	1.26 (0.52–3.04)	0.612	2.95 (0.48–18.1)	0.242
			Trend: $P = 0.589$		Trend: $P = 0.153$	
Black tea						
Non-drinker	18 (39)	84 (51)	1		1	
<1500	16 (35)	42 (25)	2.60 (1.05–6.48)	0.040	2.31 (0.48–11.0)	0.295
≥1500	12 (26)	40 (24)	2.08 (0.71–6.16)	0.184	2.58 (0.46–14.5)	0.283
			Trend: $P = 0.146$		Trend: $P = 0.265$	
Green tea						
<10 000	13 (28)	68 (41)	1		1[d]	
10 000–19 999	14 (30)	34 (21)	3.42 (1.24–9.38)	0.017	60 096 (0.04–8.6×10^{10})	0.128
≥20 000	19 (41)	64 (39)	2.85 (1.12–7.22)	0.028	1 367 280 (0.10–5.9×10^{13})	0.091
			Trend: $P = 0.024$		Trend: $P = 0.101$	
After first identification of liver disease[c]						
Coffee						
Non-drinker	27 (37)	59 (23)	1		1	
<5000	27 (37)	117 (46)	0.51 (0.26–0.98)	0.043	0.48 (0.18–1.29)	0.144
≥5000	19 (26)	77 (30)	0.57 (0.28–1.15)	0.116	0.42 (0.15–1.22)	0.110
			Trend: $P = 0.112$		Trend: $P = 0.098$	
Black tea						
Non-drinker	28 (38)	111 (44)	1		1	
<5000	26 (36)	81 (32)	1.13 (0.62–2.05)	0.698	1.48 (0.60–3.68)	0.397
≥1500	19 (26)	61 (24)	1.12 (0.55–2.28)	0.763	0.81 (0.26–2.53)	0.722
			Trend: $P = 0.729$		Trend: $P = 0.959$	
Green tea						
<10 000	22 (30)	114 (45)	1		1	
10 000–19 999	24 (33)	82 (32)	1.54 (0.78–3.06)	0.217	1.67 (0.58–4.80)	0.338
≥20 000	27 (37)	57 (23)	2.33 (1.19–4.56)	0.014	2.69 (0.77–9.38)	0.120
			Trend: $P = 0.014$		Trend: $P = 0.119$	

[a] Model includes: duration from first identification of liver disease, body mass index at first identification of liver disease, disease severity at first OCUH visit (US score, platelet count, aspartate aminotransferase, albumin, alphafetoprotein, fasting blood sugar), family history of liver disease, interferon therapy, smoking, alcohol drinking, and other caffeine-containing beverage.
[b] Only subjects whose relevant infection was known (case: 46, control: 166).
[c] All subjects (case: 73, control: 253).
[d] Model did not converge because of much explanatory variables.

These adjustments for alternative variables are likely to have at least partially compensated for missing data on the time point of infection.

The third limitation is an information bias resulting from imperfect memory of distant past history of coffee consumption. However, it is hard to believe that cases and controls have a different level of recall stimulus, since the hypothesis that coffee is related to HCC or chronic liver disease is not generally recognized. Thus, this information bias, if any, can be regarded as a non-differential misclassification in which erroneous report of coffee drinking habit similarly occurs among cases and controls. Such misclassification leads to an underestimate of the association because of the diluting effect and does not materially affect to validity of the study results [29].

It is also conceivable that other life-style characteristics can account for the protective effect of coffee. In fact, there are studies which report on the correlation between coffee drinking and other life-style characteristics, such as tobacco smoking and habitual alcohol drinking [9,26]. However, the present results were obtained after adjustment for the potential confounders (e.g., alcohol drinking, smoking, BMI, and diabetes mellitus, etc.), and the results are consistent with the previous studies conducted in different populations with different culture or life style [12,13].

As to the mechanism, several previous papers suggest that coffee drinking might improve the activity of liver enzyme [30–39], decrease the risk of liver cirrhosis [40–42] and lower the mortality from liver cirrhosis [25–28]. Thus, it seems quite probable that coffee acts to mitigate the inflammation

of liver cells, suppress the aggravation of liver disease and, as a result, prevent the development of HCC. Regarding the principal ingredients, the brewing method of coffee seems to be important. Previous studies showing results similar to ours were performed in countries where most individuals drink filtered coffee [12,13]. It is therefore likely that the key substances are included in filtered coffee. However, no further discussion is meaningful, since we did not obtain any information on the type of coffee drunk. In the present study, neither green tea nor black tea showed an inverse association with HCC. It is therefore possible to infer that ingredients other than caffeine are responsible.

The positive association between green tea and HCC, although statistically insignificant when considering cumulative consumption (Table 3), was an unexpected finding in the light of previous reports. These studies have suggested no relation of green tea to liver enzyme level [31,32], HCC [9,10] and any cancers [43]. Our results can possibly be criticized because of the following two weaknesses. First, the categorization of the frequency of consumption was inappropriate for green tea, although appropriate for coffee or black tea. The highest open-ended category of $\geq$3 cup/day was too broad to separate heavy users of green tea, whereas most of previous studies draw a boundary at $\geq$5 cup/day as the highest level. Furthermore, there were too few non-drinkers to serve as the reference category for calculating OR. Second, a reverse causality or an information bias might have affected the present results, since many Japanese recognize that green tea is good for health. It is therefore quite likely that patients with severe disease drank more green tea or have greater recall about past green tea consumption than controls. Thus, it seems sensible to have reservations about this positive relationship.

In conclusion, the present study shows that coffee intake decreases the risk of HCC among patients with chronic type C liver disease. This negative relationship could not be explained by reduced consumption due to liver disease progression. This effect might be attributable to substances other than caffeine, since neither black tea nor green tea had the same effect. It is still premature to recommend drinking coffee to patients with chronic type C liver disease, since a limited number of studies of coffee and HCC risk have so far reported from few countries. Thus, further studies are needed to confirm this association.

References

[1] Stewart BW, Kleihues P, editors. World cancer report. Lyon: IARC Press; 2003.

[2] Srivatanakul P, Spriplung H, Deerasamee S. Epidemiology of liver cancer: an overview. Asian Pacific J Cancer Prev 2004;5:118–25.

[3] Lee C, Gong Y, Brok J, Boxall EH, Gluud C. Effect of hepatitis B immunisation in newborn infants of mothers positive for hepatitis B surface antigen: systematic review and meta-analysis. Br Med J 2006;332:328–36.

[4] Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. Gastroenterology 2004;127:1372–80.

[5] Wada I, Hara T, Kajihara S, et al. Population-based study of hepatitis C virus infection and hepatocellular carcinoma in western Japan. Hepatol Res 2002;23:18–24.

[6] Nishiguchi S, Kuroki T, Nakatani S, et al. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet 1995;21(346):1051–5.

[7] Gonzalez-Peralta RP, et al. Interferon alfa-2b in combination with ribavirin for the treatment of chronic hepatitis C in children: efficacy, safety, and pharmacokinetics. Hepatology 2005;42:1010–8.

[8] Heathcote EJ, Shiffman ML, Cooksley WG, et al. Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. N Engl J Med 2000;343:1673–80.

[9] Inoue M, Yoshimi I, Sobue T, Tsugane S. Influence of coffee drinking on subsequent risk of hepatocellular carcinoma: prospective study in Japan. J Natl Cancer Inst 2005;97:293–300.

[10] 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.

[11] Kurozawa Y, Ogimoto I, Shibata A, et al. Dietary habit 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.

[12] Gelatti U, et al. Coffee consumption reduces the risk of hepatocellular carcinoma independently of its aetiology: a case–control study. J Hepatol 2005;42:528–34.

[13] Gallus S, Bertuzzi M, Tavani A, et al. Does coffee protect against hepatocellular carcinoma? Br J Cancer 2002;87:956–9.

[14] Kuper H, Tzonou A, Kaklamani E, et al. Tabacco smoking, alcohol consumption and their interaction in the causation of hepatocellular carcinoma. Int J Cancer 2000;85:498–502.

[15] Vecchia CL, Ferraroni M, Negri E, et al. Coffee consumption and digestive tract cancers. Cancer Res 1989;49:1049–51.

[16] Cheng WSC, Murphy TL, Smith MT, Cooksley WGE, Halliday JWH, Powell LWP. Dose-dependent pharmacokinetics of caffeine in humans: relevance as a test of quantitative liver function. Clin Pharmacol Ther 1990;47:516–24.

[17] Desmond PV, Patwardhan RV, Johnson RF, Schenker S. Impaired elimination of caffeine in cirrhosis. Dig Dis Sci 1980;25:193–7.

[18] Lauer GM, Walker BD. Hepatitis C virus infection: review article. N Engl J Med 2001;345:41–52.

[19] Poynard T, Bedossa P, Opolon P, for the OBSVIRC, METAVIR, CLINIVIR and DOSVIRC groups. Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet 1997;349:825–32.

[20] EASL international consensus conference on hepatitis C: consensus statement. J Hepatol 1999; 30:956–61.

[21] Fukushima W, Tanaka T, Ohfuji S, et al. Does alcohol increase the risk of hepatocellular carcinoma among patients with hepatitis C virus infection? Hepatol Res 2006;34:141–9.

[22] Habu D, Nishiguchi S, Enomoto M, et al. Ultrasonographic diagnosis of degree of chronic type C liver disease. Hepatogastroenterology 2005;52:1820–4.

[23] Kurioka N, Asai H, Harihara S, Yamamoto S. Ultrasonic diagnosis of hepatic cirrhosis—a comparison with peritoneoscopic and histological findings. Kan Tan Sui 1985;10:383–9 [in Japanese].

[24] Ohtake K. Evaluation of criteria on liver cirrhosis used for ultrasonic mass survey. Osaka city Med J 1991;40:173–94 [in Japanese].

[25] Tverdal A, Skurtveit S. Coffee intake and mortality from liver cirrhosis. Ann Epidemiol 1995;13:419–23.

[26] Klatsky AL, Armstrong MA. Alcohol, smoking, coffee, and cirrhosis. Am J Epidemiol 1992;136:1248–57.

[27] Klatsky AL, Armstrong MA, Friedman GD. Coffee, tea, and mortality. Ann Epidemiol 1993;3:375–81.

[28] Kurozawa Y, et al. Coffee and risk of death from hepatocellular carcinoma in a large cohort study in Japan. Br J Cancer 2005;93:607–10.

[29] Rothman KJ, Greenland S. Nondifferential misclassification. Mod Epidemiol 1998:127–32.

[30] Sharp DS. Re: "Alcohol, smoking, coffee, and cirrhosis" and "coffee and serum gamma-glutamyltransferase: a study of self-defense officials in Japan": letters to the editor. Am J Epidemiol 1995;141:480–2.

[31] 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.

[32] 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.

[33] 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.

[34] 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.

[35] Nilssen O, Forde OH. Seven years longitudinal population study of change in gamma-glutamyltransferase: the Tromso study. Am J Epidemiol 1994;139:787–92.

[36] Nilssen O, Forde OH, Brenn T. The Tromso study: distribution and population determinants of gamma-glutamyltransferase. Am J Epidemiol 1990;132:318–26.

[37] 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.

[38] Casoglia E, Spolaore P, Ginocchio G, Ambrosio GB. Unexpected effects of coffee consumption on liver enzymes. Eur J Epidemiol 1993;9:293–7.

[39] Poikolainen K, Vartiainen E. Determinants of γ-glutamyltransferase: positive interaction with alcohol and body mass index, negative association with coffee. Am J Epidemiol 1997;146:1019–24.

[40] Corrao G, Zambon A, Bagnardi V, D'amicis A, Klatsky A, Collabrative Sidecir Group. Coffee, caffeine, and the risk of liver cirrhosis. Ann Epidemiol 2001;11:458–65.

[41] Gallus S, Tavani A, Negri E, Vecchia C. Does coffee protect against liver cirrhosis? Ann Epidemiol 2002;12:202–5.

[42] Corrao G, 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.

[43] Nagano J, Kono S, Preston DL, Mabuchi K. A prospective study of green tea consumption and cancer incidence, Hiroshima and Nagasaki (Japan). Cancer Causes Contr 2001;12:501–8.

Hepatology Research 2008; 38: 860–868 doi: 10.1111/j.1872-034X.2008.00355.x

Original Article

Does a late evening meal reduce the risk of hepatocellular carcinoma among patients with chronic hepatitis C?

Satoko Ohfuji,[1] Wakaba Fukushima,[1] Takashi Tanaka,[4] Daiki Habu,[2] Tadashi Takeda,[2] Akihiro Tamori,[2] Hiroki Sakaguchi,[2] Shuichi Seki,[2] Norifumi Kawada,[2] Shuhei Nishiguchi,[5] Susumu Shiomi[3] and Yoshio Hirota[1]

Departments of [1]Public Health, [2]Hepatology and [3]Nuclear Medicine, Osaka City University Graduate School of Medicine, [4]Department of Internal Medicine, Hoai Hospital, Osaka, and [5]Departement of Hepatobiliary and Pancreatic Disease, Hyogo Medical College, Hyogo, Japan

Aim: Some studies have suggested that nutritional support might protect against the recurrence of hepatocellular carcinoma (HCC) among postoperative HCC patients. However, no epidemiological studies have evaluated the effect of nutritional support on HCC incidence. This study aimed to investigate the association between a late evening meal and HCC.

Methods: We conducted a hospital-based, case-control study comparing 73 cases with HCC to 253 matched controls among patients with chronic hepatitis C. A questionnaire elicited information on the consumption of a late evening meal, which was defined as a snack or meal within 2 h before bedtime. The odds ratios (OR) and 95% confidence intervals (CI) were calculated by the conditional logistic regression model.

Results: After adjustment for potential confounders, patients who consumed a late evening meal had a lower OR as compared to those who did not consume one (OR, 0.08; 95% CI, 0.01–0.48). In terms of frequency of intake, a clear inverse exposure–response relationship was observed (trend $P = 0.009$). In addition, a negative association between a late evening meal and HCC was more pronounced among patients with an α-fetoprotein level of less than 20 ng/mL and those with a body mass index of less than 25 kg/m^2.

Conclusion: A late evening meal might protect against HCC, particularly among patients with a normal α-fetoprotein level and who are not obese, although these relations might be accounted for other factors, including total energy intake. Further studies with larger study sizes are needed to corroborate these findings.

Key words: case-control study, hepatitis C virus, hepatocellular carcinoma, late evening meal, risk factor

INTRODUCTION

PROTEIN–ENERGY MALNUTRITION is often observed in patients with advanced liver cirrhosis because of nutritional and metabolic abnormalities.[1-3] Several previous papers suggested that protein–energy malnutrition is significantly associated with the development of life-threatening complications and increased mortality.[4-7] In particular, nocturnal starvation in those with liver cirrhosis seems to be an important problem because a severe catabolic state is present overnight.[8] One study showed that nocturnal starvation might be a potential risk factor for the aggravation of liver disease.[9]

To improve nocturnal starvation, current guidelines recommend late evening snacks for patients with cirrhosis,[1,10] and therefore, the administration of branched chain amino acids (BCAA) or divided meal, partly consumed as a late evening snack, is now often prescribed. Previous studies have consistently demonstrated that BCAA administration corrects malnutrition in patients with cirrhosis.[11,12] Administration before bedtime seems to be most effective in terms of nutritional metabolism.[13-15] Recent studies have also suggested that BCAA might decrease mortality among patients with liver cirrhosis.[16] Before BCAA prescription for patients with cirrhosis became popular, carbohydrate-rich snacks were considered as a late evening snack. Carbohydrate-rich snacks also improve nitrogen balance and abnormal fuel metabolism in patients with cirrhosis.[8,17-19]

Correspondence: Dr Satoko Ohfuji, Department of Public Health, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan. Email: satop@med.osaka-cu.ac.jp

Received 21 July 2007; revision 8 December 2007, 16 January 2008, 17 February 2008; accepted 19 February 2008.

© 2008 The Japan Society of Hepatology

A previous study indicated that a late evening meal, including carbohydrate-rich snacks, had the same effect as BCAA administration,[8] although a recent randomized, controlled trial suggested that the impact of BCAA administration on the improvement of nutritional parameters was superior to that of ordinary food containing matched daily energy and protein intake.[20]

However, few studies have investigated the long-term effect of nutritional support on hepatocellular carcinoma (HCC) development. To the best of our knowledge, only two studies have evaluated the effect of nutritional intervention on the risk of HCC recurrence among postoperative HCC patients.[21,22] In these studies, the intervention group had a significantly lower recurrence rate when compared to the control group. These reports suggested that nutritional support might act to prevent HCC occurrence. Thus we conducted a case-control study to examine the hypothesis that nutritional support might reduce the risk of HCC incidence. The present study took special notice of a late evening meal as a nutritional factor since this has been considered to be one of the most effective approaches for improvement of nocturnal starvation. In Japan, 80% of HCC cases are caused by hepatitis C virus (HCV) infection,[23] so the source population was restricted to patients with chronic type C liver disease.

METHODS

Selection of cases and controls

THE METHOD OF the present study has been described elsewhere.[24,25] We identified all consecutive patients with chronic hepatitis C who visited the Department of Hepatology of Osaka City University Hospital (OCUH; Osaka, Japan) for clinical follow up between 1 November 2001 and 31 January 2002 (the recruitment period). The following patients were excluded: patients with other types of liver disease (e.g. co-infection with hepatitis B virus, primary biliary cirrhosis, autoimmune hepatitis, and idiopathic portal hypertension), referred patients who had already been diagnosed with HCC at other hospitals, and patients in poor health (e.g. liver failure and terminal stage of HCC). This resulted in 1159 patients who were regarded as a source population.

From the source population, 86 patients were first diagnosed with HCC between 1 November 1998 and 31 March 2002. The diagnosis of HCC was based either on a histopathological examination or a positive result in at least one imaging study (computed tomography, magnetic resonance imaging, angiography) combined with an elevated serum á-fetoprotein level. For each HCC case, we selected 1–5 control patients, matching for age (±2 years), sex, and the date of the 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.

Information collection

From 1 June 2002 to 31 December 2002 (the study period), the physician-in-charge explained this study to the candidate cases and controls each time they underwent regular medical examinations. After obtaining informed consent verbally, the physician-in-charge gave the patients a self-administered, mail-back questionnaire. We mailed reminders to non-respondents twice at 1-month intervals. The questionnaire included items on demographic factors; past medical history; age of first identification of liver disease (e.g. abnormality of liver enzyme level or positive results for HCV infection); family history of liver diseases; smoking; alcohol drinking; dietary habits, including a late evening meal; occupation; physical exercise; and reproductive history. A late evening meal was defined as a snack or meal within 2 h before bedtime. The habit of eating a late evening meal after first identification of liver disease was investigated retrospectively by reporting a dichotomous answer (yes or no). Patients who answered "yes" also reported the average weekly frequency of eating a late evening meal and the major food items they consumed.

We also collected the findings of abdominal ultrasonography and laboratory data at the first OCUH visit from medical records. At OCUH, the findings from the abdominal ultrasonography had been scored to show disease severity on a semiquantitative scale called the "US score." This score is the sum of the five leveled scores (0, 0.5, 1.0, 1.5, and 2.0) for five variables (liver deformity, nature of the liver edge, nature of the liver surface, coarsening of intrahepatic 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.[26] While US scores ≥3.5 indicated chronic liver disorders and US scores >5.0 indicated liver cirrhosis, the sensitivity and specificity of this approach to classifying the presence or absence of liver cirrhosis were estimated to be 83–97% and 91–96%, respectively.[27,28] Laboratory data included white blood cell, red blood cell, platelet

© 2008 The Japan Society of Hepatology

count, total bilirubin, aspartate aminotransferase, alanine aminotransferase, total protein, albumin, α-fetoprotein, virus titer of HCV-RNA, and fasting blood sugar. Information about interferon therapy was obtained from medical records.

Data analyses

The frequency of intake of a late evening meal was recategorized into three levels according to the distribution of controls, with category boundaries that were drawn to make the size of groups as equal as possible. The χ^2-test and Wilcoxon rank sum test were used to compare selected characteristics between cases and controls. To consider the presence of confounding, the distribution of potential confounders was compared between patients who consumed a late evening meal and those who did not only among the control patients using χ^2-test or Wilcoxon rank sum test. The conditional logistic regression model was used to calculate the odds ratios (OR) and 95% confidence intervals (CI) for HCC risk. Variables that showed *P*-values less than 0.1 or seemed to correlate with the late evening meal were considered to be potential confounders for adjustment.

We performed an additional analysis to consider the effect of possible confounding variables, such as markers of progression of liver disease, potential malnutrition, obesity, and treatment with interferon. In the additional stratified analyses, patients were divided into two groups according to the following cut-off point: median level of US score, presence or absence of suspected liver cirrhosis (ratio of aspartate to alanine aminotransferase >1.0,[29-31] platelet count $<10 \times 10^4/\mu l$[29]), normal level of α-fetoprotein, median level of serum albumin, and presence or absence of obesity.[32] In the stratified analyses, the unconditional logistic regression model was used to calculate OR and 95% CI of a late evening meal for HCC. Each model included three matching factors (i.e. age, sex, and duration from first OCUH visit) and the potential confounders other than stratified factors. The homogeneity of OR across stratified categories was tested as the *P*-value of the interaction term between a late evening meal and each stratified variable.

All statistical analyses were performed using SAS version 8.2 (SAS Institute, Cary, NY, USA).

RESULTS

AMONG THE 419 enrolled patients, 51 were excluded. Ten patients were subsequently found to be ineligible (e.g. co-infection with HBV and complete recovery from HCV infection) and 41 patients did not visit OCUH during the study period. There were 23 non-respondents (6%) for the following reasons: death (4 patients: 3 cases and 1 control), poor health (6 patients: 3 cases and 3 controls), and refusal to participate (13 patients: 1 case and 12 controls). Eventually, 326 patients (73 cases and 253 controls, 73 matched sets) maintained the initial matched combination and comprised the patients for the analysis.

Table 1 shows the selected characteristics of the cases and controls. Cases and controls were well matched for age, sex, and duration from the first OCUH visit until the beginning of the study. A significant difference between cases and controls was observed in the duration from first identification of liver disease until the beginning of the study period (17 *vs* 13 years). Cases had more family history of liver diseases and received less interferon therapy with marginal significance. Laboratory data and US scores at the first OCUH visit indicated that cases had more severe disease condition than controls during the 7 years before the beginning of the study period.

Table 2 provides the distribution of selected potential confounders between patients who had consumed a late evening meal and those who did not among the control patients. No measurable differences were found in the distribution of potential confounders, including liver disease progression, body mass index, and interferon therapy, across the groups who did or did not consume a late evening meal.

Table 3 shows the OR for HCC according to late evening meal, adjusted for duration from first identification of liver disease, disease severity at the first OCUH visit (US score, platelet count, aspartate aminotransferase, α-fetoprotein, and fasting blood sugar), and interferon therapy. The group who consumed a late evening meal had a reduced risk of HCC as compared to those who did not consume one (OR, 0.08; 95% CI, 0.01–0.48). In addition, higher frequency intake of a late evening meal was associated with lower OR with a significant dose–response relationship (trend $P = 0.009$). Thus a late evening meal was associated with a lower risk of HCC.

The inverse associations of a late evening meal with the development of HCC did not differ between groups with or without possible liver cirrhosis (Table 4). When the study patients were divided according to the absence or presence of possible liver cirrhosis (e.g. a platelet count of less than $10 \times 10^4/\mu l$ or ratio of aspartate to alanine aminotransferase of more than 1.0), no measurable difference was observed in the inverse association

© 2008 The Japan Society of Hepatology

Table 1 Comparison of selected characteristics between cases and controls†

Characteristics	Case ($n = 73$)	Control ($n = 253$)	*P*-value‡
Age (years)	69 (65–73)	69 (65–72)	0.389
Sex (%)			
Male	47	52	0.434
Duration until beginning of the study (years)			
From first identification of liver disease	17 (12–26)	13 (10–21)	0.011
From first OCUH visit	7 (4–9)	7 (4–9)	0.289
Family history of liver diseases (%)			
Present	38	27	0.069
Interferon therapy (%)			
Present	25	36	0.072
Body mass index (kg/m^2)	22 (21–26)	23 (21–25)	0.986
Platelet count (×10^4/μL)	11 (8–15)	16 (12–20)	0.000
Aspartate aminotransferase (IU/L)	86 (59–112)	67 (43–101)	0.003
Albumin (g/dL)	3.8 (3.6–4.1)	4.1 (3.9–4.3)	0.000
Alpha-fetoprotein (ng/mL)	15 (7–36)	5 (4–11)	0.000
Fasting blood sugar (mg/dL)	100 (94–118)	98 (92–108)	0.066
US score	4.0 (3.0–5.5)	3.0 (2.0–3.5)	0.000

†Data are expressed as median (inter-quartile range) unless otherwise indicated. ‡χ^2-test and Wilcoxon rank sum test were used where appropriate. OCUH, Osaka City University Hospital; US score, ultrasonography score.

Table 2 Comparison of selected characteristics between patients who consumed a late evening meal and those who did not among control patients†

Characteristics	Patients who consumed a late evening meal ($n = 46$)	Patients who did not consume a late evening meal ($n = 207$)	*P*-value‡
Age (years)	68 (64–74)	69 (65–72)	0.960
Sex (%)			
Male	52	52	0.953
Duration until beginning of the study (years)			
From first identification of liver disease	16 (11–26)	13 (10–21)	0.152
From first OCUH visit	7 (4–9)	7 (4–9)	0.974
Family history of liver diseases (%)			
Present	28	27	0.868
Interferon therapy (%)			
Present	37	36	0.877
Body mass index (kg/m^2)	23 (21–25)	23 (21–25)	0.842
Platelet count (×10^4/μL)	17 (11–21)	16 (12–19)	0.677
Aspartate aminotransferase (IU/L)	66 (41–96)	67 (47–101)	0.482
Albumin (g/dL)	4.1 (3.8–4.3)	4.1 (3.9–4.3)	0.352
α-Fetoprotein (ng/mL)	5 (4–10)	6 (4–11)	0.452
Fasting blood sugar (mg/dL)	99 (93–110)	98 (92–107)	0.330
US score	3.0 (2.0–4.0)	3.0 (2.5–3.5)	0.471

†Data are expressed as median (interquartile range) unless otherwise indicated. ‡χ^2-test and Wilcoxon rank sum test were used where appropriate. OCUH, Osaka City University Hospital; US score, ultrasonography score.

© 2008 The Japan Society of Hepatology

Table 3 Odds ratio (OR)† for hepatocellular carcinoma according to frequency of intake of a late evening meal

Characteristics	Level	Case ($n = 73$) n (%)	Control ($n = 253$) n (%)	Univariate			Multivariate‡		
				OR	(95% CI)	*P*-value	OR	(95% CI)	*P*-value
Late evening meal	Never	66 (90)	207 (82)	1			1		
	Intake	7 (10)	46 (18)	0.47	(0.20–1.07)	0.071	0.08	(0.01–0.48)	0.005
Frequency	Never	66 (90)	207 (82)	1			1		
	<4 times/week	6 (8)	26 (10)	0.70	(0.28–1.75)	0.440	0.12	(0.02–1.02)	0.052
	≥4 times/week	1 (1)	20 (8)	0.16	(0.02–1.19)	0.073	0.06	(0.01–0.57)	0.015
					(Trend $P = 0.041$)			(Trend $P = 0.009$)	

†Calculated by the conditional logistic regression model. ‡Model includes: duration from first identification of liver disease, body mass index at first identification of liver disease, severity of liver disease at first Osaka City University Hospital visit (ultrasonography score, platelet count, aspartate aminotransferase, albumin, α-fetoprotein, fasting blood sugar), family history of liver disease, and interferon therapy. CI, confidence intervals.

of a late evening meal with HCC across the groups, although valid estimates could not be calculated in the assessment of the ratio of aspartate to alanine aminotransferase of more than 1.0. As for the α-fetoprotein level, the relationship between a late evening meal and HCC was demonstrated with smaller OR among patients with a normal α-fetoprotein level. Regarding albumin level or obesity, inverse associations of a late evening meal with HCC were more pronounced in patients with an albumin level less than 4 g/dL and those with a body mass index less than 25 kg/m^2. Furthermore, the interaction between body mass index and a late evening meal for HCC was statistically significant ($P = 0.022$ for the homogeneity of OR). A late evening meal indicated a smaller OR for HCC risk irrespective of the absence or presence of a history of interferon therapy, although the relationship reached statistical significance only in patients without a history of interferon therapy.

The results from stratified analyses suggested that the interaction between a late evening meal and α-fetoprotein level, albumin level, or body mass index existed. In that case, it may be more appropriate that the interaction terms were included in the overall multivariate analyzes. Thus we conducted additional multivariate analyses in which each interaction term was added as an adjustment. When the interaction term between a late evening meal and α-fetoprotein was included in the multivariate analysis, OR of a late evening meal was almost similar with the results in Table 3 (OR, 0.02; 95% CI = 0.00–1.21; $P = 0.062$). Considering the interaction with albumin level, OR were nearly the same as the results in Table 3 (OR, 0.09; 95% CI = 0.01–0.62; $P = 0.014$). When we included the interaction term between a late evening meal and body mass index, the model did not converge because there was only one case who consumed a late evening meal and had a body mass index of less than 25 kg/m^2. Thus we could not simultaneously consider these three interactions. In order to consider these interactions, further large-scale studies are needed.

DISCUSSION

THE PRESENT RESULTS support the hypothesis that a late evening meal may decrease the risk of HCC. This finding is consistent with those of previous studies in which nutritional intervention was associated with a lowered recurrence rate of HCC among postoperative HCC patients.[18,19] In addition, a past experimental study indicated that higher administration of a nutritional

© 2008 The Japan Society of Hepatology

Table 4 Adjusted odds ratio (OR) of late evening meal intake for hepatocellular carcinoma stratified according to selected potential confounders

Stratified category	Proportion of late evening meal intake		OR†‡	(95% CI)	*P*-value	Homogeneity of OR across stratified categories§
	Case n/N (%)	Control n/N (%)				
US score (Severity of liver disease)						
<3.5¶	0/20 (0)	27/162 (17)	–	–	–	0.951
3.5+	6/51 (12)	19/90 (21)	0.39	(0.11–1.37)	0.142	
Ratio of aspartate to alanine aminotransferase						
<1.0	6/57 (11)	33/194 (17)	0.50	(0.17–1.47)	0.209	0.947
1.0+¶	1/16 (6)	13/59 (22)	–	–	–	
Platelet count ($\times 10^4/\mu L$)						
10	4/42 (10)	36/225 (16)	0.36	(0.08–1.66)	0.191	0.563
<10	3/29 (10)	10/28 (36)	0.26	(0.04–1.53)	0.136	
α-Fetoprotein (ng/mL)						
20.0+	5/25 (20)	6/37 (16)	0.75	(0.10–5.62)	0.779	0.067
<20.0	2/44 (5)	37/194 (19)	0.02	(0.001–0.36)	0.007	
Albumin level (g/dL)						
4.0+	3/30 (10)	25/167 (15)	0.72	(0.16–3.23)	0.665	0.148
<4.0	4/43 (9)	21/86 (24)	0.13	(0.02–0.68)	0.016	
Body mass index (kg/m^2)						
25.0+	5/18 (28)	10/54 (19)	1.59	(0.22–11.2)	0.644	0.022
<25.0	1/53 (2)	36/198 (18)	0.05	(0.01–0.44)	0.008	
History of interferon therapy						
Absent	6/55 (11)	29/162 (18)	0.27	(0.08–0.99)	0.048	0.672
Present	1/18 (6)	17/91 (19)	0.15	(0.01–2.76)	0.203	

†Calculated by unconditional logistic regression model. ‡Model includes three matching factors (age, sex, and duration from first OCUH visit) and the following potential confounders other than stratified factor: body mass index at first identification of liver disease, severity of liver disease at first OCUH visit (US score, platelet count, aspartate aminotransferase, albumin, α-fetoprotein, fasting blood sugar), family history of liver disease, duration from first identification of liver disease, and interferon therapy. §Homogeneity of OR across stratified categories was tested as the *P*-value of the interaction term between a late evening meal and each stratified variable. ¶Model did not converge because there were no cases or too limited cases who consumed a late evening meal. CI, confidence intervals; OCUH, Osaka City University Hospital visit US score; ultrasonography score.

factor prevented human HCC cells from increasing.[33] Thus it seems reasonable to infer that a late evening meal has a protective effect against HCC.

It is important to clarify the optimal timing of nutritional support. Some studies have indicated that starting nutritional support in the early stage of cirrhosis may be useful in improving nutritional parameters.[34,35] In the present stratified analyses, the protective impact of a late evening meal was observed irrespective of the presence or absence of possible liver cirrhosis. The inverse effect of a late evening meal for HCC development was more pronounced among patients with an α-fetoprotein level of less than 20 ng/mL. About this association, we considered the following: (i) patients with a higher α-fetoprotein level might have a higher risk for HCC development. This background caused difficulty in the detection of the negative association with a late evening meal among these patients, but ease in determining the relationship among those with a normal α-fetoprotein level; (ii) potentially undetectable HCC cells might be developed among patients with a higher α-fetoprotein level. A late evening meal might no longer operate on the prevention of HCC among these patients. Contrary to this, the impact of late evening meal might be more easily demonstrated among those with a normal α-fetoprotein level. Further studies with larger study sizes are needed to corroborate these findings in order to consider the underlying mechanisms.

As for the interaction between a late evening meal and body mass index, the inverse associations of a late evening meal with HCC were further pronounced in

© 2008 The Japan Society of Hepatology

patients with a body mass index less than 25 kg/m^2. It was recently indicated that obesity might be a risk factor of HCC development. Thus it brought about difficulties in the detection of the negative association with a late evening meal among the obesity group, but ease in demonstrating the decreasing OR of a late evening meal for HCC among patients with a body mass index less than 25 kg/m^2. A recent randomized, controlled trial among patients with decompensated liver cirrhosis demonstrated that the impact of BCAA in reducing the risk of liver cancer is superior to that of the ordinary food group among patients with a body mass index of more than 25 kg/m^2, although there was no difference in the risk of HCC between BCAA and ordinary food among those with a body mass index below 25 kg/m^2.[36] Taken together, these findings seem to indicate that a late evening meal has a preventive effect against HCC to the same extent as BCAA administration among patients who are not obese, while the effect of a late evening meal for HCC prevention is less than that of BCAA among those who are obese. It is therefore likely that BCAA and a late evening meal exert their effects by different mechanisms among patients who are obese.

The effect of a late evening meal was found to be statistically significant only in patients without a history of interferon therapy. However, point estimates of the effect of a late evening meal were similar in the absence or presence of a history of interferon. Thus decreased statistical power in the category of the presence of interferon therapy (i.e. only a small number of patients had experienced interferon therapy) might be responsible for the lack of statistical significance.

Regarding the mechanism of a late evening meal in HCC prevention, several previous studies indicated that malnutrition, including nocturnal starvation, is related to a poorer prognosis of liver cirrhosis[4–9] and that a late evening meal or BCAA supplement before bedtime improves protein–energy nutrition, imbalance of amino acids, or glucose tolerance.[13–15,17–19] In addition, some reports have indicated that a nibbling pattern of food intake, including a good breakfast and a late evening meal, would be preferable in order to have shorter episodes of catabolism during the day.[37–39] Some intervention studies have suggested that nutritional supplementation with oral BCAA is useful in preventing progressive hepatic failure and improving surrogate markers and perceived health status.[40–42] Thus it seems quite probable that a late evening meal acts to counteract malnutrition or nocturnal starvation, suppress the aggravation of liver disease, and as a result, prevent the development of HCC.

The strength of the present study is that the source population was restricted to patients with chronic type C liver disease, which enabled us to make a straightforward interpretation regarding any risk factors for HCV-associated HCC. In addition, we could analyze the data allowing for differences of background factors between the compared groups (e.g. severity of liver disease and the duration from first identification of liver disease).

However, due to the case-control study design within a very special population, that is, patients with chronic hepatitis C, the following three limitations may be present. First, selection bias might be introduced since the source population consisted of patients who had survived to the recruitment period. Patients who developed HCC but died before the recruitment period were not included in the case series, although cases were defined as those patients who had been first diagnosed with HCC in the recent past, that is, within 3 years. However, previous studies have reported that the mortality rate was significantly lower among a nutritional intervention group than among a placebo group.[40,43] It is therefore likely that patients without nutritional support have a higher risk of death. If, hypothetically speaking, cases excluded because of death had been included in this study, the prevalence of never consuming a late evening meal would increase in the hypothetical case series and the OR would decrease. Thus this selection bias may operate to bias the association toward the null, but not lead to exaggerated results.

A second limitation is an information bias resulting from imperfect memory of distant past history of consuming late evening meals. However, the hypothesis that a late evening meal is related to HCC or chronic liver disease was not generally recognized. Thus all patients would receive similar recall stimuli about past late evening meals. The misclassification due to such information bias, if any, is probably non-differential and would not affect the plausibility of the results.

Reverse causation is a third limitation for the observed association, although most retrospective studies suffer from this limitation. The habit of a late evening meal may change over time. However, this results of this study were interpreted from the information of a late evening meal at only one point without considering the potential changes in a late evening meal associated with liver dysfunction. Since more than 30 years may elapse between HCV infection and developing HCC, a late evening meal in the recent past may be affected by already manifested liver dysfunction. A long induction period in HCC can bring about the apparent causative associations, and exposure might be

© 2008 The Japan Society of Hepatology